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EVALUATION OF SWEET CHESTNUT SELECTIONS FROM SEMI – SPONTANEOUS FLORA OF NORTHERN OLTENIA

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Keywords: sweet chestnut, European chestnut, Castanea sativa

ABSTRACT

The sweet chestnut populations from semi-spontaneous flora were studied in Dăești. Bistrița and Horezu localities from Vâlcea county and in Polovragi locality from Gorj county regarding type of flowering and growth characteristics.

A number of 13 sweet chestnut biotypes were selected in Dăești. 6 in Horezu area, 8 in Bistrița and 40 in Polovragi. In terms of phenology differences were noted between biotypes of analyzed locations. Thus, in Dăești, sweet chestnut bud break starts on May 11th and flowering at male flowers began on June 15th and lasted until June 29th comparatively with biotypes of Horezu, Bistrița and Polovragi where flowering began on May 31st and ended on July 7th.

From the point of view of growth. the vigor and tree habitus, trunk circumference and crown diameter varies depending on the biotype, location and age of the plants.

INTRODUCTION

Sweet chestnut or European chestnut (*Castanea sativa* Mill.) is a valuable fruit crop because of its rich nutrients important for the human consumption and for the economical potential (Cociu et al. 2007).

Castanea sativa has been long recognized as a multi – purpose species (Aravanopoulos 2005), because is widely cultivated for timber and nut production and because it represents an integral part of economy in many areas, particularly in rural regions (Diamandis & Perlerou 1996).

Besides the value and quality of its wood the sweet chestnut produce fruit which containing rich nutrients: 52.41 - 56.20 mg/100g - water; from 2.1 - 5.6 mg/200g - total sugar; 16.72 - 18.46 mg/100g - protein, 0.76 - 0.92 mg/100g - cellulose, phosphorus, potassium. calcium, magnesium, iron, vitamins, amino acids, etc. (Radu, 1985 cited by Botu et al., 1999). Taking into account these characteristics, sweet chestnut was the subject of many research studies.

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In case of sweet chestnut the phenotypic diversity is studied using tree, flowers (usually the catkins) and fruit traits (length, width, thickness and weight of the chestnuts, length and width of hilum, number of fruits per kg, embryony, pellicle penetration, cavity, shape and color of fruits, etc) along with phenological stages and molecular markers (Cutino et al., 2010; Furones-Pérez and Fernández-López, 2009a and 2009b; Martin et al., 2011; Solar et al., 2001).

Sweet chestnut is a fruit tree crop, which exploit slopes with thin and poor soils from the North – West of Romania and the depression area of Oltenia.

During time various contributions for determining the suitable areas of culture, selection of valuable biotypes from the spontaneous flora for naming local cultivars, establishing the chestnut breeding technology and modern culture, identification of the specific chestnut diseases and their control (Cociu et al., 2007).

MATERIAL AND METHODS

Vâlcea and Gorj counties have areas with specific microclimates where sweet chestnut finds favorable conditions for growth and fructification (Horezu, Bistrița and Dăești centers in Vâlcea county and Polovragi and Tismana centers in Gorj county).

The biological material investigated in this study includes the local sweet chestnut biotypes identified in the Gorj and Vâlcea counties.

Observations and measurements referred to bud break and flowering times, trees age, growth characteristics, productivity, frequency and intensity of specific diseases attack (mainly chestnut blight caused by *Cryphonectria parasitica* fungus).

RESULTS AND DISSCUSION

In the semi – spontaneous flora of the Northern Oltenia there are many biotypes of sweet chestnut (*Castanea sativa* Mill). The localities of Dăești, Bistrița, Horezu and Polovragi known for their sweet chestnut populations were screened. A number of 67 biotypes were identified during this study and 25 of them are presented in this paper (Table 1).

These biotypes are characterized by different tree vigor and habitus. Depending to the circumference of the trunk which goes from 1.37 m for GJ 1339 selection located in Polovragi (Gorj county) up to 5.30 m to VL 1205 H selection located in Horezu (Vâlcea county). Based on circumference of trunk the trunk cross sectional area (TCSA) was determined (Table 1). The lowest TCSA was recorded for GJ 1339 P (1494 mm) and the highest for (22,365 mm).

Crown diameter of trees varies from 5.60 m in case of VL 1103 B selection up to 15.10 m for VL 1105 B.

The tree height values of the biological material under study ranged from 34 m (GJ 1335 P), 32 m (GJ 1337 P), 29 m (GJ 1338 P and GJ 1336 P), 27 m (VL 1206 H and VL 1202 H), 18 m (GJ 1302 P, GJ 1301 P and VL 1013 D), 13 m (VL 1009 D and VL 1010 D), 15 m (VL 1108 B and GJ 1303P) to 8 m (VL 1103 B), table 1.

The approximate age of the trees was estimated between 20 years in the case of VL 1103 B selection to 350 years at VL 1205 H. The tree vigor varies from low for VL 1008 D and VL 1103 B to high for most studied biotypes (VL 1005 D, VL 1009 D, VL 1013 D, VL 1106 B, VL 1201 H, VL 1205 H, GJ 1303 P, GJ 1304 P and GJ 1340 P). The spread and semi-spread habitus is displayed for all studied biotypes (Table 2).

Table 1

			Trunk cross		
		Trunk	sectional	Crown	Height of
No	Specification	circumference	area	diameter	trees
		(m)	(TCSA)	(m)	(m)
			(mm)		
1	VL 1005 D	2.55	5177	7.50	11
2	VL 1008 D	3.60	10318	13.0	10
3	VL 1009 D	2.90	6696	12.0	13
4	VL 1010 D	2.40	4586	11.0	13
5	VL 1013 D	2.00	3185	11.50	12
6	VL 1102 B	5.10	20709	10.75	22
7	VL 1103 B	1.65	2168	5.60	8
8	VL 1105 B	2.70	5804	15.1	24
9	VL 1106 B	2.60	5382	15.0	16
10	VL 1108 B	4.70	17588	13.1	15
11	VL 1201 H	2.60	5382	12.9	29
12	VL 1202 H	2.60	5382	11.7	27
13	VL 1203 H	2.30	4212	12.40	25
14	VL 1205 H	5.30	22365	15.0	25
15	VL 1206 H	3.40	9204	12.0	27
16	GJ 1301 P	3.00	7166	12.7	12
17	GJ 1302 P	2.40	4586	10.4	12
18	GJ 1303 P	2.74	5977	6.6	15
19	GJ 1304 P	2.40	4586	8.05	20
20	GJ 1335 P	2.60	5382	10.1	34
21	GJ 1336 P	2.10	3511	13.1	29
22	GJ 1337 P	2.60	5382	9.60	32
23	GJ 1338 P	1.70	2301	8.25	29
24	GJ 1339 P	1.37	1494	7.40	20
25	GJ 1340 P	2.00	3185	10.25	22

Growth characteristics of sweet chestnut biotypes selected from Northern Oltenia

Medium and high productivity is present to all selections except for VL 1010 D and GJ 1304 P where the productivity is low. The behavior to main diseases is good. Some of the Horezu and Dăești selections present dead branches due to chestnut blight caused by *Cryphonectria parasitica* fungus (Table 2).

In terms of phenology, some differences were noted regarding the stages timing. In Dăești area, the bud break to chestnut began on May 11 and the male flowers were blooming started to June 15 and lasted until June 29 in opposition with biotypes of Horezu, Bistrița and Polovragi where the bud break started on May 31.

No.	Specification	Tree	Tree	Approximate age	Productivity	Reaction to diseases
		vigor	habitus	of the trees		
1	VL 1005 D	High	Semi-spread	70	High	Less susceptible
2	VL 1008 D	Low	Semi-spread	100	High	Susceptible to Cryphonectria parasitica
3	VL 1009 D	High	Semi-spread	70	Medium	Susceptible to Cryphonectria parasitica
4	VL 1010 D	Medium	Semi-spread	70	Low	Susceptible to Cryphonectria parasitica
5	VL 1013 D	High	Semi-spread	60	High	Less susceptible
6	VL 1102 B	High	Semi-spread	270	Medium	Less susceptible
7	VL 1103 B	Low	Semi-spread	15	Medium	Less susceptible
8	VL 1105 B	High	Spread	180	Medium	Less susceptible
9	VL 1106 B	High	Semi-spread	100	Medium	Less susceptible
10	VL 1108 B	High	Semi-spread	300	Medium	Less susceptible
11	VL 1201 H	High	Spread	190	High	Susceptible to Cryphonectria parasitica
12	VL 1202 H	High	Spread	190	Medium	Susceptible to Cryphonectria parasitica
13	VL 1203 H	High	Spread	200	Medium	Susceptible to Cryphonectria parasitica
14	VL 1205 H	High	Spread	350	Medium	Susceptible to Cryphonectria parasitica
15	VL 1206 H	High	Spread	190	Medium	Susceptible to Cryphonectria parasitica
16	GJ 1301 P	High	Semi-spread	200	High	Less susceptible
17	GJ 1302 P	High	Spread	100	Low	Less susceptible
18	GJ 1303 P	High	Semi-spread	100	Medium	Less susceptible
19	GJ 1304 P	High	Spread	80	Low	Less susceptible
20	GJ 1335 P	Medium	Semi-spread	180	Medium	Less susceptible
21	GJ 1336 P	Medium	Spread	100	Medium	Less susceptible
22	GJ 1337 P	High	Semi-spread	150	High	Less susceptible
23	GJ 1338 P	Medium	Spread	90	High	Less susceptible
24	GI 1339 P	Medium	Spread	60	High	Less susceptible
25	GJ 1340 P	High	Semi-spread	120	High	Less susceptible

Growth and production characteristics to selected biotypes

Table 2

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Table 3

No.	Specification	Bud break time	Beginning of flowering time	Ending of flowering time	Catkin type	Catkin abundance
1	VL 1005 D	11.05-15.05	15.06	29.06	longistaminate	Medium
2	VL 1008 D	11.05-15.05	15.06	29.06	mezostaminate	Medium
3	VL 1009 D	11.05-15.05	15.06	29.06	mezostaminate	Medium
4	VL 1010 D	11.05-15.05	15.06	29.06	longistaminate	Low
5	VL 1013 D	11.05-15.05	15.06	29.06	mezostaminate	Abundant
6	VL 1102 B	25.05-01.06	24.06	07.07	astaminate	Abundant
7	VL 1103 B	25.05-01.06	24.06	07.07	mezostaminate	Medium
8	VL 1105 B	25.05-01.06	24.06	07.07	mezostaminate	Medium
9	VL 1106 B	25.05-01.06	18.06	01.07	mezostaminate	Abundant
10	VL 1108 B	25.05-01.06	18.06	01.07	mezostaminate	Abundant
11	VL 1201 H	25.05-01.06	26.06	10.07	longistaminate	Medium
12	VL 1202 H	25.05-01.06	26.06	10.07	mezostaminate	Abundant
13	VL 1203 H	25.05-01.06	30.06	15.07	mezostaminate	Medium
14	VL 1205 H	25.05-01.06	26.06	10.07	longistaminate	Abundant
15	VL 1206 H	25.05-01.06	26.06	10.07	mezostaminate	Medium
16	GJ 1301 P	25.05-01.06	24.06	07.07	longistaminate	Medium
17	GJ 1302 P	25.05-01.06	24.06	07.07	mezostaminate	Abundant
18	GJ 1303 P	25.05-01.06	24.06	07.07	mezostaminate	Abundant
19	GJ 1304 P	25.05-01.06	24.06	07.07	astaminate	Medium
20	GJ 1335 P	25.05-01.06	24.06	07.07	longistaminate	Medium
21	GJ 1336 P	25.05-01.06	24.06	07.07	mezostaminate	Abundant
22	GJ 1337 P	25.05-01.06	24.06	07.07	mezostaminate	Abundant
23	GJ 1338 P	25.05-01.06	30.06	15.07	astaminate	Medium
24	GI 1339 P	25.05-01.06	24.06	07.07	mezostaminate	Abundant
25	GJ 1340 P	25.05-01.06	30.06	15.07	longistaminate	Abundant

Flowering characteristics of studied sweet chestnut selections

Flowering started on June 24 and ended on July 7 (Table 3). Catkin type varies depending of the biotype: two selections have astaminate catkins and the rest show mezostaminate catkins.

CONCLUSIONS

Local populations of sweet chestnut (*Castanea sativa* Mill) identified in the Gorj and Vâlcea areas are formed from natural and agrobiological valuable hybrids.

The selections presented in this study are interesting because of their fruit and tree traits that might be used after further investigations in the sweet chestnut breeding programs.

In order to have available these selections it is necessary to collect biological material to propagate them through grafting and to introduce them in the germplasm collection from University of Craiova - SCDP Vâlcea.

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EFFECT OF STORAGE TEMPERATURE IN THE OXIDATIVE STABILITY OF SEVERAL NUTS OILS

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Keywords: antioxidant activity, food quality, lipid oxidation, nuts, oil

ABSTRACT

Nuts oils are rich in unsaturated fatty acids susceptible of suffer lipid oxidation that limits their shelf life. The oxidative stability of the oils measures the effectiveness against these reactions that produce unpleasant odours and aromas, and may be used as an indicative of shelf-life of oils. To evaluate the effect of storage temperature in the oxidative stability of nuts oils, samples of Brazil nut, cashew, macadamia nut, peanut, roasted almond, roasted hazelnut and walnut were extracted and stored at room temperature and under refrigeration (4°C). The oils stored at room temperature showed a decrease in the oxidative stability values at the end of the storage period. However, when the oils were stored for 1 year under refrigeration conditions, the values of oxidative stability of all the oil samples analysed remained unchanged. Thus, refrigeration storage may help to increase the shelf life of nuts oils.

INTRODUCTION

Nuts are an energy-rich food predominantly composed of fat, which contains an ample range of phytochemicals included in nutrient databases, like carotenoids, phytosterols or polyphenolic compounds (Bolling et al., 2011). The oil extracted from nuts is mainly composed of unsaturated fatty acids (mono or polyunsaturated). In addition, nut oils contain bioactive and health promoting components (Alsalvar & Pelvan, 2011), which make them a valuable alternative to include in the diet. However, the abundance of unsaturated fatty acids originates a fast lipid oxidation during storage, in a free radical chain reaction between unsaturated fatty acyl groups with oxygen chemical species. The lipid oxidation affects many quality attributes of the oils, originating the release of undesirable odours and aromas that compromises the nutritional quality of nuts oils and may cause rejection and large losses of products (Castello-Branco& Torres 2009, Martinez et al., 2011).

The oxidative stability, expressed in terms of induction time, is an important parameter for the quality assessment of oils. It is related to the free radical scavenging capacity of oils (Arranz et al., 2008), and may be used as an indicative of shelf-life of oils.

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In this paper, the oxidative stability pattern during storage of several oils extracted from nuts is analyzed under two storage conditions (room temperature and refrigeration).

MATERIALS AND METHODS

Nuts samples (Brazil nut, cashew, macadamia nut, peanut, roasted almond, roasted hazelnut and walnut) were purchased at local providers. The moisture was measured to ensure the proper oil extraction. In all the nut samples, the moisture was lower than 5 %.

The oil was extracted with an oil expeller (Komet Oil Press CA59G, IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany). The oil samples were subjected to a centrifugation step at 11,000 x g for 5 minutes to eliminate the solid residues. Three replicates were done for each type of nut oil. The oil samples were stored in dark glass bottles under two temperature conditions: room temperature and refrigeration (4°C).

Oxidative stability was evaluated during 1 year by the rancimat method (Gutiérrez 1989). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 743 apparatus (Metrohm Co., Basel, Switzerland). An oil sample of 3.5 g was used, warmed to 100°C under an air flow of 10 L h⁻¹. Three replicates were done for each oil sample, and the measurements were performed every 2 months.

RESULTS AND DISCUSSION

Lipid oxidation is a problem to the food industry and the consumers, and is one of the factors limiting the shelf life of many food products (Jacobsen, 1999). Oils are composed mainly by fatty acids susceptible to suffer these reactions, especially when they have a high degree of unsaturation. The oxidative stability of the oils measures the effectiveness of antioxidants present in the oil to avoid lipid oxidation reactions that produce unpleasant odours and aromas, and may be considered as a quality parameter of the oils. Figure 1 shows the values of oxidative stability of the nuts oils during 1 year stored at room temperature and under refrigeration conditions (4°C). The initial values of oxidative stability expressed as induction time were higher in the nut oils with a large proportion of monounsaturated fatty acids. Macadamia nut and hazelnut oils have been reported to have about 80% of these fatty acids, mainly oleic acid (Madawala et al., 2012). The induction time at the beginning of the experiment was 57.06 h for the macadamia nut oil and 47.83 for the roasted hazelnut oil. When these values are compared with the olive oil, which is the most studied oil worldwide, macadamia nut and hazelnut oils showed stability values similar to olive oils from the varieties Arbequina or Negrilla (Pardo et al., 2013). However, they are considerably lower than the values obtained for olive oils from the varieties Picual or Cornicabra, that provide highly stable oils (Pardo et al., 2011).

On the other side, the lowest value was obtained for walnut oil, which has been reported to have the highest levels of polyunsaturated fatty acids among nuts. Cashew, peanut, roasted almond and Brazil nut oils showed intermediate oxidative stability values (between 12.42 h and 32.84 h). There is a clear relationship between the fatty acids proportion found in the literature for each oil type (Arranz et al. 2008, Costa et al. 2010) and oxidative stability values, as the ratio of fatty acid oxidation increases with the degree of unsaturation. However, other components found in the oils like tocopherols, polyphenols and other antioxidant compounds may also play an important role in the lipid oxidation and contribute to the stability values (Miraliakbari & Shahidi, 2008, Alsalvar & Pelvan 2011).

When the oils were stored at room temperature, the oxidative stability of all the oil samples showed a clear decrease, even after two months. The oils with higher values of oxidative stability (macadamia nut and roasted hazelnut) suffered a linear decrease during the storage period, finishing with a descent of near 50 % at the end of this period. The oils

with a greater proportion of polyunsaturated fatty acids (walnut, Brazil nut or peanut) suffered even greater descents in the oxidative stability values, which in the case of walnut reached 98 %. The walnut oil showed clear rancid odours after two months of storage, a clear indication that lipid oxidation is being produced in the oil.



Figure 1. Oxidative stability of nuts oils during storage at room temperature and refrigeration.

However, when the oils were stored under refrigeration conditions, the oxidative stability values remained unchanged for the period of 1 year (Figure 1). Even the walnut oil, that is the most liable to suffer lipid oxidation, showed no significant differences in the oxidative stability values after one year of storage under refrigeration.

Several strategies have been proposed to increase the oxidative stability of nuts oils, such as the addition of antioxidants (Marttinez et al., 2013) or the search of those cultivars that provide highly stable oils (Wall, 2010). Other simple strategy is the storage under refrigeration conditions. Refrigeration may contribute to increase the shelf life of these products, especially those with a higher proportion of polyunsaturated fatty acids, where lipid oxidation occurs quickly producing undesirable odours that may cause the loss of the product. The refrigeration conditions limit the oxidation processes in the oils, and it is an interesting alternative for long storage periods of nuts oils, avoiding the use of other techniques such as the application of antioxidants that may interact with the sensory characteristics of these oils, or the bottling under modified atmospheres.

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EFFECT OF PRESSURE EXTRACTION SYSTEM ON QUALITY PARAMETERS OF VIRGIN PISTACHIO OILS

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Keywords: food quality, nuts, oil, pistachio

ABSTRACT

Pistachios are a good source for oil extraction, as they contain about 50 % of oil rich in unsaturated fatty acids and other bioactive components like polyphenols and phenolic compounds. The yield and quality parameters of oil extracted from four batches of pistachios with different size were analyzed. Two different pressure systems (screw press and hydraulic press) were used for oil extraction. The yield was higher when the screw press was used, especially when the highest quality pistachios (larger pistachios) were used to extract the oil (40 ± 2.12 %). With the hydraulic press, the yield was around 30% for all pistachio types. The colour of the oils extracted with the screw press was darker than the oil extracted with the hydraulic press in all types of pistachios used. No significant differences were found in the acidity, K₂₇₀, and K₂₃₂ values when high quality pistachios were used. However, when lower quality pistachios (smaller pistachios) were used, the values of these three parameters increased in comparison with the largest pistachios, especially when the screw press was used. The oxidative stability was significantly higher in the samples of oil from high quality pistachios, with no differences in regard to the extraction system.

INTRODUCTION

Among nuts, pistachio (*Pistacia vera* L.) is an increasingly important crop, and one of the favourite nut trees in the world. It is widely cultivated in arid zones of the eastern Mediterranean and the US, and recently it is being introduced successfully in hot dry regions of Spain. Pistachios contain about 45-57% of oil, with a high proportion of unsaturated fatty acids like oleic, linoleic and linolenic acids (Arena et al., 2007; Tavakolipour et al., 2010; Tsantili et al., 2010). In addition, pistachios are a rich source of phytosterols and phenolic compounds (Phillips et al., 2005; Tomaino et al., 2010). These components have beneficial effects on human health, as phytosterols have been shown to reduce blood cholesterol and decrease the risk of certain types of cancer (Awad & Fink, 2000; Ostlund, 2004), whereas the phenolic compounds show antioxidant properties (Gentile et al., 2007).

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In this work, the oil was extracted from four batches of pistachios with different quality according to their size by means of pressure. Two pressure systems were used: screw press (also called oil expeller) and hydraulic press. The yield of both methods and the quality characteristics of the pistachio oil extracted with the two systems were analyzed in order to determine possible changes occurring in the oil derived from the extraction system.

MATERIALS AND METHODS

Sampling

Pistachios were kindly provided by "Pistachos de la Mancha" factory (Balazote, Albacete, Spain) from orchards surrounding the factory. Four batches of pistachios (*Pistacia vera* L., var. Larnaka) were used for this analysis: a commercial batch (labeled as Larnaka), and three batches of pistachios non suitable for commercialization due to the small size or the presence of defects like dark spots or broken kernels, labeled as A, B, and C. In the Table 1 the average size of the different batches of pistachios are shown.

Moisture content was calculated by the difference in weight of 5 g sample before and after complete drying at 105 $^{\circ}$ C overnight using a dissication oven with forced ventilation.

Table 1

Туре	Weight (g)	Width (mm)	Length (mm)
Larnaka	0.59 ± 0.10	8.80 ± 0.71	16.3 ± 1.64
А	0.54 ± 0.11	8.45 ± 0.60	16.2 ± 1.55
В	0.42 ± 0.08	8.10 ± 0.57	15.4 ± 1.78
С	0.28 ± 0.04	7.00 ± 0.53	13.5 ± 1.27

Weight, width and length of the different type of pistachios used. The values represent the average and standard deviation of 100 pistachios.

The oil was extracted with two different pressure systems (Figure 1): hydraulic press (Hidráulica Dumont S.A., Santiago, Chile) and screw press Komet Oil Press CA59G (IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany). For the hydraulic press, 200 g of pistachios were previously grinded and subjected to a pressure of 60 kg/cm² during 10 min. For the screw press, 200 g of pistachios were introduced directly in the press. In both cases, 3 replicates were prepared for each type of pistachio.

After oil extraction, a centrifugation step was carried out in order to eliminate the remaining solid residues from the samples. The oil samples were stored at 4°C in dark glass bottles to avoid oxidation until their analysis.

Analytical determinations

The colour of the samples was measured with a Minolta CR-200 colourimeter (Minolta Co., Ltd., Osaka, Japan). The illuminant used was D65, with a viewing angle of 2°. The tristimulus values obtained were used to calculate the CIELAB chromatic coordinates L* (lightness), a* (redness), and b* (yellowness), and color attributes C* (chroma) and h* (hue angle), as recommended by the Commission Internationale de l'Eclairage (CIE, 1986; McGuire, 1992).

Free acidity, given as % of oleic acid, was determined by titration of a solution of oil dissolved in ethanol/ether (1:1) with 0.1 M potassium hydroxide ethanolic solution (EEC, 1991).

 K_{270} and K_{232} extinction coefficients were calculated from absorbance at 270 and 232 nm, respectively, with a UV/VIS spectrophotometer Jasco V-530 (Jasco Analitica Spain, Madrid, Spain), and a path length of 1 cm. For K_{270} , a 1% solution of oil in cyclohexane was used, while for K_{232} a 0.5 % solution of oil in cyclohexane was used (EEC, 1991).

Statistical analysis

Significant differences among samples were determined by an analysis of variance which applied a Duncan test with a 95% significant level (P < 0.05), using the SPSS programme, release 11.5 for Windows.

RESULTS AND DISCUSSION

Yield

The yield for both extraction systems was determined (Figure 2). The highest yield was obtained for the sample Larnaka with the screw press ($40 \pm 2.12\%$). In general, when both extraction methods were compared, a slight increase in the yield was observed for the screw press, although this difference was significant only when the oil was extracted from the pistachios labeled as "Larnaka". When pistachios of quality A, B, and C, were used for oil extraction, the difference in the yield for both extraction systems was not significant. In addition, the yields obtained with the screw press showed more variation. Although the screw press is considered as a cold extraction system, it requires an increase in the temperature of the tip to obtain the best results. An increase in the temperature produces a better separation of the oil, which may affect the extraction yield. In some screw presses, the temperature is difficult to control, and therefore the yield presents greater variations. The hydraulic press is easier to control, as the only parameters that influence the process are: the pressure (which can be easily measured by a manometer installed in the press), the time, and the amount of pistachios used.

Colour

The colour parameters were expressed as L^* (colour intensity or lightness), a^{*} (redness), b^{*} (yellowness), C^{*} (chroma, or intensity of colour), and h^{*} (hue angle). Figure 3 shows the values of L^{*}, b^{*}, C^{*}, and h^{*}, where significant differences between both extraction methods were found for all the samples. The oil extracted with the screw press showed lower values of the 4 parameters, indicating darker-greener oils. A similar pattern has been observed when roasted seeds are used to produce oil (Lee et al., 2004; Park et al., 2011). As stated above, the screw press requires an increase in the temperature for a correct oil extraction. It originates the change in the color of the oil to dark, probably due to the appearance of products from the Maillard reaction, caramelization or phospholipids degradation Durmaz & Gökmen, 2011; Vaidya & Choe, 2011).

In the case of the oils obtained with the hydraulic press, lower values of L^* , b^* , C^* and h^* were observed when lower quality pistachio samples were used (Figure 3). The

quality of the pistachios, especially the presence of dark spots in the kernel, is a parameter that may influence the colour of the oil when a cold extraction system with no increase in the temperature is used. In this aspect, it is important to make a selection of the pistachios in order to obtain lighter and more attractive colours in the oil. When the screw press is used, the effect of the quality of pistachio is hidden due to the appearance of Maillard reaction products, originating more similar colour in the oils extracted from the different types of pistachios.

Acidity, K270, K232

The acidity, measured as % of oleic acid, and the extinction coefficients K_{270} and K_{232} are parameters used to estimate the quality of the oil. The values obtained for acidity, K_{270} and K_{232} are shown in Figure 4. Lower values of acidity indicate the freshness of the samples, the good quality of the oil, and no chemical or enzymatic hydrolysis of glycerolipids. The highest values of acidity were observed in sample C for both extraction systems. In regard to the extraction effect, the oils obtained from lower quality pistachios (B, C) showed significant higher values of acidity when the screw press was used. Indeed, acidity may be affected by temperature, and thus, the oils obtained with the screw press present higher proportion of free fatty acids hydrolyzed from the glycerolipids. However, when high quality pistachios were used (Larnaka, A), the acidity values were similar independently of the extraction system used. Hence, we cannot clearly exhibit the effect of temperature on the variations of acidity.

As for the ultraviolet spectrophotometric analysis, it indicates the degree of oil oxidation, being its values expressed as specific extinction coefficients. The K_{232} and K_{270} are mainly indicative of the conjugation of dienes and trienes respectively. Figure 4 shows the values obtained for K_{270} and K_{232} in the oil samples extracted with the different systems. The oils extracted with the screw press present higher values of both attributes for all the samples except for the pistachios of the best quality (Larnaka), where the values are similar. In addition, differences were also observed depending on the sample analyzed. The highest values were obtained when the pistachios of the lower quality (B, C) were used to extract the oil.

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Figure 1. A) Screw press Komet Oil Press CA59G (IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany). B) Detail of oil extraction with the screw press. C) Hydraulic press (Hidráulica Dumont S.A., Santiago, Chile). D) Detail of oil extraction with the hydraulic press.



Figure 2. Yields of pistachio oil extracted with the screw press and the hydraulic press. Values represent the average and standard deviation of three replicates. Different letters on the bars indicate significant differences (p<0.05) among samples (Duncan test)



Figure 3. Colour characteristics of the pistachio oil samples extracted with the screw press and the hydraulic press. L*: lightness; b*: yellowness; C*: chroma; h*: hue angle. Values represent the average and standard deviation of three replicates. Different letters on the bars indicate significant differences (p<0.05) among samples (Duncan test)







Figure 4. Acidity and spectrophotometric analysis (K_{270} , and K_{232}) of pistachio oil samples extracted with the screw press and the hydraulic press. Values represent the average and standard deviation of three replicates. Different letters on the bars indicate significant differences (p<0.05) among samples (Duncan test)

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THE INFLUENCE OF THE CLONE AND ROOTSTOCK ON THE RED WINE COMPOSITION FROM THE VINEYARD SÂMBUREȘTI

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Key words: vineyard, clone, quality, red wine

ABSTRACT

Cabernet Sauvignon and Merlot are the main varieties for red wine grown in the vineyard Sâmbureşti and they are part of the old and the new assortment of vineyard. Sâmbureşti vineyard prestige is due largely of quality of red wines made from two varieties, recognized worldwide for their ability to give high-class wines. Research conduced in 2010, 2011 and 2012 in the Sâmbureşti vineyard show that some clones of Cabernet Sauvignon and Merlot newly introduced in assortment have a high capacity to accumulate sugar and polyphenols, the main groups of constituents responsible foe red wines quality. Combination clone/rootstock has a significant influence on the main parameters of chemical composition and colour of red produced in the same technology conditions.

INTRODUCTION

The fame of pleasant peculiarity of Romanian wines is given not only by extremely high value of some local varieties which have given renown wine centers but also the most reputable foreign varieties that made famous some other regions and countries, being grown in Romania give wines often equal or surpassed by the generosity and the smoothness of their home countries (Popa A. & Dicu C., 2010).

Winegrapes are a climatically sensitive crop whereby quality production is achieved across a fairly narrow geographic range. In addition, winegrapes are grown largely in mid-latitude regions that are prone to high climatic variability that drive relatively large vintage differences in quality and productivity (Jones G.V. 2010).

Sâmburești is one of the most famous Romanian vineyards for quality red wines (Băducă C. et al. 2012). At Sâmburești, Cabernet Sauvignon, the reddish-brown forest soils, allow some of the most reputable red wines in Romania (Popa A., 2008).

The Samburesti Cabernet is distinguished from other wines produced from this variety by an additional force by its robustness and firmness, by his great personality, given the wealth of compounds involved in the formation of color, aroma and flavor (Macici M.,

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1996). Its qualities put it on the prestigious place in the hierarchy of Romanian wines (Cotea V.D. et al., 2003).

Along with Cabernet Sauvignon, Sâmburești Merlot, part of traditional assortment of vineyard contributed to the fame of vineyards (Băducă Cîmpeanu C., 2008).

Sâmburești Merlot wine is enticing and bears the embodiment of characteristics due to the variety and vineyard known as favoring the production of high mark red wines (Cotea D.V. et al. 2003).

MATERIAL AND METHODS

The work has been accomplished on the basis of physico-chemical analyzes carried out on red wines produced in the years 2010 and 2011 from different clones of Merlot and Cabernet Sauvignon recently introduced in the new range of vineyard Sâmbureşti. The wines from this study were obtained at the Faculty of Agriculture and Horticulture, University of Craiova through micro-winemaking of grapes from the vineyard Sâmbureşti. Each year, the grapes of different clones of the two varieties were harvested on the same day, were processed at the same time by the same scheme for winemaking, so the only difference between them is their own potential quality of each clone, and each combinations clone / rootstock.

Wines were subject to composition analysis at Oenology Laboratory of the Faculty of Agriculture and Horticulture 3 months after vinification. Analyzes on phenolic composition and color structure of the wines were made in the Laboratory of Physico-chemical analysis of horticultural products from the Faculty of Agriculture and Horticulture in Craiova, in several stages, in order to track the evolution in time. Combinations clone / rootstock in the study will be presented along with results.

Vineyards from which the grapes came were established in 2008, which means that the 2010 wines come from a third year plantation, while the 2011 wines come from a the first year of production plantation.

RESULTS AND DISCUSSIONS

In the case of Merlot, It has been studied same clones in 2010 as well as in 2011. These clones are: 181 (grafted on 2 rootstock - 1103P and SO₄), 337 and 343. In the case of Cabernet Sauvignon, It has been some differences between the clones studied in two years period of time. The data on the chemical composition of Merlot wine are set out in Table 1 and the chemical composition of Cabernet Sauvignon wine are set out in Tables 2 and 3.

The analisys of the chemical composition of the wine shows obvious superiority of the Merlot clones comparing to Cabernet Sauvignon in terms of alcoholic strength of wines. Thus, in the case of 2010 wine, the clones obtained from Merlot have, on average, 2% vol, more alcohol, while in case of 2011 wine the difference is less, an average of 1% by volume. This is surprising because the traditional assortment of Sâmbureşti vineyard, Cabernet Sauvignon has always been top quality of Merlot. In this case of new clones planted in 2008, the Merlot has a greater capacity to accumulate sugars, at least for the first harvest. It is important to note that these differences are not due to delayed harvesting of the grapes, as in the two-year study material for experiments grapes were harvested almost simultaneously, on consecutive days, each time clones of Merlot was harvested the day before those of Cabernet Sauvignon.

Meanwhile, it is noted that the clones of Merlot wines obtained from clone 181 were always more than 14% vol alcohol. Regarding Cabernet Sauvignon wines, it appears that in 2011 the alcoholic strength was approximately 1% volume higher than 2010, under

similar climatic conditions. This increase of the most important parameter of composition can be attributed clearly to the fact that plantation came in bearing, vines have grown and strengthened, leading to a greater capacity to accumulate sugars. One argument for this is the fact that in 2011, the wine with the highest alcoholic strength was obtained from a 20 year old plantation, located in Roşuleşti point. The wines made from new clones introduced range of 1-2% alcoholic strength by volume lower, which is an important difference. Most alcoholic strength of the wine of the new clone is 13.7% volume from clone 685/SO₄, the same clone from which in 2010 the wine had an alcoholic strength higher by 1 - 1.5% more volume than the other clones and the only one with more than 13% vol alcohol.

The second parameter analyzed composition is glycerol, the second chemical constituent of wine, after alcohol in terms of quantity and also the main byproduct of alcoholic fermentation. Glycerol is an important constituent for the balance of the wine taste as they participate in the sensation of smoothness and softness of the wine, especially red wine, which should mitigate the hardness and roughness of tannins, which occur in abundance in young wines. Analysis of this parameter shows significant differences between the wines from 2011 and 2010, regardless of the variety and clone. A single clone of Merlot, 337/1103P, showed a slight decrease in glycerol content in 2011 compared to 2010, the rest were increases of 0.6 to 1.1 g/L clones of Merlot and up 2 g/L Cabernet Sauvignon clones. Most spectacular growth was recorded at 685/SO₄ clone of Cabernet Sauvignon, from 10.8 to 12.8 g/L, given that this clone ethanol content increased only by 0.3 g/L. Large increases were recorded at combinations wines of Cabernet Sauvignon clone rootstocks 169/SO4 (from 10.2 to 12.1 g/L) and 169/R110 (from 10.1 to 12.0 g/L). The highest glycerol content was recorded at the 2011 Cabernet Sauvignon wine from old plantation, 13.2 g/L at an ethanol content of 14.8% vol, also the largest.

Residual sugar content show that for all clones of Merlot, the wines from 2010 are semidry, with content over 4 g/L, while all the 2011 wines are dry, with content between 3.2 and 3.8 g/L. In the case of Cabernet Sauvignon clones, only 3 wines from 2010 have had over 6 g/L, the rest of 2010 wines and all of 2011 wines have been dry wines with less than 4 g/L.

Table 1

Clone	Alcohol,		Glycerol,		Residual		Total		Volatile		Free SO ₂ ,		
	% vol.		g/L		sug	sugar,		acidity,		acidity,		mg/L	
			g/L		g/L H ₂ SO ₄		g/L acid						
									ace	etic			
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	
181/1103P	14,3	14,1	11,9	12,5	5,2	3,8	3,23	4,7	0,38	0,36	16	12	
181/ SO4	14,2	14,4	11,7	12,8	8,8	3,6	2,38	5,1	0,44	0,41	15	12	
337/1103P	14,8	13,7	12,4	12,0	4,8	3,5	2,49	4,8	0,41	0,40	12	14	
343/ SO4	14,0	14,1	11,6	12,4	7,1	3,2	2,65	4,6	0,36	0,42	18	15	

The chemical composition of Merlot wines

The next parameter analyzed is the total acidity composition. Analysis of data on the total acidity of the wines show dramatic increases in clones of Merlot in 2011 compared to 2010, in some cases taking place even a doubling of values (eg, clones $181/SO_4$ from 2.38 to 5.1 g/L H₂SO₄ and 337/1103P, from 2.49 to 4.8 g/L H₂SO₄). And clones of Cabernet Sauvignon wine is noted that in 2010 the total acidity content between 3.41 and 4.18 g / L H2SO4 while the 2011 wines the content between 4.5 and 5.3 g/L H₂SO₄. It is

important to note that the significant increase in total acidity place in conditions that increased the alcohol content. This evolution of the composition parameters is explained by the age and development of vines, which allowed increased physiological and biochemical processes that led chemically much better grapes came bearing plantation compared with that contained in the third of existence.

Table 2

Clone	Alcohol, % vol.	Glycerol, g/L	Residual sugar, g/L	Total acidity, g/L H ₂ SO ₄	Volatile acidity, g/L acid acetic	Free SO ₂ mg/L
169/Fercal	12,5	10,4	4,8	3,41	0,54	11
169/ SO4	12,0	10,2	4,1	4,11	0,62	14
169/R140	12,1	10,2	6,1	4,16	0,58	12
169/R110	12,0	10,1	2,8	4,08	0,64	16
337/Gravesac	12,6	10,5	6,4	4,18	0,48	10
341/ SO4	12,4	10,3	6,5	3,91	0,51	17
685/ SO4	13,4	10,8	3,8	3,80	0,36	14
685/R110	12,5	10,3	3,5	3,34	0,42	12

The chemical composition of 2010 Cabernet Sauvignon wines

Volatile acidity is the most important indicator of health status and biological stability of wines. The analysis of data on volatile acidity of wines from Merlot shows that their values between 0.36 and 0.44 g/L are very good while under Romanian legislation provides for a maximum of 1.2 g/L acetic acid for red wines under two years, as the case of these wines. Therefore, these values of volatile acidity, almost 3 times below the upper limit indicate that all wines are healthy and there is no danger of short and medium-term biological instability. In addition to the volatile acidity values the organoleptic qualities are not affected. For the wines of 2011 Cabernet Sauvignon, the volatile acidity is also well below the limit set by the law of viticulture and wine. On the other hand, for the 2010 wines, only 3 out of 8 samples have values below 0.5 g/l acetic acid, and the two wines exceed 0.6 g/L acetic acid. For these wines, even if the values are well below the legal limit, olfactory characteristics begin to be in danger.

In both varieties, some clones are grafted on different rootstocks, resulting in more clone/rootstock combinations, among which there are differences in the parameter composition of wine. On Merlot, the two combinations of clone 181 with 1103P and SO4 rootstocks, the differences were small for all the parameters of composition. On the other hand, the 2010 Cabernet Sauvignon, between the four combinations of clone 169 the differences were up to 0.5% vol in the alcoholic strength, up to 0.3 g/l to the glycerol content and up to 1 to 3.3 g/l residual sugar content. In 2011, the differences were up to 0.4% in alcoholic strength by volume and 0.3 g/l to glycerol content. The largest differences occurred between the two combinations of clone 685 in 2010 to 0.9% in alcoholic strength by volume and 0.5 g/l to glycerol content in favor of SO4 rootstock combinations.

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Table 3

Wine	Alcohol, % vol.	Glycerol, g/ L	Residual sugar, g/ L	Total acidity, g/ L H ₂ SO ₄	Volatile acidity, g/ L acid acetic	Free SO ₂ , mg/ L
Roșulești	14,8	13,2	3,0	4,5	0,38	12
169/ SO4	13,3	12,1	3,2	5,0	0,42	16
169/R110	13,1	12,0	3,1	4,7	0,36	20
169/Gravesac	13,5	12,4	3,6	5,2	0,40	15
337/Gravesac	12,8	11,5	3,4	5,3	0,33	22
685/SO4	13,7	12,8	3,5	4,9	0,42	14

The chemical composition of 2011 Cabernet Sauvignon wines

The largest differences occurred between the two combinations of clone 685 in 2010 to 0.9% in alcoholic strength by volume and 0.5 g/l to glycerol content in favor of SO4 rootstock combinations.

CONCLUSIONS

The analysis of the chemical composition of wines produced in the years 2010 and 2011 from clones of Cabernet Sauvignon and Merlot of Sâmbureşti vineyard, newly introduced leads to the following conclusions.

Merlot and Cabernet Sauvignon varieties are part of traditional assortment of Sâmburești vineyard. Currently, there are several clones of the two varieties grafted on different rootstocks in the structure of newly established plantations.

The chemical composition of wines produced in the years 2010 and 2011 shows very good composition parameters as for high-class wines. On Merlot, the best results were obtained from clone 181 and the rootstock SO4, the wines made from these combinations of clone or the rootstocks detaching from others. For Cabernet Sauvignon, the clone 685 made best worth on SO4 rootstock and clone 169.

Both in 2010 and in 2011, the Merlot clones showed a higher capacity for accumulation of sugar compared to clones of Cabernet Sauvignon. The alcoholic strength of wine is higher, on average 2% vol in 2010 and 1 % volume in 2011. These differences were found in both varieties while harvesting at the same time but harvesting at different times, the results may change.

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INFLUENCE FOLIAR FERTILIZATION IN CONJUNCTION WITH FRUIT THINING ON APPLE PRODUCTIVITY IDARED VARIETY

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Keywords: Fertilizer, Idared, Thinning, Urea.

ABSTRACT

Fruit thinning is a major influence on high production next year, extra quality fruit this

year.

The company's apple orchard S.A. Zubresti have been investigated by a combination of fruit thinning of 10 - 12 and 16 - 18 mm in the central flower of the application of foliar fertilization with 46% Urea N in doses ranging from 0.4 - 1.2%, Polyfeen (19 : 19:19 + 6 elements) at a dose of 0.1%, CaCl 2 at a dosage of 0,5 - 0,7%.

The number of the variety of Idared fruit research during the years increased from 155 units to 233 units followed by growth until harvest per hectare to 57.2 t in variant with foliar fertilization with the concentration of 0.6%, 0.9%, 12% and more, when the fruit center thinning is 10 to 12 mm in diameter.

INTRODUCTION

Foliar fertilization, chemical and manual fruit thinning are significant contribution, to maintain physiological balance between growth and fruiting also the quantity and the quality of fruit. (V. Balan, 2009)

The thinning of trees and the sprinkling of flowers is accomplished during or after flowering with chemicals that destroy pollen, unfertilized flowers, stigmas and embryo of seeds from the fruit barely related.

Fruit thinning is the removal of a number of apples than the optimum number of fruits per tree. The optimal number of apples per tree allows obtaining high yields, necessary commercial aspect, color characteristic of the variety, taste and flavor appropriate and also ensures favorable conditions for the formation and differentiation of flowering buds. (Cimpoieş. Gh, 2012)

The number of apples on a fruit tree is optimal if leaves are well-developed 30-50 cm2 or a cross-sectional area of the trunk 5-7 fruits (Babuc V., Rusu. T., 1997).

The thinning apples is realized by chemical and manual thinning.

Hungary uses chemical thinning effect of auxin and ethylene. The most effective are alpha-and alpha-naphthylacetamide naphthylacetic (Babuc V., 2012).

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The chemical thinning reduces the need of expensive labor force, but also the use products of polluting chemical thinning. Another disadvantage is that the crown fruit thinning is uniform.

Hand thinning of fruit is the safest and the most effective method. It provides a uniform thinning of fruits, and an optimal number of apples. Also it's not polluting method, but this method is very thorough, requiring 20-40 days / ha for a short time (Cimpoieş Gh, 2012).

The formation in apple harvest takes place over two growing seasons separated by a period of rest. In the second growing season linking occurs, growth and fruit maturation. Linking fruit going through the reserve substances accumulated in the tissues of the previous year because of the trees bloom before the leaves form aces or when they are poorly developed (Balan V., et al. 2001).

MATERIALS AND METHODS

We studied the interaction of foliar fertilization and fruit thinning, as key determinants of the quantity and quality of apple fruit.

Table 1

Nr.	The period of effect foliar	Folia	ge fertilizer	concent	ration			
	fertilization	V1f	V2f	V3f	V4f			
	Urea 46 %N active s	ubstance						
1	After bloom (when the 75% where in	water	0.4	0.5	0.6			
	bloom)							
2	When the fruit has the size of one nut	water	0.7	0.8	0.9			
	(fruit have 10-12 mm in diameter)							
3	When the fruit has the size of one	water	1.0	1.1	1.2			
	walnut (fruit have 25-30mm in							
	diameter)							
	Polyfeed (N19:P19:K19)							
4	When fruits has the ripen stage (20-	water	0.1	0.1	0.1			
	30 July)							
	Calcium chloride(CaCl2)							
5	With 20-30 days before harvest	water	0.5	0,6	0,7			

Scheme applying foliar fertilizers apple variety Idared

The influence the study of fruit load thinning processes of growth and fruiting in apple orchard covered the period of 2011-2012 organized in a apple orchard in SA "Zubresti" Străşeni district. We studied Idared apple variety, grafted on rootstock M 26, planted in 2003, at a distance of 4 m between rows and 2 m between trees in a row. Treatments were applied with 46% N urea Concentrate from 0.4% to 1.2% by adjustable single phase of growth (Tab. 1) when the temperature was 16-180C. Each variant consisted of four repetitions of each three trees, arranged by randomized block system. Chemical fruit thinning was achieved when the central fruit has 10-12 mm in diameter (Tab 2) the preparation Bioprzerzedzacz 060SL is consuming 0.075% by 7.5 ml per 10 liters of water for 12 trees and 1000 l solution per hectare respectively.

The hand thinning of fruit is usually made in June until July 5 to 10 that is no later than 30 days after the bound. This thinning has a role in improving fruit size and quality. Thinning is leaving one fruit inflorescence 10-15 cm from each other. Remove the first small fruit, deformities, diseases and then to normal attack.

The stabilities for each tree harvest is separate from the production weighing 24 trees and making the arithmetic mean. The average weight of the fruit is determined by weighing with a balance of the 100 apples, which are both formed and the degree of maturity.

The experiment was mounted on the plane of rotation in accordance with the method of organizing experiences factorial (foliar fertilization, thinning chemical and mixed manually fruit) and included variants with the following scheme for the use of foliar

Table 2

Variant	Fruit thinning methods					
V _{1r} (control)	Untreated.					
V_{2r} (chemical thinning).	Managing chemicals when the fruit diameter central inflorescence is 10-12 mm.					
V_{3r} (chemical thinning + manual thinning). Managing chemicals when fruit diameter of the centre inflorescence is 10-12 mm. Fruit thinning is performed manually by physiological fall when the fruits have 16-18 m in diameter.						
V_{4r} (manual thinning).	Fruit thinning is performed manually by physiological fall when the fruits have 16-18 mm in diameter.					

Fruit thinning methods.

fertilizers and methods of thinning the fruit: V1 (V1f + V1R), V2 (V1f + V2R) V3 (V1f + V3r) V4 (V1f + V4R), V5 (V2F + V1R) V6 (V2F + V2R) V7 (V2F + V3r) V8 (V2F + V4R) V9 (V3f + V1R), V10 (V3f + V2R) V11 (V3f + V3r) V12 (V3f + V4R) V13 (V4f + V1R) V14 (V4f + V2R) V15 (V4f + V3r) V16 (V4f + V4R);

RESULTS AND DISCUSSIONS

In 2011 the number of fruit per cultivar Idared was included in a range of 115 units in version V 3f - 4r and 186 units in version V3f -1r.

The fruit number in 2012 increased in all variants. The smallest increase was recorded in control with an increase of 29 units and fruit was 184 and the highest increase was recorded in the variant V 4f-1r with 233 units with an increase of more than 38 units in 2011. A significant increase was recorded without fertilization and fruit thinning where no fruit has greatly increased from 20 units with manual fruit thinning V 1f-4r and amounted to 36 units to 200 units invariant V1f - 2r with chemical thinning fruit and constituted 202 units.

In under variants with foliar fertilization notice that the number of fruit is lower than the version where only fertilization foliar applied urea 46% N as planned experience, it is because influence thinning load bearing trees by applying the three methods of thinning fruits that are chemical thinning, mixed fruit and manual.

Even if the number of remaining fruit is the same as in the control, they are more qualitative due to the application of foliar fertilization increases leaf area needed to develop fruit from the tree.

The biggest difference between variants of fertilization and thinning fruit is variable V 3f where the sub-variants with manual fruit thinning V 3f-4r joined 167units.

The difference between the control V 1f - 1r and variants thinning and without foliar fertilization is 28% but the difference between thinning without fertilization variants is less than 1.2% of the variants V 1f - 1f 2r and variant V - 3r 4 3% of variants V 1f - 2r and manual fruit thinning variant V 1f - 4r.

In 2012, the fruit weight increased in all variants studied.

The variants without fertilization and fruit thinning, fruit weight is between 180 g chemical fruit thinning version and 188 g version with manual thinning fruit.





The variable V 2f where the concentration of urea applied is 46% N 0.4%, 0.7%, 0.7%, mean fruit weight was 152 g, but the foliar fertilization beside the normalization with

1.0%, mean fruit weight was 152 g, but the foliar fertilization beside the normalization with the application of the load bearing, greatly increased fruit weight of 197 g fruit chemical thinning with 200 g under variants V 2f-3r and V 2f-4r where applied mixed fruit thinning and thinning manual.

The highest fruit weight was recorded version V 4f-1r where the Urea 46% N concentration is 0.6%, 0.9%, 1.2% with 158 g. In under variants where to apply thinning fruit weight is 230 g in the chemical thinning fruit with the lowest fruit weight was recorded without thinning the fruit 215g.



Figure 2. The weight of fruit from the cultivar Idared apple trees by thinning fruit and foliar fertilization with mineral fertilizer application, g., (Rootstock M26, 4x2m planting distance, trees age 8-9 years, SA "Zubresti")



Figure 3. The production of fruit by thinning Idared variety of fruits and application of foliar fertilization with mineral fertilizers, t / ha. (Rootstock M26, 4x2m planting distance, trees age 8-9 years, SA "Zubresti")

The fruit production per hectare is determined by the amount of fruit on a tree, so the least amount of fruits was recorded in the control to 29.9 t / ha and the highest in version V2r - 4f 57.2 t / ha.

The fruit harvest variants of foliar fertilization without thinning fruit increases with a concentration of Urea 46% N applied as in variant V2f -1r fruit harvest was 38.4 t / ha then V4f-1r variant where the concentration of fertilizer is a maximum of 0.6%, 0.9%, 1.2% fruit crops was 46.0 t / ha.

In all variants yield is higher than in variants without fertilization and fruit thinning. In the variant with manual fruit thinning and fertilization with urea 46% N V 1f - 4r fruit harvest is 47.0 t / ha, chemical fruit thinning variant V 1f-2r harvest in 2012 was 45.4 t / ha.

In 2011 - 2012 the lowest crop per hectare was recorded in the control V 1f-1r without fertilization and fruit thinning 24.5 t / ha, and the largest in variant V4f -2r 49 8 t / ha.

CONCLUSIONS

The value of fruit depends largely on: Administration fertilizers Urea 46% as active substance polyfeed (N19: P19: K19) and calcium chloride (CaCl2) that contribute to increase the fruit yield.

The fruit thinning has also a great importance on the quantity of the production of apples. The highest yield per unit area was recorded in the V14 version with 57.70 tons hectare where the foliar fertilization was applied at a concentration of 0.6% -1.2% in the growing phase and chemical fruit thinning stage 10 -12 mm of the central fruit blossom, and the lowest yield was recorded in control V1 which did not apply any method of thinning.

The foliar fertilization and the thinning fruits are valuable agronomic processes, which determine the quantity of the production scheduled for harvest as well as stability in the future.

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A COMPARATIVE STUDY OF THE QUALITY OF G₀ GRAPEVINE INITIAL PLANTING MATERIAL

Bejan Carmen¹, Visoiu Emilia, Nedelea Gina

Keywords: grapevine propagating material, maturation, soluble carbohydrates, starch

ABSTRACT

In recent years, Feteasca neagra 6 St., Pinot noir 3 St., Cabernet Sauvignon 131 St., Feteasca alba 97 St., Feteasca regala 72 St. grapevine clones, have been officially released and recommended for propagation, at a commercial level, in the Stefanesti vineyard. The comparative analyses on the maturation degree of the cuttings collected both from the field and from the greenhouse showed the qualitative superiority of the biological material grown in the safe conditions of the greenhouse (G_0 grapevine initial planting material). The total water content ranged between 46% (field) and 52% (greenhouse) in the red wine varieties, and the total carbohydrates content (soluble sugar and starch) was between 11% (field) and 14% (greenhouse). In the white wines varieties, the same indicators registered similar values for the water content in the woody tissue of the material grown in the depositary greenhouse but it was low in the material collected in the field.

INTRODUCTION

As part of the national program for producing the grapevine propagating material, the current research undertaken in the NRDIBH Stefanesti-Arges gives a great importance to the plants obtained through the virus elimination techniques and maintained in the depository greenhouse the biological category of G_0 Initial material, the only institution in the country that undertook this type of activity since 1988 (Buciumeanu et al., 2003, Visoiu et al., 2006). Clones, quite recently approved and proposed for propagation and production expansion in the Stefanesti vineyard, were selected to be studied and analysed (Feteasca neagra 6 St., Pinot noir 3 St., Cabernet Sauvignon 131 St., Feteasca alba 97 St., Feteasca regala 72 St.).

Observations and measurements were performed to highlight the influence of the culture conditions in the depositary greenhouse on the maturation of the woody tissue, in comparison to the breeder's material grown in the vineyard. The obtained biochemical indicators were essential for the qualitative assessment of the propagation material. The assessment of the degree of maturation of the canes was performed through biochemical tests, by determining the total water and the total carbohydrates (soluble sugar and starch) content in the wood.

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MATERIAL AND METHODS

After their approval, the clones of Feteasca neagra 6 St., Pinot noir 3 St., Cabernet Sauvignon 131 St., Feteasca alba 97 St., Feteasca regala 72 St., selected for these study and analysis, were included in the program for obtaining and certifying the grapevine propagating material, as required by the legislation in force. Observations and measurements were performed on the degree of maturation of the canes both in the growing conditions of the depositary greenhouse and in the vineyard (fig.1a÷b).



a-Depositary greenhouse (G₀ Initial material)



b-Vineyard (Breeder's material)

Figure 1. The biological material- original

The assessment of the maturation degree of the canes was performed by determining the bound water, total water, soluble sugar and starch contents in the wood immediately after the leaf fall.

The samples to be analyzed were composed of fragments of cane (including nodes and internodes) taken on the entire length of the cane. The basic, middle and top parts of the cane were analyzed separately, the end result being the average of the three measurements.

The measurement of the water content in the wood involved several working stages. In order to assess the free water content, the plant material was dried at 40°C up to a constant weight, and to determine the total water content, the plant material was dried at the temperature of 105°C.

The measurement of the soluble sugars and starch in the woody tissue was done using the colorimetric method with anthrone. The results were expressed in percentage glucose of dry matter (Scott and Melvin, 1953).

RESULTS AND DISCUSSIONS

The wood maturation marks the transition of the grapevine from the active to the dormant period, transition that occurs when the air average temperature falls below 10°C, as a process influenced by the environmental factors.

The woody tissue maturation corresponds to the period when the total sugar reached a maximum value and its degree of variation is depending on the vigour of the grapevine variety, loads of fruit, and growing conditions etc. The sugars are very important substances for plants, as major energy substances. The reserve substances such as the sugars are easily used by the cells and tissues to ensure the energy necessary to their physiological processes (Burzo et al., 1999).

The comparative analysis of the maturation process in the biological material revealed a high accumulation of the total carbohydrates in the woody tissues of the grapevine varieties grown in the depository greenhouse (Fig. 2 a;d; Fig. 3 a;f).



Figure 2. Biochemical indicators of woody material belonging to the two investigated white grapevine varieties

In the case of the Feteasca regala 72 St., Feteasca neagra 6 St., Cabernet Sauvignon 131 St., and Pinot noir 3 St. genotypes, the increased content of the total carbohydrates was the response of the higher significant translocation of the soluble sugars from the leaf towards the shoot in the depositary greenhouse. Unexpected results were obtained with Feteasca alba 97 St. clone, in which the soluble sugars content was constant in both culture conditions (6%). A high content of sugars were found with Feteasca regala 72 St (8%) and Feteasca neagra 6 St (9%) varieties. Under optimal conditions in the depositary greenhouse, the starch accumulation reached 9% with Pinot noir 3 St. and 10% with Feteasca alba 97 St. The main characteristics for plant material from the depository greenhouse were: an earlier start of the shoots maturation with one month, and a longer period for woody tissue maturation in comparison to vineyard conditions.

It is highlighted that, per total carbohydrates, all the clones studied in greenhouse conditions accumulated higher amounts of reserve substances (14% - Cabernet Sauvignon 131 St. and Pinot noir 3 St., 15% - Feteasca regala 72 St. 16% - Feteasca alba 97 St. and



Cabernet Sauvignon 131 St (vineyard)



U

f



e



Feteasca neagra 6 St.). The same clones grown in the vineyard accumulated lower amounts of total carbohydrates with 11-12%.

The variation of the water content in the grapevine shoots, in direct correlation with that of the dry matter content, is one of the most characteristic features of the grapevine maturation.

Along with the dry matter accumulation during the cutting of the wood, there is a decrease in the water content in the grapevine shoots.

The measurements on the total water content (free water and bound water) in the studied clones highlight values of this indicator varied between 50 and 54% in the plants grown in the depositary greenhouse and between 42 and 47% in those grown in the vineyard.

The variation of the total water content is due, in fact, to the different free water content of the wood taken from different culture media.

The bound water content remains almost constant (2-3%) regardless of the culture conditions, while the amount of free water, acting as solvent and carrier of substances accumulated in the plant, varies with genotype and culture conditions.

Regarding the representation of other chemical compounds present in the wood tissue (lignin, cellulose, phenolic compounds etc.) having a lower importance in assessing the quality of the propagating material, it is noted that, in the vineyard conditions, they are in a higher proportion (28-31%) compared with those in the depositary greenhouse (14-19%).

This difference can be explained by the influence of the higher ambient temperatures in the greenhouse, which could slow down the process of biosynthesis of these compounds in the intermediary metabolism.

As a whole, the obtained results proved the superiority of the quality of wood in all the grapevine clones grown in the depositary greenhouse.

CONCLUSIONS

- The biochemical analyses performed on grapevine biological material collected from Feteasca neagra 6 St., Pinot noir 3 St., Cabernet Sauvignon 131 St., Feteasca regala 72 St. clones maintained in the greenhouse depositary proved the superior quality of wood material from G_0 biological category, being under standards in force;

- All the studied clones from greenhouse conditions accumulated higher amounts of reserve substances, between 14 and 16%, in comparison to plant material harvested from vineyards which accumulated only 11-12%;

- The higher values of the total water content (free water and bound water) in canes harvested from greenhouse depository is a guaranty for a better viability of buds used for propagation, in comparison to vineyards plant material used for the same purpose.

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STUDIES REGARDING THE IMPLEMENTATION OF FOOD SAFETY MANAGEMENT SYSTEM IN THE BOTTLING TECHNOLOGY OF WINES

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Key words: food safety, HACCP, CCP, risk-prevention

ABSTRACT

Food safety has become a component of food quality and arises from the need to protect consumers. This means that products to be sold, must be safe. In the wines bottling technology can be applied a HACCP system which allows the identification of the key elements from this process. HACCP is the abbreviation for the English expression "Hazard Analysis and Critical Control Points". Using the HACCP System, we tried to identify the microbiological, chemical and physical hazards existing in the bottled wines technology and storage, in order to indicate the CCP for the hygienic quality of the products. During the bottled technology, there are factors which can affect the wines safety. Our studies formulate the good hygiene requirements and work procedures that have to be fulfilled by each company specialized in the wines bottled. In order to keep under control the bottled wines technology was identified CCP-1: Presence of glass shards inside the bottle. We hope that our researches results may help the economic agents having activity in the bottling of wines.

INTRODUCTION

"The crisis is what makes us open our eyes, is a feature of human nature. The transition process involves a deep crisis caused by the changes. So, open eyes to ensure quality and food safety, if we want to live decently", said the great calitolog J. Juran (1999). HACCP, is an acronym derived from Enghlish "Hazard Analysis and Critical Control Points" and this is a systematic method to identify, assess and control significant hazards associated with food of plant and animal origin (Pardo, et al., 2005).

Hygienic-sanitary quality is the essential condition for a food to be consumed by humans (Chira, 2005). Ever, consumers want the food at their disposal to be safe in terms of hygiene and sanitary so as not to cause illness (Mencinicopschi &Raba 2005). Hygienic quality (harmless) is influenced by: microorganisms and parasites; pollution by antibiotic residues in animal products, food additives, heavy metals, pesticides, organic substances (dioxins) and - other toxic substances like allergenic, cyanogen, antimetabolites, etc.; - natural induced toxicity by: toxic plants, mycotoxins, etc. (Pardo, 2005).

The wines resulted from white or black grapes can contain mycotoxins, pesticides and heavy metals residues in very dangerous quantities to human health. If these analyzes

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were not made for the grapes, it is imperative to make from must or from wine before bottling.

During the technological process of bottling, the hygiene rules should be respected, for does not lead to loss of hygienic quality of the product.

Food safety management systems like ISO 22000:2005 and Hazard Analysis and Critical Control Point (HACCP) can assure safety wines bottling by preventing potential hazard at source points of the process. Using the HACCP System, we tried to identifie the microbiological, chemical and physical hazards existing in the bottled wines technology and storage, in order to indicate the CCPs (Critical Control Points) for the hygienic quality of the products.

MATERIAL AND METHODS

The studies were developed on the bottling technological process of wines, according the flow diagram described in figure 1. For each step of the process was performed the risk analysis, in order to identify the chemical, physical and biological hazards correlated to the product and process and also the preventive actions and control measures which are necessary to keep under control these hazards. In order to establish the Critical Control Points in all steps of the technological wines bottling (table 1), where it's possible to implement specific control measures regarding food safety, was applied the CCP decision tree (recommended by Codex). The control of each CCP, according HACCP principles are planned in the document named the HACCP plan (table 3).

The establishing and the implementation of the control measures are shown by specific records. All researches and observations were made in a private wine cellar with its own bottling line. The wine transferred to bottling is accompanied by analysis bulletin.

RESULTS AND DISCUSSIONS

Bottling of wines is usually done in cellar with bottling machines.

The first step in bottling consists in receiving and temporary storing of the wines and auxiliary materials (bottles, corks, labels, boxes, pallets, etc.).

First time the bottles are washed, then rinsed. Then the bottles are filled with wine, are corked, are put capsules, labeled, packaged, stored and then will be sent for the selling. Glass bottle is the place where the wine remains, whether is destined for immediate consumption (for young wines) or for aging. In figure 1, is presented the flow diagram of wines bottling line which includes ten steps.

By using the CCP decision tree established by Codex Alimentarius (table 2), is presented only one CCP identified, which is focused to keep under the control the bottles washed. It also could be consider CCP, the step 10- Storage - Delivery inadequate, but this phase does not affect the hygienic-sanitary quality of the product but its commercial quality and may leads to important economic loss for the company.

The HACCP Plan (table 3) is one of the most important document from food safety management system, which contain the mainly information necessary in order to implement the control measures and keep under control the identified CCPs.

CONCLUSIONS

The HACCP system implemented in any company with the wines bottling activity, is a preventive self-control, whose principles can be applied to all food producing sectors;

HACCP prevention methods, is not a methods based on the final product control (which may affect consumer's health and may lead to important economic loss).

Our studies formulate the good hygiene requirements and work procedures that have to be fulfilled by each company specialized in the wines bottled.

In order to keep under control the bottled wines technology was identified one CCP, which is focused to keep under control the "presence of glass shards inside the bottle".

The implementation of HACCP is a benefit for all the wines producing companies, to increase consumer confidence in the wines selling on the markets; in other words the company, "sell" with its wines and "trust", which will cause a customers return who will truly appreciate the quality and safety of the bottled wines.

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Figure 1. Flow diagram - the bottling technological process of wines

Table 1

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Process step	Hazard	Preventive actions/Control measures
1. Reception sent	*B-unidentified	-
from the cellar wines	*C-unidentified	-
for bottling	*F-unidentified	-
2. Reception and	*B-unidentified	-
storage of auxiliary	*C-unidentified	
materials	*F-unidentified	
	*B-microbial	Basin cleaning before storage
3 Bottled product	contamination	Microbiological control GHP measures
storage in buffer tank	*C-unidentified	Wierobiological control. Offi measures.
storage in burier tank	*E Queraoma prassura	Pressure control and flow. Demonal training
	or low flow	Flessure control and now. Fersonal training.
	*D microhial	Cleaning ninglings facilities and equipment
	B- Iniciobiai	Cleaning pipelines, facilities and equipment
1 Starila filtration		according with the procedures. Filter control.
4. Sterne miration	*C-unidentified	-
	*F-unidentified	-
	*B- microbial	Microbiological analysis of water used to
6 11 1 1 1	contamination	wash and treat it. Personal training. GHP
5. Washing bottles		measures.
	*C - chemical residues	Water alkalinity control and correction.
		Control of lye concentration in wash water.
		Personal training.
	*F - mineral residues	Determination of water hardness correction.
	*B-unidentified	-
6. Control bottles	*C-unidentified	-
washad	*E- shards of glass	Visual inspection (defectoscopy). Select
washeu	1 - sharus of glass	(dereetoseopy). Sereet
washed	inside	suppliers. Personal training.
washed	*B- Contamination with	suppliers. Personal training. Cleaning pipelines, facilities and equipment
washeu	*B- Contamination with yeast, bacteria	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological
7. Bottling of wine	*B- Contamination with yeast, bacteria	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures.
7. Bottling of wine	*B- Contamination with yeast, bacteria *B-unidentified	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures.
7. Bottling of wine	*B- Contamination with yeast, bacteria *B-unidentified *C-unidentified	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures.
7. Bottling of wine	*B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - - The reception quality of the corks under the
7. Bottling of wine	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training.
7. Bottling of wine8. Sealing bottles	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The
 7. Bottling of wine 8. Sealing bottles with cork 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the
 8. Sealing bottles with cork 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers.
 7. Bottling of wine 8. Sealing bottles with cork 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training.
 8. Sealing bottles with cork 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring.
 8. Sealing bottles with cork 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or plastic 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring. Personnel training
 8. Sealing bottles with cork 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or plastic *B-unidentified 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring. Personnel training
 8. Sealing bottles with cork 9. Hooding, labeling, 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or plastic *B-unidentified *C-unidentified 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring. Personnel training -
 8. Sealing bottles with cork 9. Hooding, labeling, packing 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or plastic *B-unidentified *C-unidentified 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring. Personnel training - -
 7. Bottling of wine 8. Sealing bottles with cork 9. Hoodihg, labeling, packing 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or plastic *B-unidentified *C-unidentified *F-unidentified *B-unidentified 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring. Personnel training - -
 7. Bottling of wine 8. Sealing bottles with cork 9. Hoodihg, labeling, packing 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or plastic *B-unidentified *C-unidentified *F-unidentified *C-unidentified 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring. Personnel training - - - -
 7. Bottling of wine 8. Sealing bottles with cork 9. Hoodihg, labeling, packing 10. Storage-Delivery 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or plastic *B-unidentified *C-unidentified *F-unidentified *E-unidentified *F-unidentified *E-unidentified *E-unidentified *E-unidentified *E-unidentified *E-unidentified *E-unidentified *E-unidentified *E-unidentified *E-unidentified 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring. Personnel training - - - Storage parameters control. Shipping in
 7. Bottling of wine 8. Sealing bottles with cork 9. Hoodihg, labeling, packing 10. Storage-Delivery 	 *B- Contamination with yeast, bacteria *B-unidentified *C-unidentified *B- microbial contamination *C - Transmission of taste and smell of cork *F-Fragments cork or plastic *B-unidentified *C-unidentified *F-unidentified *F-unidentified *F-Storage and Delivery inadeguate 	suppliers. Personal training. Cleaning pipelines, facilities and equipment according to the instructions. Microbiological analysis. Personal training. GHP measures. - The reception quality of the corks under the instruction of work. Personal training. Cleaning programme for equipments. The quality reception of the corks under the instruction of work. Select suppliers. Operators training. Defectoscopy. Maintenance. Monitoring. Personnel training - - Storage parameters control. Shipping in optimal conditions, Cleaning programme for

*B= biohazard; *C= chemical hazard; *F= physical hazard

Table 2

	1	01	<u> </u>	Ŭ	,	
		Decision tree questions				
Process step	Hazard	Q1- there are preventive measures to prevent the risk of identified hazards?	Q2- stage is specially designed for eliminating / reducing the possibilities of developing a potential hazard?	Q3- there is the possibility of contamina- tion due to a potential hazard till the acceptable level?	Q4- can a later stage to eliminate a potential hazard identified / to reduce possibility the occurrence of a potential hazard to an acceptable level?	CCP- no.
6. Control bottles washed	shards of glass inside	yes	yes	-	-	ССР -1

CCP determination during processing (in according with decision tree)

HACCP PLAN for bottling line									
				ts		Mor	nitoring		
Stage	Hazard	Nr. CCP	Control measures	Critical limi	Method	Responsa- ble	Frequency	Records	Corective actions
Control bottles washed	Shards of glass	CCP-1	Determination of the presence of integrity bottles and glass shards in.	Without shards and other foreign bodies.	Visual inspection (defectos-copy)	Operator bottling	Constant during bottling	Sheet for defectoscopy; Register incidents observed	Mentenance ; Select suppliers; Personal training.

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RESEARCH ABOUT THE BIOLOGICAL CULTURE OF CARROT

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Keywords: carrot culture, biological culture, organically fertilizers

ABSTRACT

The aim of the research was to establish the organically optimum doses of fertilizers which must be applied to carrot culture in organically system correlated with the obtaining of a superior quality and quantity. For that in 2012 was made an experience at which the organically doses applied had six degrees, respectively 2, 4 and 6 kg cow manure/ m^2 and 2, 3 and 4 chicken manure/ m^2 . The experiment was made in the Vegetable Department of University of Agricultural Sciences and Veterinary Medicine Bucharest in an unprotected solarium with a 120 m^2 surface. Biological material used was Belgrad F1 carrot. During the growing of cultivated plants were made periodical analyses regarding maintaining the technological works needed and to respect the interdiction of use of chemical fertilizers and pesticides. The highest carrot roots were obtained at variants 2 and 3 fertilized with 6 and 8 kg cow manure/ m^2 .

INTRODUCTION

Organically agriculture is an alternative of usual modern practice of agriculture, an intensive one and which have the principal objective to obtain agro food products with a high content of biological active substances, so that do not negatively influence the human health and environment. That practice have the objective to increase soil organic matter using natural organic fertilizers (manure, compost, green fertilizers) and is an important sector for Romania which have a lot of development opportunities and is a tool in the conservation of nature and revitalization of rural space (Council Regulation (EEC) nr.2092/91, Order 721 of 26 September 2003).

From the characteristics of agro food products which must be strictly verified are the nitrates, phosphorus, potassium, heavy metals and pesticides contents (Emergency Ordinance No. 34 of 17 April 2000, which defines the characteristics of organic production, Norms for the application of the Emergency Ordinance No. 34 of 17 April 2000 on organic products).

The aim of the research was to establish the optimum doses of organically fertilizers correlated with establishing the quality and quantity obtain at some vegetables cultivated in organically system.

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MATERIAL AND METHODS

The experience was realized in the Vegetable Departments from USAMV-Bucharest, on an unprotected solarium, with a $120m^2$ surface. The incorporation of the organically fertilizers was made in six doses respectively 4, 6 and 8 kg cow manure/m² and 2, 3 and 4 chicken manure/m², in the same time with the preparation of the soil and at the beginning of the culture. Every variant had $10m^2$, total surface.

The Belgrad F1 carrot was sown at 16 March 2012 and the rice was at 2 April 2012. During the development of cultivated plants were made periodical verifications regarding the technological chains necessary to apply.

During the vegetation period were applied the nursery practices from the culture technology and the first weed out was at 11 May 2012 and the second at 8 June 2012, when the roots had the caliber of a pencil. The harvest was made at 120 days from the rise at the 13 July 2012. At the harvest were made biometrical measurements, agrochemical and biochemical characteristics which could characterize the quality and quantity of the obtain crop.

The analyses made were agrochemical ones regarding nitrates, phosphorus and potassium and heavy metals. The analyses methodology used was the standard one respectively STAS 11581-83 for nitrates, phosphorus and potassium unmethabolised forms were determined by the extraction with acetic acid and photo colorimetric determination for phosphorus and flam photometric method for potassium.

Heavy metals were obtained with humid mineralization and spectrophotometer determination.

Biochemical characteristics analyzed were vitamin C (iodometric method), acidity (volumetric method with NaOH 0,01n) and sugar (Abbe method).

The crop was registered and the statistical interpretation was made.

RESULTS AND DISCUSSIONS

At the beginning of the experience, a soil sample was made from the solarium and there were determined the principal agrochemical indicators: pH, soluble salts, macro elements contents and heavy metals contents and pesticides, which could affect the crop quality. The results obtained showed a soil with a middle content of nitrogen, low contents of phosphorus and potassium (table 1).

Heavy metals contents were in normal limits because the soils of our country had Cu element in normal limits of 50ppm, Zn between 100-300ppm, Pb-20ppm and Cd between 3-5ppm. The pesticides contents is low (table 2).

Т	a	b	le	1

The analyze of agrochemical indices at the beginning of culture							
Specification	pН	Soluble salts		Content, ppm			
		%	N-NH4 ⁺	N-NO ₃ -	P-PO4 ³⁻	K+	
Soil	6.20	0.065	2.8	27.3	29.3	102	

Table 2

The analyze of heavy metals at the beginning of culture

Specification	Heavy metals content, ppm				Pesticides co	ontent, µg/kg
	Cu	Zn	Pb	Cd	DDT	HCH
Soil	27.3	76.2	5.6	0.13	undetectable	undetectable

To know the contents of nitrogen, phosphorus and potassium, which were applied in soil with organically fertilizers there, were analyzed organically fertilizers used (table 3).

Table3

The analyses of organically fertilizers used in experience

No	Specification	N%	P%	K%
1	Cow manure	0.61	0.17	0.59
2	Chicken manure	1.12	0.87	0.35

Table 4

	The measurements of carrots							
	Variant		Medium values					
		L (mm)	Φ(m	m)	n) Weight (g/plants)		
	Harvest at intermediary s	tage of veg	getation (ca	rrots with t	the dimer	nsion of a	pencil)	
		-	- 08.06.201	2-				
		L	Dif. +/-	$\Phi(mm)$	Dif.	g/plant	Relative	
		(mm)			+/-		values %	
1	Ct	115.50	-	13.63	-	6.68	100.00	
2	V1-4 kg cow manure	152.66	+37.16	15.16	+1.53	6.88	102.99	
	$/m^2$							
3	V2-6 kg cow manure	147.33	+31.83	18.10	+4.47	13.43	201.04	
	$/m^2$							
4	V3-8 kg cm*/m ²	164.16	+48.66	17.38	+3.75	10.17	152.24	
5	V4 -2 kg chm**/m ²	130.33	+14.83	15.08	+1.45	7.31	109.43	
6	V5-3 kg chm $**/m^2$	131.50	+16.0	16.46	+2.83	9.81	146.85	
7	V6-4 kg chm $**/m^2$	142.66	+27.16	15.33	+1.70	7.95	119.01	
		Final ha	rvest – 13.0	07.2012 -				
		L	Dif. +/-	$\Phi(mm)$	Dif.	g/plant	Relative	
		(mm)			+/-		values %	
1	Ct	178.3	-	7.60	-	15.48	100.00	
2	V1-4 kg cm*/m ²	178.1	-0.2	8.59	+0.99	14.95	96.57	
3	V2-6 kg cm*/m ²	212.5	+34.2	10.25	+2.65	24.27	156.78	
4	V3-8 kg cm*/m ²	239.5	+61.2	12.84	+5.24	33.19	214.40	
5	V4 -2 kg chm **/m ²	232.1	+53.8	13.02	+5.42	21.96	141.86	
6	V5-3 kg chm **/m ²	234.5	+56.2	10.90	+3.3	28.80	186.04	
7	V6-4 kg chm **/m ²	233.6	+55.3	10.50	+2.9	31.19	201.48	

*cow manure-cm

**chicken manure - chm

Biometrical measurements regarding the development of carrot plants shows the influence of nutritive elements contents of soil upon the diameter of carrot and on the length of carrots (table 4) and on the quantity of crop g/plant.

At the intermediary faze of vegetable culture, the lengths of carrots (mm) vary between 115.5mm at control and 164.16mm at variant 3 fertilized with 8kg cow manure $/m^2$. The effect of organically fertilizers could be shown on the medium length of carrot because the values of control were under the values of other experimental variants fertilized with cow and chicken manures. The same thing was observed also in the case of medium diameter of the carrots which were between 13.63mm at control and 18.10mm at variant 2 fertilized with 4kg cow manure $/m^2$.

From the results could be observed that the fertilization with cow manure favorable influenced the development of carrots in diameter and lengths in variants 1, 2, 3.

These variants are over the variants fertilized with chicken manure.

The medium weights at the pencil dimension of carrots varied between 6.68g/roots at control and 13.43g/root at variant 2 fertilized with 4kg cow manure $/m^2$. It could be observed that the weights carrots were obtained at variant 2 and 3 fertilized with 6 and 8kg cow manure $/m^2$.

At final harvest at 13 July 2012, the medium lengths of carrots registered values between 178.1mm and 239.5mm. The medium lengths of roots were over the control value (178.3mm) with the exception of variant fertilized with 4kg cow manure $/m^2$ (178.1mm) and the longest carrots were at variant fertilized with 8kg cow manure $/m^2$ (239.5mm).

The medium diameter of roots at final harvest varied between 7.6mm at control and 13.02mm at variant 4 fertilized with 2 chicken manure/ m^2 . At all variants, the medium diameter of carrot roots was over the medium diameter from control variant.

The medium weight of carrots varied between 14.95g/root at variant² fertilized with 4kg cow manure /m² and 13.19mm at V3 fertilized with 8kg cow manure /m².

The analyses regarding the carrots quality for consume were presented in next table (table 5).

Table 5

	Variant	Content(ppm)				
	varialit	NO ₃ -	P-PO4 ³⁻	\mathbf{K}^+		
	Harvest at intermediar stage of veget	ation (carrots with	the dimensio	on of a pencil)		
	- (08.06.2012-		_		
1	Ct	115	64.4	2020		
2	V1-4 kg cow manure /m ²	117	108.0	2300		
3	V2-6 kg cow manure $/m^2$	151	110.0	2220		
4	V3-8 kg cow manure $/m^2$	126	92.8	2140		
5	V4 -2 kg chichen manure /m ²	154	86.8	1860		
6	V5-3 kg chichen manure /m ²	158	71.2	2060		
7	V6-4 kg chichen manure $/m^2$	173	64.8	2100		
	Final harv	rest - 13.07.2012 -				
1	Ct	141	313.6	2340		
2	V1-4 kg cow manure $/m^2$	107	385.2	2980		
3	V2-6 kg cow manure $/m^2$	167	387.6	3440		
4	V3-8 kg cow manure $/m^2$	187	312.8	2220		
5	V4 -2 kg chichen manure /m ²	183	292.4	2660		
6	V5-3 kg chichen manure /m ²	166	298.8	1880		
7	V6-4 kg chichen manure /m ²	308	176.8	1600		

Contents of nutritive elements unmethabolised form from carrot

The nitrates presents a restricted factor for quality, carrot as specie which accumulates nitrates in the roots and high quantities of that component in human eat could cause health problems, especially at child and old persons.

The nitrates absorption had a high intensity from the intermediary faze of analyze, the contents in nitrates varied between 115ppm at control to 173ppm at V6 fertilized with 4 chicken manure/ m^2 . The level of nitrates in carrots were under the admissible limits presented by the scientific literature, a 400ppm content.

At final harvest the nitrates content in carrots had values between 107ppm in variant 1 fertilized with 4kg cow manure $/m^2$ and 308ppm at variant 6 fertilized with 4 chicken manure/ m^2 . In that faze the content was high but the fertilization with organically fertilizers determined the methabolization of nitrates in protein and so the nitrates contents

were under the admissible value presented by the "Order no.1 from 3 January 2002 for fresh vegetables and fruits" respectively 400ppm.

Carrots accumulated nitrates in the central cylinder and the quantities increase with the vegetation period correlated with the nitrates quantities from the soil. The accumulation rate in plants increase at the high values of soil nitrogen, so it is necessary an equilibrate fertilization with organically fertilizers.

The phosphorus content of carrot in the intermediary faze of harvest varied between 64.4ppm (control) and 110.0ppm at variant 2 fertilized with 6 kg cow manure $/m^2$.

Till at final harvest the absorption of phosphorus was intensify, the phosphorus content varied between 176.8ppm at variant 6 and 387.6ppm at variant 2.

Potassium accumulation in the intermediary period of harvest was realized in the limits of values between 1860ppm at variant4 and 2300ppm at variant 1.

At final stage the accumulation of potassium presented values between 1880ppm at variant 5 and 3440ppm at variant 2, the potassium content was high at the two stages of harvest, which influence the period of maintenance of carrots.

At final stage of harvest there were made also biochemical analyses regarding sugar, vitamin C and acidity contents. Sugar contents was slightly influence by the fertilization system, the values of that indicator been a characteristic of the specie and varied in small limits between 5.468% at control and 5.843% at variant 2.

Vitamin C values were low because the variability of those characteristics was between 0.015 and 0.025me/100g fresh material. The acidity values were low with variability between 0.1013% at variant 6 and 0.1519% at variant 2.

Table 6

biochemical characteristics of carlots at final harvest						
		Sugar	Vitamin C	Acidy		
V	ariant	%	me/100g fresh	%		
			matter.			
1	Ct	5.468	0.015	0.1157		
2	V1-4 kg cow manure /m ²	5.531	0.0225	0.1447		
3	V2-6 kg cow manure $/m^2$	5.843	0.015	0.1519		
4	V3-8 kg cow manure /m ²	5.593	0.025	0.1302		
5	V4 -2 kg chichen manure $/m^2$	5.718	0.015	0.1447		
6	V5-3 kg chichen manure /m ²	5.781	0.0225	0.1302		
7	V6-4 kg chichen manure /m ²	5.593	0.0225	0.1013		

Biochemical characteristics of carrots at final harvest

Table 7

Heavy metals contents from carrot at 13.07.2009									
Variant		Conte	ent , ppm						
	Cu	Zn	Pb	Cd					
Ct	4.2	11.2	Trace	-					
V1	3.7	10.9	-	-					
V2	4.6	10.3	-	-					
V3	3.9	9.6	-	-					
V4	4.1	11.2	-	-					
V5	3.8	9.3	-	-					
V6	4.2	10.6	Trace	-					

The analyses of toxic elements, heavy metals, total forms revealed a normal content. Cu values were between 3.7 and 4.6ppm comparatively with 5.0ppm maximum admissible limits for that element. Values of Zn analyzed varied between 9.3 and 11.2ppm

comparatively with maximum admitted limit of 15,0ppm. Lead and cadmium did not present values, which could be detected. The maximum admitted limits were from "Order no.1 from 3 January 2002 for fresh vegetables and fruits".

Statistical interpretation of carrots group

Table 8

	Statistical interpretation of carrots crop							
Variant	Medium	Diferences	Crop	Significant				
	crop/variant	+/_	increase					
	Kg/m ²		%					
Ct	2.301	Ct	100	-				
V1	2.582	0.281	112.21	Ns				
V2	2.987	0.686	129.81	***				
V3	3.021	0.720	131.29	***				
V4	2.564	0.263	111.43	Ns				
V5	2.651	0.350	115.21	Ns				
V6	2.321	0.020	100.87	Ns				
$\overline{01}$ 5% -0.39	kg/m2 D	1% - 0.53 k	σ/m^2 DI 0	1% - 0.62 kg/m				

DL 1% =0. 53 kg/m2 DL 0.1%=0.62 kg/m2 DL 5%= 0.39 kg/m^2

Carrot crop was very significantly influence by the fertilization with 6 and 8 kg cow manure/m².

CONCLUSIONS

The variants values of carrots medium length were over control (178.3mm) with the exception of variant 1 fertilized with 4 kg cow manure $/m^2$ (178.1mm);

The highest medium length of carrots was registered in variant 3 fertilized with 8 kg cow manure $/m^2$ (239.4mm);

At all variants medium diameters of carrots were over the medium diameter of control;

The nitrates contents of carrots varied between 107ppm at variant 1 fertilized with 4 kg cow manure $/m^2$ and 308ppm at variant 6 fertilized with 4 kg chicken manure $/m^2$. The nitrates contents were at all variants under the maximum admitted limits of 400ppm (Order no.1 from 3 January 2002 for fresh vegetables and fruits);

Sugar content was little influenced by the fertilization system values of that indicator varied between 5.468% at control and 5.843% at variant 2. Vitamin C content was low at all variants and varied between 0.015 and 0.025mg/100g fresh material. The acidity values were also low values between 0.1013 % at variant 6 and 0.1519% at variant 2;

The analyses of toxic elements, heavy metals, from carrots revealed a content which did not over the maximum admitted limits;

Crops of carrots were significantly influenced by the culture fertilization with 6 and 8 kg cow manure $/m^2$.

REFERENCES

***Council Regulation (EEC) nr.2092/91.

***Emergency Ordinance No. 34 of 17 April 2000, which defines the characteristics of organic production.

***Norms for the application of the Emergency Ordinance No. 34 of 17 April 2000 on organic products.

***Order 721 of 26 September 2003.

***Order no.1 from 3 January 2002 for fresh vegetables and fruits.

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BIOTECHNOLOGICAL ISSUES ENCOUNTERED IN OBTAINING THE LOW ALCOHOL BEER

Căpruciu Ramona1*

Keywords: biotechnological, foam, low-alcohol beer

ABSTRACT

This study aims to obtain low-alcohol beer in the laboratory through the vacuum distillation process using the Heidolph evaporator, based on samples of normal beer (blonde and brown ale). The normal beer samples were subjected to organoleptic and physical measurements in order to achieve a comparative framework with the parameters of the low-alcohol beer. Tracking how the normal beer (blonde and brown ale) changes the organoleptic and physical properties upon distillation in order to obtain a type of beer with a low alcohol content is important in terms of biotechnology in order to determine its quality and suitability to storage.

It has also been determined in a comparative way the carbon dioxide content, the foam index, the elements that influence the activity of the enzymatic processes both during obtaining the beer and during the storage of the finished product.

INTRODUCTION

The low-alcohol beer is a special category within the beer industry, which includes the beers that cannot be classified as normal beer, mainly because of the different chemical composition (especially in terms of the alcohol content).

The biotechnological issues of obtaining the beer refer to the activity of the yeasts. From the yeasts category there are important (in terms of utility) those belonging to *Saccharomycetaceae* family, *Saccharomyces* (*Meyen*) *Rees* genus which include alcooligene yeasts used in the brewing, wine and bread industry, and are characterized by the fact that the fermentation power exceeds the breathing power (Rosendahl, I. et al. 1987, Banu C. et al., 2000, Popa A. et al. 2002, Baxter E. et al. 2007).

The technological process of obtaining the beer involves: malt polishing and grinding, brewing, saccharified mash filtering, washing and depletion of draff, filtering the wort with hops, wort clarification, cooling, seeding and fermentation of wort. After the wort fermentation, the beer will clarify after sedimentation, centrifugation and filtration. Before being delivered, the beer is stabilized (Banu C. et al. 2000, Camelia Cioban 2004).

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MATERIAL AND METHODS

This study aims to observe the physical and organoleptic transformations of the normal beer (blonde and brown ale) samples. It is important to observe the mode and dynamic of the organoleptic and physical properties transformation of the normal beer during the vacuum distillation in order to obtain the low-alcohol beer.

In this respect it was intended to obtain two types of low alcohol beer (blonde and brown ale), the obtained analyzes being compared to the initial beer samples (brown and blonde ale with a normal alcohol content). Obtaining the low alcohol beer through simple distillation involves using the Heidolph rotary evaporator with vacuum. The principle of this method is the removal of alcohol from beer thrpugh evaporation under vacuum at the boiling temperature, in a short period, greatly reducing the risk of undesirable changes in the beer components. The beer is positioned in the evaporating flask in a film of 0.1 mm thickness due to the centrifugal force, on a heat exchange oval surface. From this thin film the alcohol and some of the water contained in the beer evaporate. The dealcoholised beer is cooled by direct contact of the flask with the water at a normal temperature. The alcohol and water vapors separated from the beer are condensed in a condenser.

In order to achieve a certain concentration of ethanol in beer, it is necessary to determine, using special graphs, the evaporation rate at a given temperature. Another important aspect in the industry is to reduce the carbon dioxide before being introduced into the distillation unit. At high concentrations of CO_2 in beer, particles are entrained in the distillate which becomes turbid. In the laboratory, this was done by hand and energetic stirring. The principle of the method of the determination of free carbon dioxide is based on its ability to fix as sodium bicarbonate by treating with solution of sodium carbonate. The excess of sodium carbonate is titrated with hydrochloric acid. The method for determining the colour of the beer by spectophotometry is the European EBC (European Beer Colour or the European Convention of Beer which is based on measuring the absorbance at 430 nm (represents the absorption of the yellow colour of the beer) in a 1 cm vat. Determining the beer foam quality was done with the Hartong method, the method principle relying on pouring the beer into a recipient and determining the appearance, persistence and adherence of the formed foam.

RESULTS AND DISCUSSIONS

The low alcohol beer is a drink liked by a wide range of consumers (youth, women, drivers, etc.), with a significant treatment effect on the body and a calming effect due to the chemical compounds content of high biological value.

For the organoleptic examination, the beer is poured immediately after decapsulation in clear glass cups, thoroughly washed, with a capacity of approx. 250 cm³.

In assessing the beer appearance, smell/odour and taste, the beer is poured in special tapered glasses without foam. The tasting is done immediately after pouring the beer in glasses, one drinks normally about 100 cm³ of the product. Tasting the samples is done in special rooms without foreign odour and with low light intensity.

Checking the foam is done by using a thin, colourless, well washed and degreased glass with special shapes.

The beer cooled at 10/12 °C is poured into the glass, so that the stream to fall on its axis from a height of 30 mm above the top edge of the glass. The foam is considered appropriate if immediately after pouring the beer, has a height of 30-40 mm, and the time to its complete disappearance is of 3 minutes.

The foam volume of the normal beer (blonde and brown ale) and of low-alcohol beer was compared, analyzing the foaming index (Table 2 and 3).

The beer colour has to be for the two analyzed types of beers following: pale yellow to yellow (blonde ale) and brown yellow for the brown ale. In addition, for an accurate determination of the colour it was used the spectophotometric analysis, the result being expressed in EBC units (figure 1).

Table 1

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The concert on	OLVOID OT	LOW ALCOHOL	hoor in	comparison t	o como	other tunes	ot boor
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Types	Blond ale	Brown ale	Low- alcohol beer	
			From blond ale	From brown ale
Appearan ce	Clear liquid, free from impurities or sediment	Dark brown liquid, without impurities or sediment	Clear, bright yellow liquid, without impurities or sediment	Dark brown liquid with a slight degree of turbidity
Odour	Specific to the analyzed type, pleasant, without foreign odour (mold, dirt, etc.) with hop flavour	Specific to the analyzed type, pleasant, without foreign odour, with persistent hop aroma	Pleasant odour, without foreign odour, with hop aroma	Characteristic odour, with perceptible hop aroma
Taste	Specific to the type, bitter, pleasant, without foreign taste	Specific to the type, bitter with persistent nuances, pleasant, without foreign taste	Bitter, intense taste, without foreign taste	Persistent bitter taste, without foreign taste
Foam	It forms a compact, persistent foam, with CO ₂ emitted strongly, it forms intensely the pearling	It forms a compact, persistent foam, with CO ₂ released intensely, forming an intense pearling	It forms less compact and persistent foam, the CO ₂ is emitted slowly, less pearling	It forms less compact and persistent foam, the CO ₂ is released slowly, low degree of pearling

The spectophotometric analysis shows an obvious difference expressed in EBC units between the brown and blonde ale. Regarding the low alcohol beer produced through vacuum distillation, its colour is less intense than the original samples, as shown in figure 1. If for the normal blonde ale, the colour has 2.832 EBC units, for the low alcohol blonde ale the value decreases to 2.511 EBC units.

Table 2

Determination of foaming at normal beer blonde/brown

Beer Time		Foam volu	ime (ml)	Report	VB/VS (ml)	I.S(ml)		
volume	(min.)	beer blonde	beer	beer	beer brown	beer	beer	
(ml)			brown	blonde		blonde	brown	
20	3	16,0	20,0	1,25	1,00			
60	3	22,5	26,4	2,66	2,27	0,83	0,70	
100	3	28,0	30,5	3,57	3,27			

VB= Beer volume; VS= Foam volume; I.S= The foaming index.

Table 3

Beer	Time	Foam volume (ml)		Report VB	/VS (ml)	I.S (ml)		
volume	(min.)	beer	beer	beer	beer	beer	beer	
(ml)		blonde	brown	blonde	brown	blonde	brown	
20	1	2,0	3,0	10,0	6,66			
60	1	2,5	4,0	24,0	15,0	24,43	14,62	
100	1	3,0	4,5	33,3	22,22			

Determination of foaming light beer blonde/brown

VB= Beer volume; VS= Foam volume; I.S= The foaming index.



Figure 1. The color low-alcochol beer compared to the normal

An obvious decrease is recorded for the brown ale sample which from 40.321 EBC units reaches after the distillation 33.911 EBC units. Regarding the colour of the analyzed beer types, it can be said that after the distillation process for obtaining low-alcohol beer, it loses its intensity.



Figure 2. Determination of carbon dioxide in the finished beer

In the literature there are known issues such as the fact that carbon dioxide is a component of the beer which results from the fermentation and has the following effect on beer: it improves the taste, forms the foaming, preserves the beer preventing the development of foreign micro-organisms, which are harmful to the beer. After the primary fermentation the beer contains about 0.2% dissolved carbon dioxide, in comparison to the finished beer (before extraction) – which contains 0.35-0.45%. It results that during the secondary fermentation an amount of 0.25% carbon dioxide has to accumulate in the environment. Taking into consideration these issues in the study, it resulted an amount of carbon dioxide (after the distillation of the normal beer) lower than normal (0.20 g/100 ml min. for low alcohol blonde ale, and 0.28 g/100 ml min. for the low alcohol brown ale) - figure 2.

This indicates a poor pearling of low alcohol beers and the decrease of the taste property.

CONCLUSIONS

Using the evaporation under vacuum for the distillation of normal beer leads to the aroma loss for the low-alcohol beer, so in the industry it is recommended the usage of aroma recovery systems.

In terms of colour, purity, foam and carbon dioxide content, the low alcohol beer should be:

- low-alcohol blonde ale - pale yellow, lighter than the original sample, clear liquid with specific shine without sediment or impurities; the white foam and average pearling of carbon dioxide (visual determination);

- low-alcohol brown ale - lighter brown yellow than the original sample, clear liquid, without sediment or impurities; foam and pearling of carbon dioxide (visual determination);

The colour of the analyzed types of beer after the distillation process for obtaining low-alcohol beer loses its intensity. The spectophotometric analysis shows an obvious difference expressed in EBC units between the brown and blonde ale;

Regarding the foaming, the following conclusions were obtained: the normal blonde ale is included in the insufficient foaming category; the normal brown ale is included in the good foaming category; the low alcohol blonde and brown ale, the both data for the foaming indices exceeding the corresponding foaming category, are included in the category of very low foaming beer. For this reason, in the industry it is required to attach a facility for the carbonation of the beer, immediately after distillation, or to use other methods to decrease the alcohol concentration (the use of specific enzymatic preparations);

The low-alcohol beer taste is defined by fullness, more intensely perceived bitter flavour due to poor pearling, without foreign taste;

The smell is typical for the low-alcohol beer, pleasant, without foreign smell (mouldy, sour), and with the hops flavour and harmonious defined malt;

After the distillation of the normal beer it was obtained a quantity of carbon dioxide much lower than the original sample, which led to a poor pearling, reducing also the flavour degree of the analyzed type of beer.

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ANALYSIS OF THE MAIN CHEMICAL COMPONENTS OF SOME TOBACCO VARIETIES ACCORDING TO THE LEAVES POSITION ON THE PLANT

Căpruciu Ramona1*

Keywords: total alkaloids, albumins, tobacco leaves

ABSTRACT

Smoking as a harmful factor is based on multiple causes, but the most important one is related to the nicotine dependence, alkaloid existing in all the forms of commercial presentation of the products obtained from the tobacco leaves. From a quantitative perspective the alkaloids and albumin concentration is different depending on the leaves position on the plant (bottom, middle, and top). It has been shown that also due to factors such as soil type and its fertilization, the climate, the harvesting method, etc., the leaves on the plant accumulate different chemical substances.

This study aims to analyze some chemical substances on the leaves of Virginia and Burley tobacco varieties in order to determine the degree of their harmfulness.

INTRODUCTION

Smoking itself is usually widespread throughout the world and is practiced equally by men and women, children and adults and affects the lives and health of millions of people (Nicholas Hodisan, 2006).

In 1882, Passell and Reinmann - quoted by Constantin Bolcu , 2007, have separated for the first time the nicotine in the tobacco leaves.

The psycho-activity maintained by the nicotine is due to the effect it exerts on the central nervous system. With the inhalation of cigarette smoke, the smoker has the impression that he is more energetic, better describes the ability to concentrate, he is more careful and even in good mood, thanks to the discreet euphoric effect of nicotine (Constantin Bolcu, 2007).

The eloquent undertaken studies in this respect both nationally and abroad (Abraham P.et al. 2005 studies on skin, A. Somesan et al. 2007 studies on the digestive and kidney system damage, Nicoleta, Antigona Trofor et al. 2004, 2008, J. Seglera, 2000 studies on the harmful effects on pregnancy and lungs, etc.) mention the harmful effects of smoking on the human body.

MATERIAL AND METHODS

This study aims to observe the accumulation of chemical substances in the tobacco

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plant (foliage of Virginia and Burley varieties), depending on the position of cultivation. In this respect a chemical analysis was undertaken of the leaves from three position floors (base, middle, and top) in terms of total alkaloids and albumin content. The nicotine extraction from the leaves of the analyzed varieties was achieved by water vapour induction because the nicotine is a volatile alkaloid. As equipment we used the vacuum evaporation system. The purification was carried out by using organic acids because of the ease of movement of the alkaloids base in the alkaloid salt followed by the extraction with the nonpolar solvent.

The identification was made through precipitation reactions with iodine, the general precipitation reagents consisting of complex iodine. The reagent used is Bouchardat, being an aqueous solution of iodinated iodine.

The obtained precipitate from the reaction was orange-brown. For the determination it was used the spectrophotometric method using the electromagnetic radiations in the UV region (185-400 nm).

To determine the albumins there was considered that the albumins are soluble in water and in diluted solutions of electrolytes (such as acids, bases, salts).

The used method was the precipitation with neutral salts, sodium sulphate in a saturated solution.

RESULTS AND DISCUSSIONS

Some of the main chemical compounds with direct relevance regarding smoking and human health are the albumins and alkaloids. The chemical composition determines the product quality, being influenced by the variety characteristics, environmental factors (climate and soil), the plant age, the leaf position on the stem etc. and can be improved in various ways.

In this study we are interested in a quantitative determination of total alkaloids and albumins from the leaves collected from the main position floor of the studied plants (bottom, middle and top), leaves of varieties designed to provide high-quality cigarettes (Virginia and Burley).

The accumulation of nitrogenous substances depends very much on the amount of nitrogen in the soil and if it is available to the plant. The top leaves and the youngest on the plant accumulate more nitrogen. As the leaves mature, the nitrogenous substances content decreases because they migrate to the younger leaves on the upper floors and to the inflorescence. The alkaloids undergo many changes during the fermentation-drying process. During the plant growth the biochemical synthesis and decomposition of nicotine undergo a continuous metabolic process. After harvesting the leaves, during the fermentation-drying process, the nicotine transformations can continue.

During the fermentation-drying process of the tobacco leaves, the albumins (protein substances with large molecules) are degraded by the action of proteolytic enzymes. This phenomenon is favourable for the quality of tobacco; the accumulation of these substances in large quantities can have a negative influence.

As a result the content of total alkaloids, albumins and other chemicals substances depends on the variety of tobacco, on the conditions and methods of culture, as well as on the harvested leaves position on the plant. For these reasons, there are quite large variations of the chemical content of the different types of cigarettes on the market.

The analysis of the total alkaloids and albumins according to the leaves position on Virginia and Burley varieties is presented in Table 1.

Chemical parameters Virginia Burley Middle Bottom Middle Bottom Top Top 0.64 0.97 Total alkaloids 0.54 1.03 0.71 1.23 4.25 5,28 6.32 5.66 6.22 Albumins 7.26

The determination of some chemical parameters of the Virginia and Burley tobacco varieties depending on the harvesting position floor

Analyzing the data from Table 1, it is observed for Virginia variety a higher total alkaloids content in the leaves harvested from the top floor (1.03%), which decreases with the floor (0.64% in the middle in comparison to 0.54% at the bottom). And in terms of the albumins content it is observed a maximum quantity at the top leaves (6.32%), decreasing as in the case of total alkaloids to the bottom (5.28% at the middle leaves and 4.25% at the bottom leaves). Comparing the studied varieties it is observed that also for the Burley variety the amount of the analyzed substances decreases from the top to the bottom with the observation that in this variety leaves both the total alkaloids and the albumins are in higher amounts than those recorded by Virginia variety. Thus, the top of the plant contains a total alkaloids content of 1.23% which conveys the Burley variety to be considered harmful. And the leaves taken from the middle of the plant maintain a substantial amount of total alkaloids (0.97%).

Regarding the albumins amount, the top foliage is characterized by a high content (7.26% compared to 6.32% for the Virginia variety), conferring to the finished product obtained from this raw material a bitter, choking taste. In terms of both total alkaloid content and in terms of albumins content, the Burley variety recorded higher contents, aspect that conducts to the use of these varieties in the tobacco industry. Thus, the Virginia variety, being more chemically balanced can be used alone or in blends for the manufacturing of high quality cigarettes.

The raw material derived from the Burley variety cannot be used in the manufacture of specific products but it can be used only in blends with chemically poorer varieties in order to form a necessary balance for forming the cigarettes.

CONCLUSIONS

The content of total alkaloids and albumins depends on the harvested leaves position for both Virginia and Burley variety.

It is observed for Virginia variety a higher total alkaloids content in the leaves harvested from the top floor, this content decreases with the floor. The decrease occurs in the same direction as for the Burley variety (from the top to the bottom). Following the laboratory determinations it was achieved a higher content of total alkaloids and albumins for the Burley variety compared to Virginia variety, taking into account that both varieties were grown in the same orographic and climatic conditions.

Based on these data we can establish the main direction of production if we want to achieve a more balanced tobacco, with chemical and organoleptic qualities that recommend it for the Superior and first quality class. The impact of the analyzed substances on health is known. The undertaken study demonstrated that the leaves with the lowest toxic potential are located on the lower floors, those with the higher toxic potential are located on the top floor. There was also observed that the foliage floor with a balanced chemical composition is the one in the middle. The products obtained from the raw material taken from this floor are balanced in terms of composition and organoleptic characteristics. Obtaining cigarettes from the bottom floor leaves would lead to fade products without chemical and organoleptic harmony. Therefore it is recommended to use them with the top foliage, being able to create a balanced composition, the leaves from the top floor of the plant having for both varieties superior chemical elements in comparison to other floors (especially for the Burley variety). The cigarettes obtained from the raw materials coming from the top floor, are peppered, choking products which cannot be consumed, so it is recommended that the leaves from this floor to be used in blends.

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NECESITY AND POSSBILITY OF CULTIVATING VINE KINDS FOR TABLE GRAPES NEARLY THE BALNEARY AND CLIMATIC RESORTS FROM THE DEPARTMENT OF VALCEA

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Keywords: conveer areas, energetical out-turn, lucrativeness

ABSTRACT

Due to the researches we have performed from 2009 to 2012 concerning the necessity and possibility of cultivating vine kinds for table grapes near by the balneary climatic resorts from the department of Valcea, we have pointed out some matters which lead us towards a positive answer. The researches we have performed have pointed out the fact that, in the vicinity of these resorts, some areas do exist where the offered pedo-climate conditions are favourable to cultivating vine kinds for table grapes. Among other things the obtained harvests could provide a quantity of grapes for consumption in the fresh status lasting for the whole year. But, for this purpose, we should take into consideration to create some spaces provided with a controlled atmosphere, where table grapes should be preserved during winter. Apart from the sctrictly business-related issues, the practice of this production line could offer us the opportunity to protect the hygienical and alimentary value of the grapes offered for consumption, while we know that, now a days, such a desideratum may not be accomplished for the grapes that are transported towards other destinations from Australia, from the Republic of South Africa, from Chile, Italy or Turkey.

INTRODUCTION

For a given place, the climate is defined as the multi-annual regime of the occurring meteorological processes. It is variable, following the site's position upon the planet the detailed geographical situation that it owns, its respective relief's altitude and configuration. The most important item required by the genesis of the climate is the sun's radiations. The energy issued by the sun through its radiations brings light to the earth and warms it up, it turns the sea waters into vapours, producing clouds filled with ready-to-fall rain, it provides to chlorophyll and to other pigments the energy they need in order to carry on their numerous and complex synthesis processes.

The decisive circumstances for the plant's life and activity-upon which, together with the air, depends essentially the synthesis of the large number of chemical compounds which exist within the grape's bacca are: the duration of the sun's brightness, the surrounding environment's temperature and the precipitations from the atmosphere. Yet, they are also, for one given region, essential assets of its respective climate. A tight connection also exists between the soil's quality and the one of its viticultural production. For the soil and the underground, their chemical features but, most of all, their physical assets, are ultimately important. It is them which regulate the regime of waters, of the air and of temperature, as well as the soils' perviousness and their capacity of retaining waters underground. Within the chlly viticultural regions, the graveled soils do ensure a good

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draining for the excessive amounts of water and become warm very easily. Through better retaining the heat, these soils allow the grapes to acquire a more effective maturation.

Taking into consideration the climate's decisive influence upon the vocation of a viticultural area for a superior quality, together with the one of the soil, which is equally essential a lot of studies and researches were carried on and new ones keep being initiated, in order to elucidate the modalities through which these influences are expressing themselves and therefore, to find out how the viticultural farmer might draw benefits from these naturally happening phenomena (Condei Gh. and all., 2003, Dejeu L.C., 2011, Ratona J., 1997, Popa A., Condei Gh., 2006, Popa, A., 2007, Popa, A., Dicu, C., 2010, Tonietto, Carboreanu A, 1999, Teodorescu, St., 1987, Teodorescu, St., Popa, A., Sandu, Gh., 1987, Cichi Daniela Doloris and al., 2012, Oslobeanu, M., and all. 1991, Renzo Angelini and all 2010).

MATERIAL AND METHODS

In order to point out the main items which define the vocation of a viticultural area to produce table grapes, it was necessary to become aware of how the meteorological parameters do act upon it and to examine the types of soil which exist within the respective area. We have established the level held by the various meteorological indices by making use of the data recorded by the Romanian an specialized institutions. These data contained information concerning: the duration of the effective insolation; the maxima, average mean and minima temperatures; the exact number of frost days; the average amount of precipitations; the wind'speed; the number of fog days. The levels of these meteorological parameters were analyzed in respect to the requisements of the vine kinds for table grapes. We acknowledged the soil types and their respective assets with the support of OSPA Ramnicu Valcea and we employed the laboratory procedures that were recommended to us by the Soil'Science National Society.

RESULTS AND DISCUSSIONS

The Institute of Meteorology and Hydrology (pertaining to the National Agency for Meteorology and Hydrology) has gathered recordings of meteorological parameters at Ramnici Valcea for 100 years long. Concerning the meteorological parameters which are essential for the culture of vine (Table 1), we have performed a process of calculus and of synthesis which has led us to a series of conclusions which bears a peculiar importance for the possible culture of the vine kinds for table grapes.

During a whole year, the sun shines for 1990 hours. It is enough to provide adequate conditions for the physiological, biological and chemical processes, which happen within the vine plant. This latter benefits from a longer duration of sun's brightness starting by May (174.9 hours), reaching to 277.9 hours in July, then maintaining itself at good values (256,6 hours in August, 205,5 hours in September and 168,7 hours in Octomber). Therefore, during the periods when it goes through the processes of growth, then of maturatio, the plant does dispose of enaugh energetical resources able to ensure for it an active and efficient metabolism. The air's temperature, should it be absolute maximum, an average mean one or the absolute maximum, does record levels which sustain the development of the plant's processes either physiological, biological or chemical ones. Not even during the period of the vegetation's pause, namely during winter the negative temperatures do not endanger the plant, with, sometimes, the exception of the monts January and February. It is remarkable to notice that, during the vegetarion's period especially, the average mean air's temperatures do not record spectacular changes from one month to another, neither from day to night. At Ramnicu Valcea, during the vegetation period (either at its beginning or at its end), the number of frost days is extremely small. In most of cases, the frost days do not exist at all. The annual average amount of precipitations

do reach to 733 l/m^2 , with a relatively uniform situation in time during the respective year. The less days with precipitations are to be recorded from August to October. This fact is a benefit for the grapes, because so they are able to achieve their repening process. When they appear, winds do not cross over the average limit of 30-40 km/h. The existing number of fog days not lither bring prejudices to the processes of growth and maturation. Table 2 presents the levels of some climate's parameters during the vegetation period. They were recorded during the recent decade.

The average main amount of precipitations per month reach to 468 mm, with a relatively uniform distribution. There are more of them during the growth stage of twigs and of grapes, and less of them during the stage of the grape's ripening.

The air's average monthly temperatures during the decisive months involving the processes of growth and maturation (from June to September) do not diminish below 19,2°C and do not present significant variations from one month to another. Even in Jully, the air's relative humidity does not decrease below 66%. Foe each month, the sun shines for a sufficient number of hors: in July it reaches to 283 hours, then in August in maintains at 272 hours, at 217 in September and at 181 in October.

Taking into consideration the requirements of the vine kinfs for table grapes and comparing them to the present meteorological data, we have to notice that the formers are indeed fully accomplished, especially for the kinds present an extra-early ripening, an early one, a middle-type one or even a late ripening.

Table 3 presents the 5 (five) types of soils that may be found within the surface owning a viticultural potential and sited around the balneary climatic resorts from the department of Valcea.

Due to the parental materials (marls, clay-minyled marls, marl-mingled clays on materials issued from them), at the profile's basis a C horizon is separating itself a pseudorendzinic car one which is specific to the phaeozem type of soils. The Cpr horizon is averagely constitued of marls, clay-mingled marls or marl-mingled clays, usualy containing more that 3390 of clay and more than 12% of carbonates. These types of soils have fine texture, argillaceous or larm-mingled argillaceous. They are rich in calchreous humus and argillaceous amounts, their structure is well developed either glomerule-shaped, granulated serve of calcium fed humus or polyhedronal. Their redoes reach to 300-500 t/hl, their level of saturation in bases is of 80-100%, their pH lies between 6 and 8 (their reaction is: welkly alkaline, neutral, weakly acid). They are well endowed with nutritive substances and their microbiological activity is well carried.

The calcareous rhaego-soil has a weakly developed AoC profile. The Ao horizon, generally almost not shaped, is thick of about 10-40 cm. The C horizon which follows, represents the parental material, composed of not consolidated rocks.

There are not specific new formations within this profile. Its texture is sand-shaped till argillaceous. These soils are lither not structured or, if it should exist, their structure would be a weakly developed granulated or polyhedronal one. They are poorly or medium-sized endowed with nutritive substances and humus. Their reaction stands between acid till weakly basical.

Podzolic soils have a diverse texture, most of times it is middle rough or middlesized, with no profile-shapped differences. They are soil with a granulated structure in their superior horizon and not structured for the rest of the profile. Yet, they are well drained and they do not show humidity in excess (by water) unless they should be situated in depression zones or plateau-shaped ones. Their contents in humus (mostly in raw acid one) is very much decreasing while going in deep within the profile. Their level of saturation in bases and their pH are among the lowest ever met (V might diminish below 5%, while the

Meteorolog	ical parameter						Мо	onts						Annually
		Ι	II	III	IV	V	VI	VII	VIII	IX	Х	XI	XII	
Duration of in (he	nsolation's time ours)	75,0	80,1	138,9	174,9	216,5	243,8	277,9	256,6	202,5	168,7	91,1	64,5	1990,5
Air's temperature	Absolute maximum	14,3	19,2	26,0	28,1	33,5	34,9	37,3	36,8	33,5	30,5	25,2	16,0	37,3
(°C)	Average mean	-2,1	0,6	4,9	11,1	15,8	19,1	20,6	20,0	16,0	10,4	5,8	0,3	10,2
	Absolute minimum	-27,0	-18,8	-15,3	-3,3	3,0	3,9	7,5	7,0	-5,8	-5,8	-10,8	-16,9	-27,0
Number of	of frost days	27,5	27,2	14,1	1,4	0,0	0,0	0,0	0,0	0,2	2,9	10,3	22,3	99,9
Average me precipita	ean amount of tions (l/m ²)	39	39	37	54	95	91	101	70	57	52	55	43	733
Nember o precipita	of days with tions $\geq 0,1$	10,2	11,3	9,7	12,5	15,2	13,3	11,7	9,2	7,1	7,5	10,7	10,4	128,8
Speed of	wind (m/s)	10,5	8,8	10,6	9,3	11,7	10,7	10,4	10,1	8,6	9,7	9,1	9,4	9,9
Number	of fog days	9,9	8,6	4,9	1,3	1,2	0,8	0,6	0,7	11	3,6	7,2	11,6	51,5

Levels of the main climate's elements for a hundred years at Ramnicu Valcea (1886-1996)

Note: The wind's speed does express the average mean of the maxima monthly values

Table 2

Table 1

Levels of some climate parameters, during the vegetation period (april 1-st - October 30-th), at Ramnicu Valcea (1990-2010)

Climate parameter	Monts								
	April	May	June	July	August	September	October		
Average mean monthly amount of precipitations (mm)	69	73	81	94	66	42	35		
Average mean monthly temperature (⁰ C)	11,2	15,8	19,2	20,9	20,3	16,4	11,0		
Average mean monthly a relative humidity (%)	-	-	69	66	69	73	74		
Average mean monthly duration of insolation's (hours)	183	222	247	283	272	217	181		

Table 3

Viticultural	Latitude	Longitude	Altitude	Average	Amount of	Monts 01.IV-30.X		Х
area				mean annual	precipitations/	Temperature	Insolation	Precipitations
				temperature	year (mm)	degrees (⁰ C)	hours	
Rm. Valcea	45°06'	24°22'	242	10,2	710	3173	1425	411

Geographical and climate's elements (from the vegetation's period) at Ramnicu Valcea (1990-2010)

Table 4

1.	Typical Phaeozem (Pseudo-rendzine)	Cpr horizon for the first 150 cm; Am horizon resulted from the alteration of the substratum
		(Cpr); A/Cpr horizon, presenting, at least within its upper side, colours owning values and
		cromes <3,5 at the material in the humid state, as well on the sides as inside of its
		structural elements; does not present the other subtypes' features
		Profile: Am-A/C-Cpr
2.	Pellic Phaeozem (Levigated Pseudo-	It is resemblant to the typical one, but with a Bv horizon. At least within its upper side, its
	rendzine)	colours present crome values <3,5 at the material in the humid state, as well on the sides as
		inside of its structural elements
		Profile: Am-Bv-Cpr
3.	Calcareous rhaego-soil (Rendzinical	Ao horizon, followed by parental material, issued from not consolidated rocks, which the
	rhaegosoil)	geological erosion maintains near
		Profile: Ao-C-Rrz
4.	Skeleton type Podzol	Au-Es-Bhs-R,R horizons. R's upper limit is situated from 20 to 50 cm depth
		Profile: Au-Es-Bhs-R
5.	Podzol upon gravels (Podzol gravels)	Aon-Es-Bhs-R, horizons R's upper limit stands between 20 and 50 cm.
		Profile: Aou-Es-Bhs-R

Types of soils at Ramnicu Valcea (in the predominance order)

pH may descend below 4). Their endowment with nutritive substances, as well as their microbiological activity, are very low.

As we could notice almost all among the kinds of soils might be useful for the culture of their microbiological activity, are very low.

Where the respective case should appear for the podzolical soils, mineral fertilizer, s ought to be added (with nitrogen, phosphorus and potassium) and, most of all things, calcarous amendaments should be applied.

CONCLUSIONS

The vine kinds for table grapes may be cultivated many amoung the areas existing in the neighourhood of the balnery, climatic and touristic resorts wich function around Ramnicu Valcea.

The climate parameters and the predominant, soil types are adequate for the development of the vine's physical, biological and chemical processes, especially in the cases of extra-early, early and middle-sized kinds.

It is necessary to draw an inventatory of the most important viticultural area's of this zone, so that a program of vine planting should be realized.

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OLTENIA DISPOSED OF AREAS OWNING A VOCATION FOR PRODUCING TABLE GRAPES

Catalinoiu (Coca) Valeria*1

Keywords: conveer areas, energetical out-turn, lucrativeness

ABSTRACT

Through applying the multiple criteria method in order to delimit the areas which own a vocation for cultivating the kinds of vine producing grapes destined to be consumed in their fresh natural status, completed by the calculus made of the oenoclimate aptitude index, we were able to spot which sites in Oltenia where could be produced table grapes of vthe best quality do exist. We have remarked that Oltenia's climate circumstances do facilitate the cultivation of table grapes' vine for a wide range of sorts, and for considerable period of time during a year. Relying upon the statistical data, in thos woark we have drawn the inventory of the areas presently occupied by vine for grape tables, and we have issued some hypotheses concerning the possible perspectives of this productive line. Our hypotheses ought as well satisfy the viticultural producers economically, and provide for the consumers the enjoyment concerning the hygenical and sanitary value of grapes, which are suitable for consumption in their fresh natural status.

INTRODUCTION

By their beauty, as well as their tasty and crispy care, grapes have attracted the human being's attention, even since he was going through an inferior stage of the human society. For a long time, grapes were exclusively served as fresh consumption by humans (Popa, A., 2012). In comparison whith many other fruits, table grapes were and are prefered by consumers of all ages. Most of aliments and vegetables are consumed after being cooked, there by their intrinsic vitamins come to be destroyed. Yet grapes, consumed as fresh fruits, do offer to the human organisms their whole endowment of necessary vitamins.

Grapes are an aliment, but also a medicine which is difficult to substitute. Their chemical composition does recommand them as an energizer for muscles and nerves, a toxins' fighter a help for mineralizing the human body they are a stimuls for the liver, a diuretic and laxative, they ease the eliminating of gall, they do cure anaemia, arthrosis and asthenical. The grapes do reopresent: the nature's miraculous pharmacy.

Throught time, especially durng the recent decades, a lot of valuable studies have been carried on, as well as researches, concerning: the creation of table grapes' specialized kinds; the adoption of the most convenient thechniques for their cultivation; the outline of the sites that could favour most the table grapes; in increase the hygenical and sanitary value of table grapes within greenhouses and solari, etc (Condei, Gh. And all. 2003; Dejeu

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L.C., 2011; Oprea St., 2001; Popa, A., 2008; Popa, A., Condei, Gh., 2006; Tonietto& Carboneanu, 1999; Olteanu, I. and al., 2002; Cichi Daniela and al., 2012; Teodorescu, C., Stefan and al., 1987; Fregoni, M., 2005; Popa, A., Dicu, C., 2010; Popa, A., 2012; Renzo Angelini and al., 2010).

MATERIAL AND METHODS

From 2009 to 2012, our purpose was to outline the Oltenian viticultural spaces owning a vocation for table grapes production. Our study was focused on the calculus of the Oenoclimate Aptitudes Index (A), the value of which is given by the sum of temperature degrees (T) and of the hours of effective insolation (I) during the vegetation period (April 1-st – October 30-th) revised through eliminating the excess of precipitations fallen from the atmosphere. But they are also decisive matters into the plant's life and in its activity. It is upon them (together with air) that depends the synthesis of the numerous chemical componds which activate within the grape's bacca. In orded to delimit in the strictest way possible the viticultural areas destined to produce table grapes, (till the lot's level), we have employed the multiclriterial method.

RESULTS AND DISCUSSIONS

The appreciation of the table grapes' quality concerns the cluster' size, the form and the situation of bacca upon the rachis; the bacca's form and size, the skins' colour, the skins' elasticity, the gustative assets, the resistance proven during transportation, the adaptability to the atmosphere and, last but not least, the nutritional value.

In order to reach for these features upon the most convenient level, the kinds destined to produce table grapes need to find, within their viticultural area, pedoclimate circumstances ofthen more generous than the ones required ofthen more generous than the ones required by the wine-producing kinds. Amoung these they require: as many days as possible with no frost, and the lack of stressing circumstaces during the blooming period (such as low temperatues or abundent precipitations); they need, during the vegetation period, to dispose of a long duration time of the sun's brightness (more than 1000 hours), of more than 300 mm of precipitations and the air's relative humidity should not diminish under 70%.

For the resolution soils, the requirements are: to be deep enough, to be easily warmed, to withhold enough water, to be enough provided with aeration, to dispose of the right ratios of nutritive elements.

Table 1 presents the geographical and climate assets owned by the viticultural areas and centers from Oltenia. Due to the geographical position they own, situated in terms of Nortern latitude, from $43^{0}46$ (Bechet) to $45^{0}11$ (Polovragi); and in regard to the sea level, at an altitude standing between 58 m (Bailesti) and 530 m (Polovragi), the annual average' mean temperatures reached to are: $9,3^{0}$ C (Polovragi) to $11,5^{0}$ C(Calafat). During one year's time, the precipitations amount does oscilate between 544 mm (Bailesti) and 893 mm (Polovraci). In all centers the vegetation period goes beyond 160 days/year. During it, the active temperatures is abundantly recorded (at Polovragi, 2938°C till 3508° C at Bailesti); the sun's hours brightness do oscillate between, at Polovragi, 1302 and at Calafat 1613 hours. During the some vegetation period, the natural precipitations rise to an amount going from 238 mm (Calafat) to 513 mm (Polovragi).

When calculating the value of the Indices for Oenoclimate's Aptitude (A), we have remarked values standing between 3978 (Polovraci) and 5003 (Calafat). This fact proves that within the viticultural centers belonging to zones A0 and A2 (Polovraci, Tg. Jiu and Ramnicu Valcea), the best result to be obtained would be white wines of superior quality. Yet, in many micr-areas within these zones, table grapes may be obtained, since

they might be considered as' tolerated zones for table grapes. In five viticultural centers (Stefanesti Arges, Corcova, Dragasani, Samburesti, Costesti) the best results are manly red wines, while some microzones from these centers are to be framed within the medium favourable zone concerning table grapes. The other (14) viticultural centers situated in the southern part of the province do own a vocation for obtaining superior quality red wines, while concerning the table grapes, they belong to the favourable zone. Table 2 presents group of 13 viticultural centers, order suiting the vocation that each of them own for table grapes. One center (Zimnicea) does offer table grapes of the highest quality (the very favourable zone). Nine centers belong to the favourable zones, while three of them fit into the middle zone. In all the other viticultural zones from Oltenia, within certain microclimate zones, table grapes may be obtained. This is why, as for as this production line is concerned they belong to the tolerated zone. We also appreciate that, due to its physical, chemical and biological assets the respective type of soil has a great influence, contributing to establish the vocation of a viticultural area for producing table grapes. Table 3 presents the soil types, pointing out their predominance order within the main viticultural centers of Oltenia. We have to remeark that does influe upon the viticultural centers' hierarch regarding their vocation on for obtaining high quality table grapes. Being ware of the climate circumstances and of the main soil types within the most important Oltenia viticultural centers also taking into consideration the agricultural and biological requirements of the main kinds of table grapes that are cultivated, we have drawn for each of the viticultural ares the sort that would be recomanded for it, establishing them as a perpective (Table 4). The largest surfaces would be held by the kinds: Chasslas Dore, Victoria et Muscat Hamburg. A less extended are should belong to the kinds: Perla of Csaba, Muscat Adda, Afuz Ali and Italy. Table 5 presents for Oltenia, the evolution of the surfaces cultivated with table grapes. We are easly able to reach for the conclusion that, for Oltenia reconstructing viticulture should also mean taking into consideration the extent of the surface destined to the culture of table grapes. The viticultural farmes who would chose to cultivate table grapes' kinds should enjoy a great economical satisfaction, because the harvests of table grapes do find themselves a lot of destination (consumption as fresh fruits, as marmalade, as stewed fruits, as juices, as sweet shop art, etc.).

CONCLUSIONS

The region of Oltenia, Romania is situated in the European perspective cultivating table grapes, at the Northern limit of the most favourable zone. Within the Carpathians'-Danubian-Gethical space, the outline of this zone contains viticultural centers owning very favourable and/or favourable ecological circumstances.

In Oltenia, most of the viticultural centers dispose of enough resources which would be required by the vine kinds for table grapes such as of enough sun's that wind and even enough water supply

The table grapes bearing a pleasant comercial appearance and especially appreciated in organoleptic terms, obtained within favourable areas, acknowledged for their vocation from kinds owning some outstanding assets of quality, may be revaluted through obtaining a Controlled Origin Denomination, particulary if it sould be supported by a long since existing tradition of viticultural practice within the respective areas.

In Oltenia, as well as through Romania it is necessary to delimit the areas which would be the most favourable for cultivating table grapes

In a not too for future the surfaces cultivated in Oltenia with table grapes' vine might reach to an extent of 3197 ha

It is necessary to cultivate kinds of vine for table grapes upon the areas mostly situated mearly the recreation and medical treatment' resorts which function in Oltenia.

may be obtained grapes for fresh status consumption (table grapes) Viticultural Northern Altitude Anual Annual Month Index of Vocation for table grapes precipitations' 01.IV- 30.X oenoclimate centers and latitude (m) average temperature amount aptitude (A) areas Temperature Effective Precipitations (°C) A=T+I-(P-250) (mm) degrees (T) insolation (mm) (P) °C hours(I) ore Tolerant zone (****) Polovragi 45°11' 530 9,3 893 2938 1302 513 3978(A₀) 210 Tg. Jiu 45°02' 10,4 3233 1450 433 4500(A₂) Tolerant zone (****) 816 45°06 242 10,2 3173 1425 411 4437(A₂) Tolerant zone (****) Rm. Valcea 710 44°52 Stefanesti-307 7,7 644 3044 1492 359 4427(A₂) Medium favourable zone (***) Arges Corcova 44°35' 150 10,7 741 3313 1546 374 4682(A₃) Medium favourable zone (***) 44°30' 182 684 3316 1576 385 4757(A₃) Medium favourable zone (***) Dragasani 10,8 44°02' 260 10,5 682 3226 1536 395 4627(A₃) Medium favourable zone (***) Samburesti Costesti 44°30' 220 10,2 654 3236 1511 357 4640(A₃) Medium favourable zone (***) 44°25' Drobeta 86 11,0 634 3388 1549 309 4878(A₃) Favourable zone (**) Turnu -Severin 130 10,7 587 3339 1550 287 Favourable zone (**) Vanju Mare 4892(A₃) Orevita Oprisor 44°13 150 10,6 637 3340 1487 338 4765(A₃) Favourable zone (**) Plenita-44°05 145 11,2 565 3448 1439 288 4843(A₃) Favourable zone (**) Orodel 44°02' 73 312 Favourable zone (**) Segarcea 10.9 575 3353 1540 4805(A₃) Tamburesti 44°28' -10,4 612 3301 1525 312 4764(A₃) Favourable zone (**) 44°29' 200 10,7 634 3278 336 Favourable zone (**) Brabova 1516 4702(A₃) 44°19' 3403 4939(A₃) Favourable zone (**) Bradesti 195 10.9 543 1574 288 44°19' 4848(A₃) Favourable zone (**) Banu 195 10.8 613 3359 1575 336 Maracine 3359 1575 336 Favourable zone (**) Iancu Jianu 210 10,8 613 4848(A₃) -Calafat 43°59' 66 11,5 494 3480 1613 238 5003(A₄) Favourable zone (**) Favourable zone (**) Bailesti 44°01 58 11,2 587 3481 1490 288 4933(A₄) Bechet 43°46' 65 11.3 544 3508 1512 304 4966(A₄) Favourable zone (**) 44°47' Caracal 112 10,9 597 3433 1609 336 4956(A₄) Favourable zone (**)

Gegraphical and climate features (multiannual dates) of the Oltenia viticultural areas and centers

Area Area Very favourable* Middle *** Tolerant**** Favourable ** Zimnicea * (Witness) Dealurile * The other viticultural centers Severinului and vineyards from Piemontal Dealurile Craiovei * and Subcarpathian Oltenia Podgoria Dacilor * Calafat * Sadova- Corabia * Draganesti Olt * Dabuleni * Poiana Mare * Cetate Stefanesti- Arges * (Witness) Dragasani *

*

Favourability level of the cultivated areas reserved in Oltenia for table grape's kinds

* Precocity by 4 weeks

Targu Jiu

**Precocity by 2 weeks

***Moment 0, in rapport with Chasselas D'ore

****Tardivity by 2 weeks

Main existing soil types within some Oltenian viticultural areas where grapes for consumption in a fresh status (table grapes) are also produced

Viticultural areas	Soil types hierarchized by their predominance order
Polovragi	Lithical soils upon calcareous skeletons; skeleton shaped Eutrycambosoil
Targu Jiu	Argycal Phaccozem; Pellical phaeozem; Calcical Preluvosoil; Typical Luvosoil upon gravel
Rm. Valcea	Argycal Phaeozem; Pellical phaeozem; Calcical Preluvosoil; Typical Luvosoil upon gravel
Stefanesti Arges	Pellical phaeozem; Argycal Phaeozem; Skelethic Podzol; Podzol upon gravel
Corcova	Chalcycal Preluvosoil; Chalcycal Preluvosoil eroded; Euthrycambosoil skeletons; Pellical phaeozem
Dragasani	Chalcycal Preluvosoil eroded; Preluvosoil eroded (skelethic); Argycal Phaeozem; Phaeozem typical; Pellical
	phaeozem; Rhaegosoil calcareous
Samburesti	Rhaegosoil calcareous; Typical Luvosoil upon gravel; Typical Luvosoil skelethic; Calcical Prelusosoil
Drobeta Turnu Severin	Calcycal Prelusosoil; Preluvosoil eroded (skelethic); Calcical Prelusosoil eroded; Luvosoil psammical
Vanju Mare Orevita	Preluvosoil psammical; Preluvosoil red eroded; Luvosoil typical upon gravel; Euthrycambosoil skeletons;
	Typical Luvosoil skelethic
Plenita Orodel	Prelusosoil red eroded; Typical Phaeozem
Segarcea	Chalcycal Cernosiom ; Typical Phaeozem; calcareous rendzine
Tamburesti	Psammosoil
Banu Maracine	Preluvosoil calcycal eroded
Iancu Jianu	Preluvosoil calcycal eroded; Typical Luvosoil upon gravel; Skeleton shaped Eutrycambosoil; Luvosoil psammical
Calafat	Psammosoils; Phaeozem typical
Bailesti	Phaeozem typical; Phaeozem psammical
Bechet	Psammosoils
Caracal	Typical Phaeozem; Typical Phaeozem eroded

**** 1 * 1	1 1 C	1 .	. 11	
Wine kinds recommend	led for	nroducing	table or	anes in ()ltenia
whick kinds recommend	acu ioi	producing	table gr	apes in Onema

Vineyards/Viticultural center/				Kinds	for table grapes			
commune	Perla	Cardinal	Victoria	Chasselas dore	Muscat Hamburg	Muscat Adda	Afuz- Ali	Italia
Samburesti			*	*				
Dobroteasa			*					
Dragasani			*	*				
Iancu Jianu			*	*				
Banu Maracine			*	*	*			
Bradesti			*	*	*			
Brabova			*	*	*			
Severin Halanga		*	*	*	*	*	*	*
Corcova					*	*		
Vanju Mare Orevita			*	*	*	*		
Scorila Drancea			*	*	*	*		
Segarcea			*	*	*			
Targu Jiu				*	*			
Poiana Crusetu				*	*			
Draganesti Olt			*	*	*	*		
Plenita	*		*	*	*		*	
Vrata	*			*	*			
Poiana Mare	*			*	*		*	
Dabuleni	*							
Potelu	*			*	*			

Table 5

Areas cultivated with table grape's producing kinds, throughout time and within a (certainly) possible perspective

Department	Year									
	1975	1980	2007	2012	Perspective					
Dolj	781	2000	25	25	1234					
Olt	347	687	83,5	83,5	718					
Mehedinti	133	250	101	101	480					
Gorj	79	100	0,00	0,00	375					
Valcea	209	200	206	159	392					
TOTAL	1549	3234	415,5	368,5	3197					
	15									

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ASSORTMENT OF TABLE GRAPE VARIETIES AND GEOGRAPHICAL DISTRIBUTION IN VITICULTURE AREAS OF DEVELOPMENT REGIONS SOUTH MUNTENIA AND SOUTH-WEST OLTENIA OF ROMANIA

Cichi Daniela Doloris¹*, Popa Camelia², Ciobotea Cristina Magdalena², Stoica Felicia¹, Costea D.C.¹

Keywords: development regions, Romanian table grapes

ABSTRACT

In time, the viticulture Romanian areas and the assortment of table grapes varieties of vines suffered a series of quantitative and qualitative adjustments, caused primarily by the social and economic changes, and of the development of scientific knowledge and practical of viticulture, ampelography and breeding the vine.

In this paper we have proposed an analytical study focused on: table grape expansion in the culture in vineyards of Romania, the structure of the assortment of table grape varieties in the viticulture areas of the development regions South - West Oltenia and South-Muntenia of Romania and potential strategies for efficient use of environmental resources and the genetic resources in these areas.

INTRODUCTION

The grapes for fresh consumption, by their chemical composition, are a very valuable and important aliment for humans. To full maturity grapes containing large amounts of sugars (glucose, fructose), easily assimilated, about 1-2% organic acids (tartaric, malic, citric acid), about 1% mineral salts (Ca, Fe, K, P), vitamins (C, B1, B2, PP, A, E), enzymes, polyphenols (Dobrei A. 2003, Dejeu L.C. 2010, Damian et al. 2011, Rotaru et al. 2011, Simion & Donici 2011). They have an important role in the growth and development of the human body, by stimulating and regulating major metabolic processes in the tissues (Popa A. 2012).

Diversification of assortment and the improvement of the varietal conveyer and of the geographical conveyer of the table grape varieties in regarding (varietal assortment): create of the new varieties, better adapted to the different ecological conditions in our country, with different periods of ripening (maturation), superior from the point of view of the productive and qualitative characteristics, that to present increased resistance to the unfavorable environmental factors, and to the main diseases and pests, represents priority

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objectives in the vine breeding and in the viticulture (Cichi et al. 2011, Dejeu L.C. 2010, Oprea & Moldovan 2007, Sestras 2004, This et al. 2006, Vintilescu 2012).

In the last two decades, literature in the field of the wine and in the field of the medical gave numerous clear evidence about the fact that some chemical constituents of grapes plays an important role in the protection of the human body to a series of the most serious diseases, some of them being on the first places regarding the mortality rate (Athina Damianaki et al. 2000, Corder R. et al. 2001, Girotti S. et al. 2002). Thus, it was demonstrated clearly that in the grape is a variety of phenolic compounds that exercising the beneficial effects on health, including against cancer (Arif Atak et al. 2011, Teissedre P.L et al. 1996, Xu C. Y. Zhang et al. 2010). Among these constituents was noted particularly a neflavonoid polyphenolic compound, resveratrol (Moreno A. et al 2008).

The trends, preferences and requirements, rapidly changing at the consumers of grapes, plus a steady decrease in recent years in the production of table grapes in Romania and import volume growth in this category of products, require a careful analysis of the areas of culture of table grape varieties, as well as the assortment of grape varieties fresh consumption in our country.

MATERIAL AND METHODS

Reconsidering assortment of vine varieties and Romanian viticulture areas established to producing table grapes, through the creation of competitive vineyards plantations technically and economically, is both a priority in the research and viticulture practice from Romania, and a important element of local identity and pride of the residents of these areas.

To this end, in this paper we have proposed an analytical study focused on: expansion in the culture of table grape varieties in Romania, the structure of the assortment of table grape varieties in the viticulture areas of the development regions South - West Oltenia and South-Muntenia of Romania and potential strategies for efficient use of environmental resources and the genetic resources in these areas.

RESULTS AND DISCUSSIONS

The current expansion in culture of table grape varieties in Romania Distribution of table grape culture in our country is the result of long observations and researches on making full use of the environmental resources of the viticulture Romanian space, of the biological and productive potential of the different grape varieties, in terms of increased economic efficiency (Constantinescu & Indreaș 1976, Ionică Mira 1999, Martin et al. 1974, Oşlobeanu M. et al. 1991, Popa et al. 2008, Popa & Dicu 2010).

Regarding the dynamics of the viticulture area cultivated with table grapes, in Romania, during 2000-2012, is found (Figure 1) continuous and a worrying decline of 70.56% in 2012 (5210 ha) compared with 2000. This is because biological aging of most existing vineyards before the Revolution of 1989 and the absence of adequate strategic measures for the recovery of these vineyards supported by the general socio-economic situation and the lack of financial support they received in exchange for growing plantations for wine grape varieties.



Figure 1. Dynamics of areas under vineyards for table grapes in Romania (thousand ha) Source-MADR, INS Romania (data processing)

In the year 2012 the total vineyard area planted with table grapes in Romania was 5912.1 ha (National Institute of Statistics, 2013). The largest share in area planted with table grapes (76.2%) is located in South-East, while the region of development South - Muntenia (345. 6 ha) is placed on third place, South- West Oltenia region with 110.5 ha are only on fifth place in the Romanian development regions (Figure 2).

According to National Institute of Statistics of Romania (2013), in terms of expansion in culture for table grape varieties in Romania, the highest rate for the surface (40.2%) is the variety Chasselas dore (2096.5 ha), followed by Afuz Ali variety with a share of 24.6% (1283.0 ha). Both Chasselas dore and Afuz Ali variety is very old varieties in culture (over five decades), they have the very heterogeneous population in regards the production and quality, physiological resistances, ripeness of wood and, therefore, it is necessary to speed up rigorous clonal selection.

The variety Victoria, much appreciated and valuable in terms of the biological, productive, qualitative aspect of commercial, had a percentage of only 1.6% of the total area under table grape varieties (Figure 2). Regarding the new varieties of table grapes (superior of old varieties in terms of the production, quality and resistance to unfavorable factors abiotic or biotic), obtained in Romania, they have an insignificant, less than 4%.

Table grape production areas from the development region of South-West Oltenia are located in 17 viticulture centres in wine growing region *Oltenia and Muntenia Hills*, respectively, in 9 centres in wine growing region of *Sands and other favourable land of the South of Romania* (tables 1 and 2).

The development region of South-Muntenia includes areas for the production of table grape located within 11 centers among wine growing region of *Oltenia and Muntenia Hills*, four centers among wine growing region of *Danube Terraces* and 5 centers in region of *Sands and other favourable land of the South of Romania* (tables 3 and 4).

Range structure of varieties in viticulture areas in developing regions South-Muntenia and South West Oltenia- It appreciates, rightly, that that the grape varieties for fresh consumption are produced much more dynamic than the wine grape varieties, this



Figure 2. Distribution area (Ha) of the vineyards for table grapes (by development regions in Romania) and the area (Ha) of the main table grapes cultivars in Romania (data processing, Source-*National Institute of Statistics of Romania*, 2013)

being due to the requirements and demands continuously changing of the niche consumer that accepting more easily the replacement a table grape variety with the another (Antonacci, A. Scienza quoted in AA.VV. 2010, Grecu V. 2010, DAI/ACED 2011). In this context, the viticulture research and producers in the field should be constantly interested in the development of scientific knowledge in the field of genetic improvement in the vine and in creating new varieties, with a high potential of the production and quality, with increased resistance to diseases, pests and unfavorable environmental conditions, with the favorable impact on the environment and to the economic agents, trough the transfer of these knowledge in economic practice.

Table 1

- Wine grow	ing re	gion (Itenia	i ana i	Muntenia	Hills .	ጥ		
					Varieties				-
Viticulture Centre, County	Augusta	Cardinal	Victoria	Azur	Muscat de Hamburg	Muscat d'Adda	Călina	Afuz Ali	Italia
Sâmburești Olt			*		*	*			
Dobroteasa Olt			*		*	*			
Drăgășani Vâlcea	*		*	*	*	*	*		
Gușoieni Vâlcea			*	*	*	*	*		
Măciuca Vâlcea			*	*	*	*	*		
Iancu-Jianu Olt			*	*	*	*	*		
Banu Mărăcine Dolj			*		*	*			
Brădești Dolj			*		*	*			
Brabova Dolj			*		*	*		*	
Segarcea Dolj			*		*	*		*	
Severin-Dealul Viilor Mehedinți		*	*		*	*		*	*
Corcova Mehedinți					*	*			
Golul Drâncei Mehedinți			*		*	*		*	
Vânju Mare Mehedinți			*		*	*		*	
Orevița Mehedinți			*		*	*		*	
Plenița Dolj			*		*	*		*	
Târgu Jiu Gorj			*		*	*			

Structure of t	he assortmen	t of tab	le grap	e varieti	es recon	nmend	led an	d autho	orized i	n the
viticu	ilture areas o	f the de	velopn	nent regi	on of Sc	outh-W	/est O	ltenia		
	** **			01	114		¥ ¥ • 11	.1.		

* In accordance with O.M. No. 225 of 31 March 2006- Minister of Agriculture and Rural Development of Romania

In ecological conditions from Romania, the maturation of table grape varieties is between 15 July and 30 October (table 5 and table 6), during seven periods of maturation (Cichi Daniela et al., 2010, Damian et al., 2011).

Synthetic analysis of the structure of the varieties assortment recommended in viticulture areas of development region South West Oltenia (according Minister of Agriculture and Rural Development of Romania, O.M. No. 225 of 31 March 2006-approving *Zoning noble grape varieties of fruitful vines admitted in culture in growing areas in Romania*), show off a limited number of varieties, respectively nine varieties: Cardinal, Victoria, Azur, Augusta, Muscat d'Adda, Muscat Hamburg, Afuz Ali and Italia (pyrene varieties for fresh consumption) and one seedless variety (Calina-for fresh

consumption and raisins), which shows an insufficient use of existing growing availability of genetic resources in Romania and green supply inefficient use of space existent in vineyards Oltenia. Regarding the market supply of grapes for fresh consumption in the development region South West of Oltenia by production obtained from varieties recommended and authorized in viticulture areas existing, this is ensured within the August 16 to October 15 (table 5), the conveyor varietal being represented by four white varieties and four red varieties (grape seeds) and a pink seedless variety (Calina).

Assortment of table grapes from viticulture areas of South-Muntenia development region includes 24 varieties, of which 13 are white and 11 varieties are varieties with red - black skin, with different shades (Table 6).

In the current context orientation of consumer preferences for grape varieties for fresh consumption by seedless (without seeds) or less seeds in berry must specify that, although South-Muntenia Development Region benefits from areas with very high and high favorability for growing seedless grape varieties (Greaca, Giurgiu, Zimnicea) assortment of varieties is limited to two cultivars in this category, namely - Otilia and Centenar de Pietroasa (table 6).

Table 2

Structure of the assortment of table grape varieties recommended and authorized in the
viticulture areas of the development region of South West Oltenia - Wine growing region
Sands and other favourable land of the South of Romania *

Viticulture Centre, County					Varieties				
	Augusta	Cardinal	Victoria	Azur	Muscat de Hamburg	Muscat d'Adda	Călina	Afuz Ali	Italia
Vrata Mehedinți					*	*		*	
Izvoare Mehedinți					*	*		*	
Jiana Mehedinți					*	*		*	
Poiana Mare Dolj					*	*		*	
Cetate Dolj					*	*		*	
Dăbuleni Dolj					*	*		*	
Tâmburești Dolj					*	*		*	
Potelu Olt					*	*		*	
Drăgănești-Olt			*		*	*		*	

* In accordance with O.M. No. 225 of 31 March 2006- Minister of Agriculture and Rural Development of Romania

Recommended and authorized assortment in viticulture areas in the South-Muntenia ensure echeloning fresh grape consumption in period 15 July to 15 October.

Range structure of recommended varieties in viticulture areas of South-Muntenia Development Region (tables 3 and 4) although it rich, many of these varieties do not satisfy the current requirements of producers and consumers and, most new table grape varieties produced in Romania (with higher productivity and quality attributes) are very poorly promoted and spread to their culture, culture area and is usually limited to the area in which they were obtained.

										Va	rieties	5									
Viticulture Centre, County	Augusta	Cardinal	Victoria	Muscat de Hamburg	Muscat d'Adda	Afuz Ali	Italia	Chasselas dore	Chasselas de Raneasa	Istrita	Centenar Pietroasa	Coarnă neagră select	Otilia	Timpuriu de Dietroasa	Transilvania	Milcov	Napoca	Select	Silvania	Tamina	Xenia
Boldesti, Prahova				*	*			*		*	*		*	*							
Valea Calugareasca, Prahova	*		*	*	*	*			*	*	*		*	*	*	*	*	*	*	*	*
Urlati-Ceptura, Prahova	*		*	*	*	*			*	*	*		*	*	*	*	*	*		*	*
Tohani, Prahova	*		*	*	*	*	*		*	*	*		*	*	*	*	*	*		*	*
Cricov, Prahova	*		*	*	*	*	*			*	*		*	*	*	*	*	*		*	*
Costesti, Arges	*	*	*	*	*			*												*	
Bucsani, Dâmbovita				*	*			*													
Valea Voievozilor, Dâmbovita				*	*			*													
Stefanesti Arges,	*	*	*	*	*	*				*	*	*	*	*					*	*	
Topoloveni Arges,	*		*	*	*	*														*	
Valea-Mare, Dâmbovita				*	*																

Structure of the assortment of table grape varieties recommended and authorized in the viticulture areas of the South-Muntenia Development Region of Romania- Wine growing region *Hills of Muntenia and Oltenia**

* In accordance with O.M. No. 225 of 31 March 2006- Minister of Agriculture and Rural Development of Romania

Structure of the assortment of table grape varieties recommended and authorized in the viticulture areas of *South-Muntenia* Development Region of Romania – Wine growing regions Terraces of the Danube and Sands and other favourable land of the South of Romania *

Table 4

									Variet	ies								
Viticulture Centre, County	Augusta	Cardinal	Muscat timpuriu de	Victoria	Muscat de Hamburg	Muscat d'Adda	Afuz Ali	Italia	Chasselas de Baneasa	Istrita	Centenar Pietroasa	Otilia	Timpuriu de Pietroasa	Select	Tamina	Xenia	Greaca	Alphonse Lavallee
				Wi	ne grow	ing reg	ion Te	rraces	of the	Danu	be							
Fetesti Ialomita	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*
Greaca Giurgiu	*	*		*	*	*	*	*	*					*	*	*	*	*
Giurgiu Giurgiu	*		*		*	*	*		*					*	*	*	*	
Zimnicea	*	*	*	*	*	*	*	*	*					*	*	*	*	
Teleorman																		
	W	ine gr	owing	region	n Sands	and oth	er fav	ourabl	e land	of the	e South	n of Re	omanic	ı				
Furculesti		*		*	*	*	*	*										
Teleorman																		
Mavrodin		*		*	*	*	*	*										
Teleorman																		
Urziceni Ialomita					*	*	*			*	*	*	*					
Suditi Ialomita					*	*	*			*	*	*	*					
Ulmu Calarasi					*	*	*	*										

* In accordance with O.M. No. 225 of 31 March 2006- Minister of Agriculture and Rural Development of Romania

Table 5

	July	Augu	ist	Septer	nber	Oc	tober
Variety	15-31 Maturity group I	1-15 Maturity group II	16-31 Maturity group III	1-15 Maturity group IV	16-31 Maturity group V	1-15 Maturity group VI	16-31 Maturity group VII
Augusta							
Cardinal*							
Victoria							
Azur			(¹ C ¹				
Muscat de Hamburg			物制				
Muscat d'Adda							
Călina				A CAL	10 10		
Afuz Ali							
Italia*							

The period of ripening of table grape varieties recommended and authorized areas in Wine growing region of Oltenia

*Unauthorized for planting from 2012 according to *the Official Catalog of varieties of crop plants in Romania for* 2012 (by Minister of Agriculture and Rural Development of Romania and State Institute for Testing and Variety Registration-ISTIS)

Currently, the Romanian market facing with the lack of the grapes varieties with a few seeds in berry or even lack thereof (high degree of seedless), the existence a limited number of grape cultivars with very early and early ripening, but especially of those with the complex biological resistance.

The Romania's current viticulture, characterized by a strong specialization in wine production, has a number of disadvantages, especially as regards the weakly promoting and enlargement in the crop of indigenous grape varieties. At this shortcoming is added and the lack of coherent national programs which to support the inventorying, collection and conservation of the viticultural germplasm fund, which over time can permanently damage the Romanian viticulture genetic patrimony, what limits the availability of genetic resources that can be used in the process of breeding.

Table 6

				<u> </u>			
	July	Au	igust	Septe	ember	Octo	ber
Variety	15-31 Maturity group I	1-15 Maturity group II	16-31 Maturity group III	1-15 Maturity group IV	16-31 Maturity group V	1-15 Maturity group VI	16-31 Maturity group VII
Centenar Pietroasa (Seedless grapes)							
Augusta Muscat Timpuriu de București							
Otilia (Seedless grapes) Timpuriu de Pietroasa							
Napoca							
Cardinal*							
Victoria		-					
Chasselas dore Istrița							
Chasselas de Baneasa				ŧ.			
Milcov							
Muscat de Hamburg Muscat de Adda Transilvania Alphonse Lavallee*							
Silvania							
Select Greaca							
Afuz Ali Italia* Xenia							
Coarnă neagră secționată Tamina							

The period of ripening of table grape varieties recommended and authorized areas in South-Muntenia Development Region of Romania

*Unauthorized for planting from 2012 according to *the Official Catalog of varieties of crop plants in Romania for 2012* (by Minister of Agriculture and Rural Development of Romania and State Institute for Testing and Variety Registration-ISTIS)

CONCLUSIONS

Even if the range of table grape varieties seems rich for our country (varieties and local and foreign clonal selections) many of the varieties included in the official Catalog of varieties of crop plants in Romania do not meet the current requirements of producers and consumers and, many of table grape varieties obtained in Romania are less promoted and spread in culture (their area of crop, usually limited to the area in which they were obtained).

Ensuring the consumption of fresh grapes as long a period of time, improving vineyards in areas established to obtain for this product and reducing / elimination of imports of grapes, can be achieved only by creating, promoting and expanding the new varieties in culture, obtained in the research, varieties with different maturation periods, higher quality of existing varieties. These new varieties should require as few phytosanitary treatments, with high adaptability to ecological conditions in Romania, varieties which have high potential to enter into direct competition with imported products with high commercialization potential in European markets.

In this context, is required a national strategy for reconsidering of the national network of inventorying, evaluation and conservation of genetic viticultural resources (including the public-private partnerships) on the one hand, but also, the reconsideration of areas of the economic exploitation of the assortment (geographic conveer) respectively, the diversification of the assortment of the table grape varieties (conveyer varietal) in relation to the needs of consumers and producers.

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BEHAVIOUR IN THE PROCESS OF GROWTH AND FRUITING OF APPLE VARIETIES IN THE SOUTH OF THE COUNTRY

Mihai Cichi¹

Keywords: vigor, production, rootstock, variety, apple.

ABSTRACT

The shortage of information on the behaviour of a particular variety in a particular area, and on the other hand, the rapid dynamics of new varieties, forcing experts from all countries and areas to investigate the behaviour of new fruit varieties in natural conditions.

Although there are currently in culture an impressive number of apple varieties, each of them manifested in the process of growth and fruiting as well in terms of quality and safety, significant deficiencies.

Good resistance at Podosphaera Leucotricha have presented the varieties Red Delicious and Sir Prize. At Endostigme Inaequalis, the reaction was different in the sense that the varieties Golden Delicious, Starkrimson, Golden Spur and showed obvious sensitivity.

On the other hand each natural area, fructiferous or centre lies in ecological conditions has certain conditions, of microclimate, an environment edafic.

These items contribute more or less display production potential of certain varieties.

In the south zone conditions, for ensuring continuous supplies of fruits, can be retained in assortment of the varieties: Golden Delicious, Jonagold, Sir Prize and the Starkrimson.

INTRODUCTION

Validation requirements for fruit species apple and internally but also to export, they did but should be extended to areas of culture.

For these reasons varieties are preferred that lend to new methods of planting in orchards, in intensive and superintensive system and which fulfils the conditions imposed by the new european requirements.

In the main countries producing apples, the assortment is changed from one decade to the next, and even after a few years in order to cope with the demands of the ever-increasing consumer and competition between the foreign and domestic producers on the domestic markets.

Eugeniu Gudumac (2008), has developed an informative guide to support small and medium-sized producers from the area Chişinău.

The guide reflects some important technological aspects at formation of young plantations superintensive, with the presentation of varieties for testing in production especially for the South of Moldova.

Bălan & Şaganean, 2006, studied the growth potential of apple trees, depending on the cutting system at four varieties Pinova, King Ionagold, Champion and Gala Must grafted on M 9.

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They highlighted the fact that the increase in foliar surface dynamics is in corelation with the dynamic growth of the shoots.

Apple varieties resistance to diseases constitute one of the main objectives of the programme of improvement.

Sina Cosmulescu (2005), noted that genetic resistance varieties to Endostigme inaequalis more widespread in the culture in different countries are: Florina, Prima, Liberty, Sir Prize, Pionier, Generos, Romus.

In Bulgaria it was shown that the number of treatments has been lower to varieties resistant to *Endostigme inaequalis* than to the neresistant, (Dzhuvinov et al., 2001).

In studying the assortment has an important role and foliar fertilization, this issue has occupied Bălan V. and Vămăşescu S., (2012).

Within the framework of the three varieties, they have studied the foliar surface in relation to the requirements of different foliar fertilizers on plants.

Remy P. (1962), specifies that the varieties Golden Delicious, Mc. Intosh are very sensitive to lack of magnesium in the soil, which has as a result a premature leaf fall.

MATERIALS AND METHODS

Research has been carried out in the period 2010-2012 within the framework of plantations established in the botanical gardens in 1993 with six apple varieties and one rootstocks (M 9). The experience was randomized blocks method located in the 2 repetitions of 10 trees in repetition, returning 20 trees per variant.

Shape crown for trees was Drapeau Marchand.

Trees were planted at a distance of 3.5 m between rows and 2.0 m on row of trees. In order to achieve this purpose, we have proposed several research objectives:

-checking of varieties in growth;

-checking the fructification varieties;

-the production obtained.

RESULTS AND DISCUSSIONS

In the study of the six varieties on the rootstock, I found the following:

Within the studied varieties early start in vegetation develops varieties: Golden spur (3 III-8 IV) and followed by the variety Sir Prize (4 III-9 IV). A late entry in the vegetation detached from 2-3 days, presents the variety Golden Delicious (5 III-10 IV), the Jonagold (5 III-10 IV), Red Delicious (6 III-11 IV) and the variety Starkrimson (6 III-11 IV). Retain a longer period leaves the following varieties: Red Delicious, Sir Prize and Starkrimson (22.X - 7.XI). Significant very significant positive growth compared with average, presents varieties Jonagold (122.2 m/ tree) and Sir Prize (120.3 m/ tree).

Reduced vegetative growth, reflecting a small vigor, presents the varieties: Golden Delicious and Golden Spur, which compared to the average registered very significant negative values, and the distinct values significantly negative has Starkrimson variety.

Compared with the Golden Spur, small increases presented the Golden Delicious variety whose values are negative, significant distinctively (108.4 m/tree). The amount of fouling increases highlight very clearly and in Figure 1.



Figure 1. The amount of fouling increases at several apple varieties (Years 2010-2012)

Note the above the average values in the varieties: Jonagold (3.80 m/tree) and Sir Prize (4.00 m/pom) whose values are significantly positive. Also, the varieties Golden Spur (3.00 m/tree) and Starkrimson (3.20 m/ tree) presents significant negative values compared to the average. In terms of the diameter of the crown, the varieties Jonagold (4.40 m/tree), Sir Prize(4.35 m/tree) and Red Delicious (4.30 m/ tree) stood out with significant values and very significant positive compared to the average.

Analyzing the growth increase in the thickness of the surface sections of the trunk, we keep that growth meant a spore done: Jonagold (190.5 cm²/tree), Red Delicious (180.0 cm²/tree) and Sir Prize (190.0 cm²/tree), which compared average, registered very significant positive values. Distinct values and very significant negative showed varieties Golden Delicious (145.0 cm²/tree), Golden Spur (140.5 cm²/tree) and Starkrimson (160.5 cm²/tree), compared to the average.

In the fructification process exist differences, but are not too high between varieties. In the varieties of apple, mixed buds start in vegetation in the first decade of March (3-7 III), or the first-second decade of April (7-14 IV). Start more early buds swelling the varieties Golden Spur and Sir Prize (3. III-7. IV). The beginning blooming is invoked at of apple in the second decade of the month of April (13.IV-16. IV) or the first decade of May (3.V - 9.V). With the early blooming stand out the varieties: Golden Spur (13.IV - 3.V) and Sir Prize (13.IV - 4.V). End of blooming takes place in the third decade of the month of April (22.IV - 25.IV) or the second decade of the month of May (13.V-19.V). The period of blooming overlaps with most varieties and thus it is still necessary to group the varieties with close or simultaneous flowering and reciprocal pollination. Ripened their fruits in the first or second decade of September the variety Jonagold, Red Delicious and Starkrimson (1.IX - 20.IX; 7.IX - 20.IX; 10.IX - 20.IX).

The varieties Golden Delicious, Golden spur and Sir Prize can be harvested during the last days of September (2. IX - 30. IX). Knowledge of the specifics of the fruition of any kind within a species has a particular relevance in indicating how cutting and even tree leadership.

Thus, the varieties Golden Spur and Starkrimson presents a short formations dominating the fruition of spikes (80-95%). This character makes use of small distances between floors, a large number of short branches and the possibility of their use within the superintensive plantations.

Dominant on long formations the following varieties: Sir Prize(45%), Golden Delicious (30%), Red Delicious (30%).

The percentage of formations of fruits in the varieties of apple fruit studied is presented in Figure 2.



Figure 2 - The specifics of the fruition of several apple varieties

The determinations made in the years 2010-2012, it is established that yields achieved an average of 43.3 kg/tree. The highlights however this varieties: Golden Delicious (55.0 kg/tree) and Starkrimson (60.0 kg/tree), as a very significant positive, compared to the average. Jonagold variety should be highlighted with an estimated production of 45.0 kg/tree larger than the average 43.3 kg/tree.

A small amount of fruit was obtained from varieties Golden Spur, Red Delicious and Sir Prize (30.0-35.0 kg/tree), proving a poorer adaptability of these varieties at the present conditions of the central zone, values being distinctly and very significant negative.

CONCLUSIONS

The vigour of growth through increases in the amount assessed by vegetative tree height, crown diameter, the increase in thickness of the trunk is large in the varieties Jonagold, Red Delicious, and Sir Prize.

Effect of growth shows the varieties Golden Delicious, Golden Spur and Starkrimson. Fruits maturation begins in the first decade of September and ends in the third decade of the month September, being considered as varieties of autumn - winter.

In the central area, in order to ensure continuous supplies of fruits, can be retained in the assortment of the varieties: Golden Delicious, Jonagold, Sir Prize and the Starkrimson.

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RELEVANCE OF THE CHOICE OF SEEDLESS VARIETIES USED AS GENITORS IN HYBRID COMBINATIONS

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Keywords: elite hybrid, parental forms, seedless variety, genetic resources

ABSTRACT

In aim to create new table grape varieties it is take into consideration the primordial role of genetic resources in improving of the viticultural assortment, as source of new traits for obtaining new genotypes highly competitive. Diversification and supplementing the existing assortment in Romania, with varieties with different degree of seedless, various use and high quality, is a desideratum of the breeding program.

To the obtaining of hybrid elites were chosen table varieties recognized for superior quality and productive potential of the crop, earliness and degree of seedless: Canner, Black Pearl. Were made the specific agrobiological and technological determination which revealed high biological quality of the varieties targeted under the conditions of the viticultural center Ştefăneşti-Arges and to the new elits at the new elites obtained in F1.

INTRODUCTION

Improving the Romanian viticultural assortment with new varieties, mainly seedless, with different use, with varying degrees of seedless and with staggered ripening represents in fact a necessity for viticulture and national economy. In recent years in Romania the assortment of varieties for fresh consumption and processing is one weak because have not been created the varieties for this segment and implemented in the areas of culture. Testimony is that in *The official catalog of varieties of crop plants in Romania for 2012* are recorded only four varieties of seedless table grape, respectively Călina, Pietroasa, Otilia and Perlette.

The practice efficiency of the works breeding can not be assured and increased, without a thorough theoretical basis, without knowledge of the phylogenetic and ontogenetic material with which they work (Neagu, 1975)

If the seedless varieties from the old assortment of the world of corresponded to the initial purpose of industrialization, where the size of berries had a less important role, today for the fresh consumption, the size of the berries becomes a problem of first order (Constantinescu & Indreas 1976, Indreas & Visan 2002, Popa Camelia et al. 2010).

The high quality standards required by the the international market determines breeders at a rigorous research of the genetic basis for the future combinations. The

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premises of the choice a some suitable genitors, wich to transmit the quality and quantity characters pursued in descendents are extremely important.

MATERIAL AND METHODS

An important objective of European viticulture is today the creation of new varieties adapted to climate and soil conditions, of table grapes with outstanding quality traits, to replace some of the existing varieties in old plantations and and having as few seeds in the berry (seedless).

As a result the intraspecific hybridizations performed with varieties of American origin: Canner și Black Pearl, at Stefanesti in 2007-2009 was obtained a rich varietal fond with elites hybrid for table grapes, pyrene and seedless.

After a studying for several years of the varieties for jam and raisins existing in the ampelographic collection, we found that the two varieties used as paternal genitors have agrobiological and technological characteristics important, that can transmit valuable traits in combination with the Victoria variety.

Canner and Black Pearl varieties were studied in a ampelographic collection at I.N.C.D.B.H. Ștefănești. Its were grafted on Kober 5 BB rootstock and planted in 1996 at a distance 2.5 m between rows and 0.9 m on row. The training system of grapevine is Guyot on half-tall strains.

Pedoclimatic conditions are specific to the vineyard Ştefăneşti. The experimental field was placed on a typical preluvisol with clay to loam-clay texture in the first 60-80 cm depth.

RESULTS AND DISCUTIONS

Within the study has been carried out observations and determinations on varieties resistance at frost in winter, vigor of the hub, deployment the main phenophases, duration of the vegetation period, were calculated the coefficients of the absolute fertility and relative, productivity indices, the quantity and quality of grape production.

Canner (Figure 1) variety is characterized by: *The mature leaf* is large, pentagonal, with large teeth, glabrous. *The bunch* is large, ramose, lax. *The* size of berry is medium-large, with narrow ellipsoid shape, color of skin is yellow greenish, rusty on the sunny side. The firmness of flesh is very firm, crispy and with small rudimentary seeds.

Black Pearl (Figure 2) variety is highlighted by: *The mature leaf* is medium, pale green, glabrous, the size of teeth is medium, with both sides rectilinear. *The bunch* is large, ramose, lax. *The size* of berry is medium, with obtuse ovoid shape and color of skin is dark red - violet. The firmness of flesh is firm, seedless.

In this paper are presents the agro biological and technological characteristics of the both seedless varieties during the three years of study (2010-2013) to demonstrate the value of combination into hybrid descendants.

The time of bud burst of the two seedless varieties have demonstrated a good adaptability to specific climatic conditions in vineyard Ștefănești (Figure 3).

The average of values (years 2010-2013) concerning the viability and fertility of buds demonstrates that the two varieties have had close values, the losses being small. The coefficients of absolute fertility had recorded values equal in both varieties; just CFR differentiates very little Black Pearl variety as against Canner. The yield per vine is high and very close at both varieties (2.5 -2.8 kg/vine), a factor for that those varieties were selected as paternal genitors in the hybrid combinations (Figure 4).









of the bud

absolute and relative

To highlight the productive value of the varieties, the productivity index were calculated: absolute (CFA) and relative index (CFR), Figure 5. Due to the the large weight of the grapes from Canner variety (479 g) the productivity indices of this variety had recorded values very high, special values for a seedless variety. The main quality traits that highlight the two seedless varieties are presented in chart 6. The size of berry, expressed by the weight of the 100 berry, in average had presented values between 368g (Canner) and 239g (Black Pearl). The weight of a bunch of grapes, was determined by repeated weighings of these per 10 vines, keeps the same ladder as in the case of the weight of berry. Thus, the largest bunches of grapes had the Canner variety (479g) followed by Black Pearl with 150 g less. Due to the higher content in the sugars of the must (204g/l), BlackPearl variety recorded higher values regarding the gluco- acidimetric index.



Due to the morphological characteristics, agro-biological and special technological, the mentioned varieties were used in hybrid combinations to obtain new genotypes for table grapes with highly degree of seedless.

CONCLUSIONS

Under the climatic conditions of viticultural center Ștefănești - Arges, the two seedless varieties (Canner and Black Pearl) have superior qualitative traits as against many existing varieties in the culture and on market place. Due to the special morphological characteristics, shape and color of the berry, size of bunch, the accumulation of sugars, the varieties have been successfully used in the process of creating new seedless grape genotypes.

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THE HIGHLIGHTING OF THE PHENOTYPIC CHARACTERS EXPRESSED IN DIFFERENT WAYS WITHIN THE SAME HYBRID COMBINATIONS

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Keywords: hybrid, phenotypic characters, intraspecific combination.

ABSTRACT

To supplementing viticultural assortment for table grapes it is take into consideration obtaining some hybrids at which due to the recombination of the genes, selection for the quality, earliness, productivity to allow identifying of the some forms superior as against the parents from that further follows obtaining some new varieties. Thus the ampelographic determinations were made to the bunch (grape) and berry descriptors at 14 elite obtained by self-fertilization of the Victoria variety. By self-fertilization performed it was take into consideration the transmitting and fixation in the vegetative lineage of some valuable traits (quantity – quality), the type of the fusion heredity (color of grape) the maximum capitalization of the environmental conditions. It was found that 13 elites have the color of berry greenish yellow and at an elite the color of berry is rose. Regarding the shape of berry it is diversified shapes from ovoid shape to cylindric shape and even horn shape.

INTRODUCTION

The new varieties and hybrids, obtained by breeders must meet a large number of characteristics and importance traits of biological and economic, expressed at a superior level. The meeting of a largest number of characters and valuable characteristics into the same variety or hybrid does not mean the creating of some varieties or universal hybrids for all regions, for all the natural and practical requirements. Possible is the realization of new forms with a biological plasticity much higher, which can be grown under different ecological conditions (Neagu 1975, Oprea & Moldovan, 2007, Ciobotea et al. 2012).

The researches made in time have revealed the fact that together with the most commonly used conventional methods to creating of new genotypes - intra-and interspecific hybridization - the method by self-fertilization is a method that not induce the diversity of genetic just puts in order the genetic heritage, which aims at improved of genotype, thus as this to manifest by the appearance of the phenotypic characters more valuable (Pena A. 1970, Oprea St., 1971).

At the grapevine, self-pollination may reveal expression of the intrinsic variability even present in the genome of some varieties that seem very homogeneous phenotypically (Sestraş, 2004). Therefore, Tamai et al., 2003 confirms the hypothesis of the polyclonal

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origin of some varieties, emitted by Rives (1961) and recommends self-pollination as a method of increasing the genetic diversity of the grapevine.

MATERIAL AND METHODS

In order to obtain some superior biotypes than those existing in the current viticultural assortment (earliness, the productivity and the crop quality, resistance to handling and transport, the size of the bunch and berry, higher biological resistance to the unfavorable environmental factors, pathogens and pests) is essential also the knowledge of the hereditary base of genitors but and establishment of the suitable methods for creating hybrids and varieties of high quality.

The hybridizations performed within the *Vitis* species at I.N.C.D.B.H. Stefănești were took into account the transmission and the fixation in vegetative lineage of intermediate characteristics between genitors (quantity – quality), the type of merged heredity (the color of the grapes), the conversion of sex with heterozygous pronounced character.

By self-pollination of the *Victoria* variety were obtained numbers of 31 hybrid elites which have got, in a particular way, a part from the characteristics of the self-fertilization variety. Of these a number of 14 elite have proved particularly in terms of shape, color, size of bunch of grapes and berry.

Within the genetic recombinations, to separate the most favorable genes and to fix the most important characters was used the selection method of the valuable descendants within the lines resulted from successive self-fertilization. In this way we tried obtaining of some hybrid elites for table grapes with high quality knowing that this characteristic is determined by several genes, with an additive action what can be genetically fixed by selffertilization.

For the knowledge of the main ampelographic characteristics of the elites, but and a knowledge of the way that the characteristics have been transmitted from the the parental varieties at the descendants varieties, was used **The methodology of the ampelographic descriptors**, established by the O.I.V., U.P.O.V. and I.B.P.G.R regarding descriptors: bunch and berry.

Data interpretation was done aiming the transmission of the traits mentioned in relation to the Victoria genitor, the representative variety for the table grapes.

RESULTS AND DISCUSSIONS

According to *The methodology of the ampelographic descriptors*, established by the O.I.V., U.P.O.V. and I.B.P.G.R, each trait is described, and the terms codified by digits, represents a level of expression, being the smallest units in the presentation of a trait. To avoid any confusion and to facilitate the determination, for the each level of the expression was established the reference varieties, to compare.

Analyzing 14 hybrid elites selected in F1 after the size, color, shape of bunch of grapes and berries, we find that these have character and traits by the dominance on the whole, of a character or trait of the Victoria variety.

Victoria - It is a complex hybrid produced by the crossing of varieties: Cardinal x (Alphonse Lavallée x Ahmeur Ahmeur) x Afuz Ali. Hybridization was performed in the Research Institute of Horti-growing (ICHV) Bucharest, by Victoria Lepădatu. The works of the selection were continued at S.C.V. Drăgășani, the variety was homologated in 1978. It is one of the most valuable works of Romanian varieties for table grapes.

In order to assess the size transmission of the bunch of grape, the genotypes obtained by self-fertilization of the *Victoria* variety (Figure 1) have shown a strong variability from the size of the bunch and berries.

It follows that the transmission of the grapes size in F1 lineage is influenced by the use of their in the hybrid combinations.



Figure 1. The average weight of the bunch and berries from 14 hybrid elites compared to *Victoria* variety

Through repeated weighings of the 100 berry and grapes we determined the weight of grapes. The *Victoria* variety, the variety used in the artificial hybridise has been exceeded by the 6 elites as regards weight of the bunch. Regarding the weight of 100 berry are distinguished three hybrid elites (A16, A19, A27) with values between 400-850 g, surpassing (even once again) the *Victoria* variety, variety recognized and appreciated for the size of berry, Figure 1.

Remarkable and interesting is the way how have been transmitted to the elites the morphological characters relating to the shape of the bunch and berry, but also the color of the berry. Table 1 Thus, 9 elites have the shape of bunch - *funnel shape*, similar with Victoria variety, while 5 elites have different shapes (*cilindrical*). The descriptor *the shape of berry* demonstrates the different way how this influenced the descendants. Only two elites hybrid have the shape of berry inherited from the parental variety – *ovoid* - the others have *obtuse ovoid* shape (4 elites), *narrow ellipsoid* (4 elites) and *cylindric* (4 elites), Figure 2.



Fig. 2 Victoria variety and the hybrid elites obtained by self-fertilization of it

And the color of berry varies to the 14 genotypes, comparative with the variety Victoria. If, majority of elites have the yellow color alike with the parental variety, have been and two elites with golden berry, one – green yelow, and distinguished, one elite with rose berry.

Table 1

		bhape and color of	the bullet and being	
No	Flite/variety	The shape of	The shape of	The color of
140.	Enter variety	bunch	berry	berry
1.	Victoria	funnel shaped	ovoid	yellow
2.	A1	cilindrical	obtuse ovoid	yellow
3.	A5	funnel shaped	obtuse ovoid	rose
4.	A6	funnel shaped	broad ellipsoid	golden
5.	A8	cilindrical	cyilindric	yellow
6.	A9	cilindrical	obtuse ovoid	yellow
7.	A15	cilindrical	narrow ellipsoid	yellow
8.	A16	funnel shaped	cylindric	yellow
9.	A17	funnel shaped	ovoid	yellow
10.	A19	funnel shaped	narrow ellipsoid	golden
11.	A25	funnel shaped	narrow ellipsoid	green yellow
12.	A26	cilindrical	ovoid	yellow
13.	A27	funnel shaped	cylindric	yellow
14.	A29	funnel shaped	cylindric	yellow
15.	A31	funnel shaped	obtuse ovoid	yellow

Shape and color of the bunch and berry

CONCLUSIONS

From genotypic analysis of hybrid grapevine plants (F1), derived from the self-fertilization of the Victoria variety, results the following conclusions:

A part of the resulted hybrid elites have the superior qualitative traits than the genitor variety, fact which demonstrates a remarkable hereditary dowry of the *Victoria* variety;

Of the 14 genotypes presented, the elite A 5 had presented the color of berry – rose - different from the self-fertilization variety whose berry is yellow character borrowed from the gene fund of the parental varieties of the genitor;

Presented elites are special and also valuable in terms of quality reason for which can compete with many varieties from the viticultural assortment.

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THE RECOVERY OF PLUM CROP THROUGH ALCOHOLIC DISTILLATES

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Keywords: raw, distilled, sugar varieties, aging

ABSTRACT

Summary of our research on exploitation through distilled plums belonging to different varieties, such as Agen, Fat Romanian, Stanley and Sweet haulm, grown in Samburesti-Dobroteasa showed that fruits of these varieties is a staple good quality distilled. Raw distillates with the most complex composition are obtained from the fruit varieties Agen and the Fat Romanian, because they have a high potential for accumulation of sugars. Made aging in oak barrels, these spirits enrich their composition, especially if we refer to esters and aldehydes, impressing by the flavor they have. Our observations also noticed that we have a raw material (marc) of good quality, but, if the alcoholic fermentation and distillation is faulty, distillates obtained ceases to be quality.

INTRODUCTION

Among the tree species, plum occupy large areas in Romania, with a long tradition in the cultivation of this species. Prunes have a high food value. In their chemical constitution, there are useful substances for human consumption (nutrition). They contain per 100 g fresh substance, water (73-86%), dry matter (26-33%), sugar (9-16%), organic acids (0, 39-2%), protein (0, 22 to 1.07%), pectin (0.35 to 0.95%), tanoide (0.6 - 0.25%), ascorbic acid (0.20 - 14%) and have energy value of between 44 - 89 per 100 g of fresh.

Plums contain a number of vitamins: provitamin A (β caroten) 0.05 -0.60 mg / 100 g, ascorbic acid (vitamin C) 1 to 23 mg / 100 g, Vitamin B1 20 - 200 y / 100 g, vitamin B2 20 - 90 y / 100 g, Biotin 0.6 y / 100 g, vitamin PP 0.2 to 0.6 y / 100 g Pantothenic acid 0.12 to 0.24 mg / 100 g. Plums contain 100 g ash: Cl (0.0015 mg), I (0.007 mg), P (22.8 mg), Na (2.2 mg), K (167 mg), Ca (13, 3 mg), Mg (13 mg), Fe (0.14 mg), Mn (0, 14 mg).

As a result of this composition they are widely used for fresh consumption, semi and industrialized. All these have been highlighted by numerous studies: Popa A, 1985, 2002, Radulescu Paula 2010; Pomohaci N. and al., 2009, 1999; Wittkowski, R. and al., 2000; Tourliere, SD, 1978, Curbelo, Garcia, AS, 2006, Bonepas, C., Gauthier, P., 2008.

Yet more than 40 - 45% of production for obtaining distillates, which are known as brandy, plum brandy, horinca, etc. These distilled, by their chemical composition and olfactogustative characteristics are superior to those obtained from cereals. Measurements and observations made by us to the plum distillates from Samburesti – Dobroteasa, Olt County, attests that.

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MATERIAL AND METHODS

Marc distillates were obtained from plum varieties belonging: Agen, Fat Romanian, Stanley and Fresh haulm, grown in the area Samburesti - Dobroteasa, Olt County. The chemical composition of raw, or shortly distillates aged in oak barrels (220 l), was determined by laboratory analysis using the methods recommended by the OIV and those in standards in Romania. Organoleptic assessment was made using compensation system points from 1 to 20. At plum marc was a double distillation.

RESULTS AND DISCUSSIONS

Results plum distillate is appreciated when raw (immediately after distillation) as one of the finest spirits. Substances contained in raw distillates of different origins plums. A part from fruits, mostly formed during alcoholic fermentation of the pulp of plum, while others are formed during distillation. Besides alcohol, water extract and raw ash plum distillate containing volatile substances such as acids (particularly acetic acid), aldehydes (especially acetic aldehyde), furfural, esters (mostly in ethyl acetate) and alcohols, butyl and amyl alcohols predominating. The quality of raw plum distillate is determined by the extent to which these substances enter the composition, as a result, they serve to characterize them. The proportion of these components depends on the plums quality - raw material, mode of fermentation and distillation mode, climatic offer made to areas where fruits grow. The alcoholic strength of raw distillate of plums, belonging to different varieties, depends on the potential for accumulation of sugars in fruits and how fermentation and distillation is made; it oscillates between 47 vol% and 55 vol% alcohol. Smaller alcoholic concentrations are found in raw distillates made of marcs of Stanley variety.

Total acidity values are between 164 and 185 mg (acetic acid) to 100 mg anhydrous alcohol. Aldehydes are found in proportions of 40 to 48 mg per 100 ml anhydrous alcohol. Plum distillate esters were determined from values of 35 to 114 mg (ethyl acetate) in 100 ml anhydrous alcohol.

Furfural, although falling within aldehydes, during the analysis of distilled spirits is metered separately. It's thought, in moderate proportions, to contribute to a more complex, pleasant flavor. When there is excessive amounts of it, it has got a negative influence.

Raw distillation of plum, belonging to different varieties contain very little furfural (0.11 to 2.06 mg per 100 ml anhydrous alcohol). Somewhat higher amounts (2.06 ml / 100 ml anhydrous alcohol) were found in the distillate derived from Fatty haulm variety plums. Higher alcohols are found in quantities ranging from 264 to 304 ml (isobutyl alcohol) in 100 ml anhydrous alcohol. The extract has values ranging between 340 and 604 mg / l.

Ashes, due to content in the extract, have got great variation limits, from 103 to 290 mg / l. Non-alcoholic coefficient, representing the sum of content in acids, aldehydes, esters, furfural and higher alcohols; the sum esters plus higher alcohols and the report higher alcohols / esters give us information about the qualitative value of raw materials, the manner in which alcoholic fermentation was conducted , when and how distillation took place, but help us, also, diagnose the qualitative value gained by aging raw plum distillate. Sensory qualities of raw distilled of superior quality from plums, should come to the organoleptic assessment as clear liquids, colorless or colored to dark yellow with characteristic taste and pleasant, alcoholic smell.

Sometimes we meet situations when, although raw plum distillates are clear and colorless, they have a bitter, metallic taste, very bad, and, if they are available in oak, they acquire a blue- black color as ink, repellent. This shows that distillates contain large

proportions of iron and sometimes copper. Excessive content of heavy metals explains the emergence of bitter, unpleasant taste, and the blue - black color, by forming ferric combed in contact with oak wood. The two heavy metals (iron and copper) can normally be found in raw distillates as traces, but excessive enrichment of iron and copper of the distillate comes on one hand from the distillation installations, on the other, from the borhots in which sulfur is found in large quantities and during the process of distillation corrodes the installations.

Copper, in amounts of 2.5 to 3 mg / l, which pass sanitary laws, does not affect sensitive the taste into bad taste, the color or degree of clarity of plum distillate, on the contrary, is a catalyst for the oxidation - reduction processes after which distillates enrich sensitively their olfacto-gustative qualities.

The presence of copper is put, in this situation, in relation to the degree of purity of the copper processing or how it is made the distillation unit, knowing that metals are corroded more so, more impure are the conditions.

Unsatisfying olfacto-gustative qualities are registered also when the plum distillates come from borhots smelling of mold, hint of lag or beginning of deterioration. The taste and smell of smoked betrays inadequate leadership of the distillation process. Table 2 summarizes the composition and organoleptic characteristics of plum distillate after 6 months (180 days) of aging in oak barrels with a capacity of 220 liters.

Since about the process of aging we will be back with another work, in this we summarize a few general issues. It can easily detach that received good marks from the judges for flavors tasting were rich coating agreeable, coming from plums from which distillate was produced, and the wealth of aldehydes and esters gained during the operation of distillation and then during aging.

Distillates from the variety Agen plums were most appreciated. Immediately after were ranked distillates from Fat Romanian plum varieties and Fresh haulm. They impressed by the accuracy and smoothness of flavor, the lack of aggressiveness of alcohol, the ideal balance between alcohol, acidity and extract, but especially by their vocation to aging. It is a testimony to the fact that when the raw material is first quality, the fermentation process was well managed and the distillation and aging process is properly controlled by the technologist, the distillates obtained are of real quality.

CONCLUSIONS

The chemical composition of raw plum distillate and their sensory attributes are complex and different, being dependent of the culture area of the trees, the plum variety, the time of harvest, the process of alcoholic fermentation and distillation.

Agen plum varieties, Fat Romanian, Fresh haulm and Stanley can be the raw material for spirits.

Distillates with a balanced and harmonious composition and that - best enjoyed organoleptic evaluation are those from fruit varieties Agen and Fat Romanian, followed closely by those from Sweet haulm fruit variety.

Plum crude distillates are suitable for aging in small oak capacity vessels (220 l), enriching - and enhancing composition and sensory characteristics.

S					In mg	per 100 mL of	f anhydrous				mg	/1
Number of sample analyzed	Variety	vol% alcoholic strength at 15 ° C	Total acidity (acetic acid)	esters (ethyl acetate)	Furzu-rol	higher alcohols (alc.izob- utility)	soft Coefficient	Sum + esters and supe- rior alcohols	higher alcohol esters	aldehydes (acetaldeh- IDA)	Extract	Ash
6	Agen	52 - 55 53	17 - 56 180	35 - 36 35	0,11-0,21 1,9	66-190 304	133,11-302,21 560,9	71-297 339	1,88-5,27 868	15-20 40	12-210 604	9-45 290
6	Fat roma- nian	51 - 53 52	16 – 47 170	38 - 41 104	0,12-0,28 2,01	64-150 291	138,12-265,28 615,01	102-191 382	1,68-3,65 2,79	20-27 48	14-106 502	8-40 104
6	Stanley	46 – 48 47	18 – 49 185	37 – 39 102	0,14-0,34 1,90	61-104 280	146,14-226,34 609,9	98-143 382	1,64-2,66 2,74	30-34 41	12-104 340	7-56 107
6	Sweet haulm	50 - 54 52	15 – 50 164	39 – 46 114	0,13-0,31 2,06	63-130 264	136,13-252,31 585,06	102-176 378	1,61-2,82 2,31	19-26 41	13-104 492	7-47 103

Medium variations and limits of oscillation of the main components of raw distillates Plum (2010 - 2011)

Table 1

			In m	n mg / l distilled In mg at 100 mL anhydrous alcohol										0
Variety	° Alcoholic title vol % at	Hq	Extract	Ash	Total	Total acidity (acetic	Aldehide (acet	Esters (etyl	Furzurol	Higher alcohols (alc.	Non- alcoholic coeficient	Esters + supe- rior alcohols	sperior alcohols / esters	Organolepti assessment notes 1-20
Agen plum distilled	53	3,80	98	8	1,36	106,30	38,40	253	0,4	343	741,10	596	1,35	19,96
Fat romanian plum distilled	52	3,60	96	10	1,84	122,10	39,40	296	0,5	359	817,00	655	1,21	19,62
Stanley plum distilled	47	3,56	90	7,5	1,25	66,40	33,19	194	0,9	205	499,49	399	1,05	18,20
Sweet haulm plum distilled	52	3,75	95	8	1,31	89,20	37,52	232	0,7	287	646,42	512	1,23	19,52

Composition and organoleptic characteristics of plum distillate after 6 months of aging in oak barrels with a capacity of 220 liters

Table 2

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RESULTS OBTAINED IN THE EXPERIMENTAL AREA THE AGRO PRODUCTIVITY STUDY OF SOME QUINCE VARIETIES IN THE FRUIT **GROWING BASIN TÂRGU JIU**

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Keywords: quince, crown shape, variety

ABSTRACT

An important factor in intensive quince cultures is the crown shape, with influence on the quantity and quality of the production and especially on the disease attack, mainly Erwinia and Monilia. From the observations it results that, no matter the variety, the quince is less productive when having either a "Simple Palm" crown or a "Bush Trunk" one. As a conclusion, the quince does not react positively to palm shape crown or to a stuffy crown as bush trunk.

As a general conclusion of the obtained preliminary results is that the form of "late bowl" and "shapeless hedge" are the most suitable for intensive plantation quince, using Moldovenesti and Aurii varieties, which have a 3-5 day later flowering compared to the other studied varieties. This way it is avoided the negative effect of some environmental factors of that time of the year (white frost, late frost, cold rain).

INTRODUCTION

Quince is one of the fruit tree species with old traditions among Romanian farmers. Using the fruit in human's nutrition comes from the distant past, as recorded in archaeological documents over 4000 years ago. (Badescu Gh. et all, 1984; Gosch, C. et all. 2011.)

The important position the quince occupied in ancient peoples, tree growing has gradually decreased, as there have been developed other species, particularly the apple and the pear as a result of their better taste qualities and characteristics of being kept fresh for consuming for a longer period of time after harvest.(Badescu Gh. et all., 1984; Drăgănescu E. – 2006).

Quinces are rich in sugars 10.1%, protide 0.4%, pectin 0.9% 0.3% fat 0.50% tannin salts K 201 mg% Ca 8 mg% 10mg% Fe 0.60 Mg mg%. (Cepoiu N., 2001; Draganescu E., 2006).

Within Tg-Jiu Horticulture Research - Development Station there have been and there still are l concerns regarding the production improvement and check of some valuable quinces varieties and biotypes, as well as the establishment of modern technological links specific to this culture. (Roman I., 1994, Tomescu I. et all., 1994).

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The experimental area is located on the third terrace of the Jiu River at the altitude of 383 m in the northern part of Targu-Jiu City on the experimental plantations established in 1987 at Targu-Jiu Fruit-Growing Research and Production Station.

The soil type is albic luvisol, pseudogleized with a percentage of up to 30% natural clay with low permeability, existing the danger of bogging while excessive rainfall, poorly stocked in nutrients, the depth of groundwater being of 1 to 1.5 m, weakly acid soil reaction.

The area is included in a temperate climate with an annual average temperature of $10,2^{0}$ C and an annual average volume of precipitation of 753 mm, unevenly distributed in the course of the year.(Ghena N., Branişte N., 2003).

During the bloom and fruit forming in 2012, more precisely between 22.04 - 07.05 there were recorded temperatures between 6 0 C at night and 20 0 C at day, without the danger of frost or other unusual meteorological phenomena.

MATERIAL AND METHODS

Agro-technical works were applied according to the technological record of this culture, in all variants, as a common factor, the spaces between rows were grassed, repeatedly mowed, the resulted organic material used as a straw cover along the rows (Cociu V., 1990; Drăgănescu E., 2006; Iglesias, I. and Asín, L., 2011).

The bear fruit – fructification cutting were applied differently according to each experimental variant, aiming to achieve well-balanced and trimmed crowns (Ion Ligia – 2007).

An important factor in intensive quince cultures is the crown shape, with influence on the quantity and quality of the production and especially on the disease attack, mainly *Erwinia amylovora* and *Monilinia* (Gosch, C. et all., 2011; Smith M. et all., 1982; Vagelas I., et all., 2011; Mihăescu Gr., 1998; Tetileanu C. et all., 1984). Setting the crown shape involves the lowest cost for cutting maintenance and harvesting, all these having as final goal the getting constant and effective annual productions.

Therefore, there have been studied the following factors:

Factor A: - The 5 stages variety, namely: 'Aurii', 'Aromate', 'Moldovenești', 'De Constantinopol', 'De Portugalia'

Factor B - Crown shaped with 4 stages: Late Flat Bowl, Simple Palm, Bush trunk, Shapeless Hedge.

RESULTS AND DISCUSSIONS

The following conclusions come from the recorded preliminary data: from Table 1 it appears that the established parameters represent a characteristic of both the crown shape and the variety. From the first tests, the "Late Flat Bowl" shape of the crown is suitable for the variety 'Aurii' and 'Moldovenesti', varieties with richer production. "Late Flat Bowl" providing a good illumination and ventilation. "Shapeless Hedge" shape of the crown also ensures optimal parameters for fruit for the two mentioned varieties.

From previous observations it results that, no matter the variety, the quince is less productive when having either a "Simple Palm" crown or a "Bush Trunk" one. As a conclusion, the quince does not react positively to palm shape crown or to a stuffy crown as bush trunk. In Tables 2 and 3 there are presented, for further details, the season phenological measurements according to the shape of the crown From the results, the biggest workload for cutting maintenance is required by bowl-shaped bush and shapeless hedge, the manual intervention being more difficult due to the arrangement of the fruit branches, like a frame. The least workload was registered at "late bowl" and "simple palm"

Var	iant	Volume of	Number	Percentage	Flowers per tree	Percentage	Fruit	Percentage
V1 - Crown Shaped	V2 - Variety	work trees	of fruit	of average		of average		of average
		per our	buds per	%		%		%
			tree					
Late Flat Bowl	Aromate	7	878	88,5	540	77,3	102	81
	Aurii	7	1070	108	782	112	142	113
	Moldovenesti	7	1138	115	827	118	161	127
	De Constantinopol	15	980	98,7	852	107	131	103,9
	De Portugalia	8	901	97,5	590	84,5	96	76
AVERAGE		6,8	<i>993</i>	100	698	100	126	100
Simple Palm	Aromate	7	898	96	480	73,8	111	101
	Aurii	7	948	101	705	108,4	112	102
	Moldovesti	9	980	104	682	104	117	107
	De Constantinopol	6	980	99,4	693	106,6	108	99
	De Portugalia	7	920	98,3	690	106	101	92,6
AVERAGE		7,2	935	100	650	100	109	100
Bush Trunk	Aromate	6	846	94	492	75,2	93	96
	Aurii	8	920	104	702	107	101	104
	Moldovesti	7	1019	113	771	117	112	115
	De Constantinopol	4	902	100	698	106	96	98
	De Portugalia	6	811	90,2	611	93,4	82	84
AVERAGE		6,2	899	100	654	100	97	100
Shapeless Hedge	Aromate	6	892	88,1	512	75,2	128	94
	Aurii	8	1110	109,6	731	107	147	108
	Moldovesti	8	1211	119	825	121	158	116
	De Constantinopol	4	1039	103	709	104	138	101
	De Portugalia	5	812	80	624	91,4	112	82
AVERAGE		6,2	1012	100	680	100	136	100

Summary of the parameters established according to the shape of the quince crown, in 2013

Table 1

crown. As far as fruit is concerned, there has been noticed that "late bowl" and "shapeless hedge" crown have the most buds and therefore the highest fruit percentage, namely 116% and 107% compared with the 4 crown variants average.

Table 2

	Average indices determinants							
	Volume of work		Number		Number of		Number of fruits	
V2-	Number trees per hour		fruit buds per tree		flowers per tree		per tree	
Crown shaped								
		%		%		%		%
		from the		from the		from the		from the
		average		average		average		average
Late flat bowl	6,8	103	993	103,5	698	104	126	107
Simple palm	7,2	104	935	97,4	650	97	109	93
Bush trunk	6,2	94	898	93,6	654	98	97	82
Shapeless hedge	6,2	94	1012	105,5	680	101	136	116
Average	6,6	100	959	100	670	100	117	100

The synthesis of the research results obtained according to the shape of the crown

Table 3

The correlation between the blossom time and percentage of fruit

V1- variety	Blossom time	Average	Number	Binding
		number flowers	fruits per	percentage
		per tree	tree	%
Aromate	21.04 - 04.05.2013	506	108	21
Aurii	24.04 - 06.05.2013	715	125	17
Moldovesti	26.04 - 07.05.2013	773	137	17
De Constantinopol	24.04 - 06.05.2013	713	118	16,5
De Portugalia	21.04 - 02.05.2013	628	98	15,5

From the made observations, it appears that there is a correlation between flowering times, varieties, average flowers per tree, number of fruit per tree. Thus, the highest binding percentage was recorded at Aromate variety, namely 21% of the number of flowers per tree although the most flowers were recorded at Moldovenesti and Aurii varieties, which is explained by the higher number of flowers in these varieties.

De Portugalia variety proved to be less suitable in the area; the results according to the parameters being inferior to the other studied varieties. Regarding the fruit production according to the shape of the crown, for the studied varieties, in Table 4, there are the following results.

CONCLUSIONS

As a general conclusion of the obtained preliminary results is that the form of "late bowl" and "shapeless hedge" are the most suitable for intensive plantation quince, using 'Moldovenesti' and 'Aurii' varieties, which have a 3-5 day later flowering compared to the other studied varieties. This way it is avoided the negative effect of some environmental factors of that time of the year (white frost, late frost, cold rain).

"Late bowl" and "Shapeless Hedge" crown shaped were found to be the most suitable for the establishment of a quince plantation, both in the intensive and the superintensive system, the production increases being of up to 16% compared to other types of crown. The recommended varieties for the hill area of Oltenia are 'Moldoveneşti' and 'Aurii'.
Table 4

Variety	Crown	Number of	Number of	Number of	Average	Produ	iction	Significa
	type	trees per	fruits	harvested	weight	Kg per	%	nce
		hectare	per tree	fruits	g per fruit	hectare		
			_	per tree				
Moldovenesti	Late Flat Bowl	625	161	108	350	23625	105	Х
	Simple Palm	625	117	98	350	21437	96	0
	Bush Trunk	1250	112	52	330	21450	96	0
	Shapeless Hedge	1250	158	57	320	22800	102	Х
Average		-	-	-	-	22326		100
Aromate	Late Flat Bowl	625	102	85	370	19656	99,4	-
	Simple Palm	625	111	83	370	19193	97,5	0
	Bush Trunk	1250	93	44	350	19250	97,8	0
	Shapeless Hedge	1250	128	47	350	20562	104	XX
Average		-	-	-	-	19665		100
Aurii	Late Flat Bowl	625	142	90	400	22750	108	XXX
	Simple Palm	625	112	82	400	20500	97,08	0
	Bush Trunk	1250	101	41	380	14475	92,2	000
	Shapeless Hedge	1250	147	47	370	21737	102,9	Х
Average		-	-	-	-	21115		100
De Portugalia	Late Flat Bowl	625	96	87	350	19031	101	-
-	Simple Palm	625	101	84	350	18375	97,7	0
	Bush Trunk	1250	82	43	340	18275	97,1	0
	Shapeless Hedge	1250	112	46	340	19550	103	Х
Average		-	-	-	-	18807		100
De	Late Flat Bowl	625	131	89	365	20303	100	-
Constantinopol	Simple Palm	625	101	85	370	19887	98,5	-
	Bush Trunk	1250	96	44	360	19800	98	0
	Shapeless Hedge	1250	138	46	360	20200	102	Х
Average		-	-	-	-	20172		100
	D	L 5% = 2%	DL 1%	= 4%	DL 0.1% = 6%	%		

The influence of the soil and crown shape on quince fruit production in 2013

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THE ESTABLISHMENT OF THE PROPORTIONS OF THE BY-PRODUCTS RESULTED FROM THE PROCESSING OF GRAPES IN THE WINE-MAKING INDUSTRY

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Keywords: grapes, bunches, peels, seeds, husk

ABSTRACT

In the wine-making industry, besides main products - wines, there results a series of byproducts or secondary products, bearer of certain constituents, some of which with a considerable value. In grapes - processing results: the grape bunches, the grape pomace and the marc of grapes; after the alcoholic fermentation there results the carbon dioxide and the deposit of leavens (yeast); in the process of purifying and preserving the wine, there results: different precipitates, sediments from gluings, tartar, the water used to wash the filters (that contain alcohol); from the distillation of wine there appears the marc.

INTRODUCTION

In many situations, especially in private small and medium viticultural exploitation, hasty or not at all processed by-products reach the environment. In this kind of situations, besides the losses of the viticultural production value, which can frequently exceed 20% (Pomohaci N. et al. 1990), they also seriously affect the environment, through their polluting potential.

Considering the fact that with the by-products resulted from processing of grapes, there are valuable constituents, such as: tannins, anthocyans, tartric salts, vegetal oils, proccupation for their extraction has existed, both in our country and in other wine - growing countries (Bulancea M. et al. 2002, Palma M. & Barroso C.G. 2002, Louli V. et al. 2004, González-Paramás Ana M. et al. 2004, Arvanitoyannis S. Ioannis et al. 2005, Duca Gh. et al. 2007).

The aim of this paper is to establish the proportion of solid parts of grapes resulted from the extraction of must.

MATERIAL AND METHODS

The study was performed during the viticultural years 2010, 2011 and 2012 on grape production belonging to the main varieties from the structure of the assortments of white and red wines, from Dragasani vineyard. The following grape varieties were aimed: Sauvignon, Italian Riesling, Fetească regală and Crâmpoșie selecționată for white wines

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and the categories: Merlot, Cabernet Sauvignon, Negru de Drăgășani and Novac for red wines.

At technological maturity, using the official scientific methods, there has been established the production of grapes for surface conventional unit, and through physicalmechanical analysis of an important number of medium samples, there have been specified the proportions of grape bunches, grape peels, seedsgrape pomace, as main by-products resulted from the extraction of must from the grapes.

RESULTS AND DISCUSSIONS

The proportions of the different solid parts resulted after the processing of grapes for white wines, reported to the productions by hectare, are presented in table 1.

The lowest production of grapes had Sauvignon (an average 7925 kg/hectare), and the greatest productions were obtained from Crâmpoșie selecționată (an average 9933 kg/hectare). Satisfactory productions were achieved by Italian Riesling (8433 kg/hectare) and Fetească regală (8858 kg/hectare). During these three years, for all grape varieties, the lowest proportions were obtained in the relatively droughty, very hot year - 2012.

The proportions of the solid parts of grapes resulted after the extraction of must, differ according to the variety of the assortment and according to the viticultural year for the same variety.

The grape bunches derived from the removing of grapes off the bunch, register (at averages) the highest proportions at the varieties with small grapes - Sauvignon and Italian Riesling (5,2%) and the lowest amounts of this by-product belong to Crâmpoșie selecționată, whose grapes are medium to big (4,1%). Little below 5% (actually 4,8%) there is the medium proportion of grape bunches for Fetească regală.

The proportions of grape peels were established directly according to the size of grapes beans, being bigger with the varieties that have small beans, where their number is considerably higher than at the varieties with big beans. In this case, Sauvignon, whose grape beans gather numbers between 770 and 780, the proportions of peels were situated between 12,7% and 13,0% (with an average amount of 12,8%). With obvious bigger beans and thinner peels at Crâmpoșie selecționată, their proportions represent an average amount of only 8,1%. With Italian Riesling and Fetească regală the proportions of peels have values of 11,3%, and respectively 11,1%.

The proportions of seeds registered values according to the number of grape beans per kilogram, but also according to the genetic nature of the respective grape variety. For this respective variety studied, the highest proportions of seeds were registered at Italian Riesling (5,5%), followed decreasingly by Sauvignon (4,5%), Fetească Regală (4,1%), Crâmpoșie selecționată (3,8%).

Gathering the proportions of solid parts (except for the rest of the pulp stuck to the peel), the quantities and proportions of grape pomace result, being the main by-product when processing the grapes. According to the productions per hectare, the proportions of grape pomace decrease, from the variety with the smallest grapes and the smallest beans to the varieties with big grapes and beans. This aspect is confirmed by the results of this study, which shows the following proportions of grape pomace: 21,6% for Sauvignon; 21,0% for Italian Riesling; 19,2% for Fetească regală; 15,5% for Crâmpoșie selecționată.

In absolute values, the quantities of husks of grapes resulted when processing white wines range from 1541 kg/hectare for Selected Crâmpoșia and 1775 kg/hectare for Italian Riesling. Sauvignon and Fetească regală also have over 1700 kg/hectare of husks of grapes.

		Due du sti su	By-products %						
Grape variety	Production year	kg/hectare	Grape bunches	Grape beans	Grape peels	Pulp	Seeds	Grape pomace	
	2010	8105	5,4	94,6	12,8	82,6	4,6	21,8	
Coursi on on	2011	7860	5,2	94,8	13,0	82,6	4,4	21,7	
Sauvignon	2012	7810	4,9	95,1	12,7	82,7	4,6	21,4	
	Average	7925,0	5,2	94,8	12,8	82,6	4,5	21,6	
	2010	8770	5,3	94,7	11,0	83,6	5,4	20,8	
Italian	2011	8540	5,1	94,9	11,2	83,3	5,5	20,9	
Riesling	2012	7990	5,2	94,8	11,4	82,9	5,7	21,4	
	Average	8433,3	5,2	94,8	11,3	83,3	5,5	21,0	
	2010	9030	4,9	95,1	10,9	85,0	4,1	19,2	
Fetească	2011	8890	4,7	95,3	11,0	85,1	3,9	18,9	
regală	2012	8655	4,8	95,2	11,3	84,5	4,2	19,6	
	Average	8858,3	4,8	95,2	11,1	84,9	4,1	19,2	
	2010	10100	4,1	95,9	8,1	88,0	3,9	15,6	
Crâmpoșie	2011	9930	3,9	96,1	8,2	87,8	4,0	15,6	
Selectionata	2012	9770	4,3	95,7	8,1	88,5	3,4	15,3	
	Average	9933,3	4,1	95,9	8,1	88,1	3,8	15,5	

The proportions of the by-products resulted after processing the white grapes according to the productions per hectare

Table 1

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Grape		Production kg/hectare	By-products %						
variety	Production year		Grape bunches	Grape beans	Grape peels	Pulp	Seeds	Grape pomace	
	2010	8160	4,6	95,4	16,0	80,2	3,8	23,5	
Maulat	2011	8005	4,3	95,7	15,9	80,5	3,6	23,0	
Meriot	2012	7840	4,5	95,5	16,5	79,5	4,0	24,0	
	Average	8002	4,5	95,5	16,1	80,1	3,8	23,5	
	2010	7050	5,1	94,9	18,8	76,3	4,9	27,6	
Cabernet	2011	6770	4,8	95,2	18,6	77,1	4,3	26,6	
Sauvignon	2012	6810	4,9	95,1	19,5	75,9	4,6	27,3	
	Average	6877	4,93	95,1	19,0	76,4	4,6	27,2	
	2010	12600	4,4	95,6	12,4	84,1	3,5	19,6	
Negru de	2011	12050	4,7	95,3	13,2	83,6	3,2	20,3	
Drăgășani	2012	11940	4,2	95,8	13,8	82,6	3,6	20,8	
	Average	12197	4,43	95,6	13,1	83,4	3,43	20,3	
	2010	13010	5,4	94,6	12,7	83,6	3,7	20,9	
Novac	2011	12830	5,1	94,9	12,1	84,5	3,4	19,8	
	2012	12790	5,3	94,7	12,8	83,4	3,8	21,0	
	Average	12877	5,27	94,7	12,5	83,8	3,63	20,6	

The proportions of the by-products resulted after processing the red grapes according to the productions per hectare

Table 2

At the varieties for red wine, the productions of grapes per hectare and the proportions of by-products resulted when processing the grapes, according to the productions, are presented, from the point of view of the value, in table 2.

The average productions of grapes place at the top of the assortment the variety Novac (12877 kg/hectare), followed decreasingly by: Negru de Drăgășani (12197 kg/ha), Merlot (8002 kg/ha), Cabernet Sauvignon (6877 kg/ha).

In relation to the mentioned productions, the grape bunch proportions are situated between 4,43% at Negru de Drăgășani and 5,27% at Novac. With these varieties there isn't a certain relation between the size of the grapes and the proportions of bunches.

The grape peels, the location of anthocyans substance, which offer the particularity of red wines, occupy different proportions according to the size and number of grape beans per kilogram. Cabernet Sauvignon, whose number of beans is frequently around 880 per1 kilogram, has an average proportion of 19%. The bigger beans of the varieties Negru de Drăgășani and Novac represent the cause of some lower proportions of peels (13,1%, and respectively 12,5%). With medium- sized beans, the variety Merlot has a proportion of peels of 16,1%, also signifying a considerable content of anthocyans.

The proportions of seeds are also determined by the number of grape beans per kilogram. For the sort Cabernet Sauvignon the seeds represent 4,6%, and for the other varieties these proportions are situated between 3,43% for Negru de Drăgășani and 3,8% for Merlot. Generally, the proportions are in agreement with those mentioned in top literature concerning other vineyards (Cotea D.V et al. 1985).

According to the proportions of already mentioned solid parts, the resulted grape pomace reach considerably higher levels when compared to white varieties. So, for Cabernet Sauvignon the grape pomace represent an average of 27,2% of the production of grapes. For the other varieties, the grape pomace decreasingly sums up proportions of: 23,5% for Merlot; 20,6% for Novac; 20,2% for Negru de Drăgășani. In absolute values, the medium quantities of grape pomace resulted after the vinification of black grapes from Drăgășani vineyard are situated between 1868 kg/hectare for Cabernet Sauvignon and 2650 for the variety Novac.

CONCLUSIONS

When processing grapes to extract the must, important quantities of grape pomace result, in the composition of which enter: the grape bunches, the grape peels, the seeds and the rest of the pulp stuck to the peels and seeds.

The amounts of grape pomace according to the productions of grapes differ: according to the grape variety in the same vineyard and viticultural year; and considering the viticultural year for the same variety and vineyard.

Generally, the proportions of the grape pomace are lower for the varieties of grapes used for white wines as opposed to those for red wines, grown in the same viticultural area.

For Drăgășani vineyard, the proportions of grape pomace are situated: between 15,5% at Crâmpoșie selecționată and 21,6% at Sauvignon – from the raw material for white wines and between 20,2% for Negru de Drăgășani and 27,2% for Cabernet Sauvignon – from the raw material for red wines.

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POSSIBILITIES OF RECOVERING THE ANTHOCYAN COLOURING SUBSTANCE RESULTED FROM FERMENTED GRAPE POMACE

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Keywords: grapes, anthocyans, technological reserve, extractibility

ABSTRACT

In the process of obtaining red wines, from the technological reserve of anthocyans of the grape beans, only a part is found in the wine, the rest remaining in the grape pomace. Depending on the grape variety, the duration of maceration- fermentation and efficiency at vinification, in the grape pomace there can remain proportions of anthocyans ranging from 60 to 65%.

Knowing the hygienic-alimentary value and as a colouring agent of anthocyan colouring substance, its recovering from the grape pomace is necessarily needed. The use at the extraction of the solutions of sulphurous acid 1 - 1,5% and of acid alcoholic solutions (50% alcohol + 1,5% acetic acid) draws a recovery of up to 90%.

INTRODUCTION

The black grapes variety have the physiological capacity to biosynthesise and store in the grapes considerable amounts of anthocyans, constituents of the great category of polyphenols. The anthocyans offer the particularity of red wines and represent one of the essential elements for the quality of these products.

During the last 2-3 decades there has been identified the great beneficial role of the polyphenols compounds in the wines in which the anthocyans occupy a leading position. So, there have been discovered the protective action of polyphenols against cirrhosis (Lappara J., 1989), against cardiovascular diseases and the reduction of tumor cells (Mattivi F. 1993, Teissedre F.L et al. 1996, Ravanello C. 1997).

In the process of vinification in red only a part of the technological reserve of anthocyans from grapes passes in the wine, the rest remaining in the grape pomace.

Knowing the great oenological and hygienic-alimentary effects of the anthocyans, high-level studies have been directed to recover the remaining contents from the byproducts (Louli V. et al. 2004, Lafka Theodora-Ioanna et al. 2007, Duca Gh. et al. 2007).

The extraction and recovery of anthocyanic substance from the grape pomace are also supported by the data found in this paper.

MATERIAL AND METHODS

The investigations were performed during the viticultural years 2011-2012, using

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the productions of the grapes variety: Cabernet Sauvignon, Merlot, Negru de Drăgășani and Novac from Drăgășani vineyard.

According to the official methodologies there have been established: the productions of grapes kg/ha; the main elements of physical- mechanical structure of the grapes; the efficiency in must- wine at vinification; the obtained volume of must- wine (l/ha); the amounts of anthocyans mg/kg grapes; the extractibility of anthocyans %; the technological reserve of anthocyans mg/kg grapes; the total content of anthocyans from the grape production kg/ha; the chromatic structure of the colouring substance; the amounts of anthocyans mg/l; the total contents of anthocyans and their proportions in wines and those remained in by-products (kg and %); the chromatic structure of obtained wines.

Regarding the possibilities of extraction of anthocyans from the grape pomace there have been used sulphurous acid solution 1% and alcoholic solution (50% + citric acid 1,5%).

The efficiency of extractive solutions has been determined through the measuring of the colouring intensity of some medium samples rigurously constituted from the experimented variants compared to the same chromatic parameter of some samples from perfectly even peels of grape pomace, from which the anthocyans were totally extracted by grinding using quartz sand or crushed glass and repeated washings.

RESULTS AND DISCUSSION

The main technological elements of grape production are illustrated in table 1. The grape productions were situated between 7200 kg/ha at Cabernet Sauvignon and 11120 kg/ha for Novac. The other two grapes variety had intermediary productions.

Table 1

Grape variety	Production of grapes kg/ha	Bunches of grapes %	Grapes (beans) %	Peels %	Efficiency in must-wine at vinification 1/100 kg grapes	The quantity of obtained must-wine l/ha
Cabernet Sauvignon	7200	5,2	94,8	20,1	67,6	4865
Merlot	8460	4,9	95,1	16,9	69,7	5916
Negru de Drăgășani	10980	4,7	95,3	14,8	71,8	7899
Novac	11120	4,5	95,5	14,4	71,9	7943

The main elements of physico-mechanical structure and efficiency of grape productions for red wines in Dragasani vineyard (the average of the years 2011 – 2012)

The grape beens represented relatively close proportions for the four grape variety, situated between 94,8% at Cabernet Sauvignon and 95,5% for Novac.

More meaningful differences were registered with the proportions of grapes peels. Considering this aspect, the grape variety Cabernet Sauvignon occupies the first place, with 20,1%, while on the last place there is the grape variety Novac with 14,4%. These different proportions will account for the different supply of anthocyans from the respective grape varieties.

The outputs in must-wine at vinification, between 67,6% at Cabernet Sauvignon and 71,8% at Negru de Drăgășani, in relation to the grape productions, justify the amount of must-wine, situated between 4865 l/ha for Cabernet Sauvignon and 7943 l/ha for Novac.

The characteristics of anthocyanic complexes in grapes results from the data in table 2.

Table 2

Grape variety	Anthogyans	Extractibi	Technologi	The total amount of	The chromatic structure of the anthocyanic complex		
	mg/kg grape beans	lity anthocya ns %	mg/kg grape (beans)	anthocyans in the used raw material kg	Yellow pigments %	Red pigments %	Blue pigments %
Cabernet Sauvignon	1435	50,8	729	9,794	29,4	61,8	8,8
Merlot	1320	51,2	676	10,620	30,7	60,9	8,4
Negru de Drăgășani	1396	51,8	723	14,610	28,9	62,0	9,1
Novac	1375	51,5	708	14,60	27,8	62,6	9,6

The main technological and compositional parameters of the anthocyanic complex in the grapes used for red wines in Drăgășani vineyard (the average of the years 2011 – 2012)

Considering the proportions of peels, the contents of anthocyans in the grapes also differ with the four grape varieties. The highest content was found at Cabernet Sauvignon (1435 mg/kg grape beans), and the lowest at Merlot (1320 mg/kg grape beans).

Even if with the new varieties Negru de Drăgășani and Novac the proportions of peels are a little lower than with Merlot, the amount of anthocyans is higher.

Considering the extractability of anthocyanic substance, ranging between 50,8% at Cabernet Sauvignon and 51,8% at Negru de Drăgășani, the technological reserves situate on the first place the grape variety Cabernet Sauvignon (729 mg/kg grape beans), placed close to Negru de Drăgășani (723 mg/kg grape beans) and even Novac (708 mg/kg grape beans).

According to the mentioned data, we observe that in the production of grapes used for vinification, the total amounts of anthocyans places on the first position the variety Negru de Drăgășani (14,61 kg), followed at a slight difference by Novac (14,60 kg). The last place is occupied by Cabernet Sauvignon, because of small productions (9,794 kg), the variety Merlot having a content of 10,62 kg.

In relation to the chromatic structures, the red pigments represent the highest proportions with Novac and Negru de Drăgășani (62,6%, respectively 62,0%).

The data recorded in table 3 illustrate that in wines obtained through macerationfermentation (until the sec) the anthocyans contents are between 663 mg/l at Merlot and 720 mg/l at Cabernet Sauvignon.

Considering the yields in vinification and the volumes of wine obtained from the raw material, the anthocyans were extracted in proportions ranging from 35,7% at Cabernet Sauvignon and 38,0% at Negru de Drăgășani, in the grape pomace the remaining contents recording proportions situated between 62 % at Negru de Drăgășani and 64,3% at Cabernet Sauvignon (table 3). In absolute values, the remained quantities of anthocyans range

between 6,294 kg at Cabernet Sauvignon and 9,06; 9,07 at Negru de Drăgășani, respectively Novac.

Table 3

The anthocyans proportions from the obtained wine and those remained from the grape
pomace in relation to the amounts of grapes and the efficiency at vinification
(the average of the years $2011 - 2012$)

	The volume of		The total	The total	The chromatic structure of the anthocyanic complex in wine			
Grape variety	wine obtained after maceration - 1 -	Anthocyan s mg/l	amount of anthocyans in wine %	remained in the husks of grapes%	Yellow pigments %	Red pigments %	Blue pigments %	
Cabernet Sauvignon	4865	720	35,7	64,3	29,0	62,1	8,9	
Merlot	5916	663	36,9	63,1	29,9	61,4	8,7	
Negru de Drăgășani	7899	702	38,0	62,0	26,8	63,8	9,4	
Novac	7943	696	37,9	62,1	26,4	63,7	9,9	

The results concerning some possibilities of recovering the anthocyanic from the grape pomace are illustrated in table 4.

The process of extraction with the used agents continues over a period of 48 hours, after which a refitting of anthocyans on the solid parts of the grape pomace can take place.

After 24 hours of contact of solvents with the grape pomace, the proportions of extracted anthocyans reach levels of 65,1%, after the activity of sulphurous acid and 67,0% when using alcohol combined with citric acid, in the case of the grape pomace of Cabernet Sauvignon and between 70,1% when sulphurous acid interferes and 73,6% at the action of alcohol + citric acid in the case of the Negru de Drăgășani grape pomace.

After 48 hours of contact with the solutions, the proportions of extracted anthocyans increase up to 88,7% and respectively 89,6% when using the husks of grapes of Cabernet Sauvignon and up to 89,6% and respectively 90,6% when using the husks of grapes of Negru de Drăgășani.

After 48 hours maintaining the contact between solvents and grape pomace is useless, in some cases occuring a reduction of colouring substance, for both extractive agents.

CONCLUSIONS

The grape varieties Cabernet Sauvignon, Merlot, Negru de Drăgășani and Novac from the assortment for red wines in Drăgășani vineyard show a remarkable anthocyanic potential ranging between 1320 mg/kg grapes and 1435 mg/kg grapes.

The extractability of anthocyans exceedes in all cases 50%, where the technological reserves exceed 700 mg/kg grapes with Cabernet Sauvignon, Negru de Drăgășani și Novac.

In the process of maceration-fermentation only a part of the anthocyans are extracted and pass into the wine, which do not drop below 35%, but which do not exceed 38% either. The rest of the colouring substance remains in the grape pomace, as main by-product at vinification in red.

The value of anthocyans as real natural dye in food industry and their sanitary importance, used in the pharmaceutical industry, has determined persistent researches to recover them from the wine-making industry by-products.

The use of the sulphurous acid solution 1% and of the alcohol solution 50% + citric acid 1,5%, in contact with the grape pomace for 48 hours can result in anthocyans recoveries ranging between 88 and 91% from the remaining contents of the by-products.

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The duration of contact with the extractive agent-hours -24 hours 48 hours 72 hours The grape The extraction agent of The colouring intensity of the extract pomace (Variety) anthocyans Absolute Absolute % Absolute value % % value value Total grinding and extraction using acid-2,12 100 2,12 100 21,12 100 alcoholic solution (M) Cabernet Sulphurous acid Sauvignon 1,38 65.1 1,88 88,7 1,88 88.7 solution 1% Alcoholic solution 67,0 1,42 1,90 89,6 1,89 89,2 50% + citric acid 1,5% Total grinding and extraction using acid-2,01 100 2,01 100 2,01 100 alcoholic solution (M) Negru de Sulphurous acid Drăgășani 1,41 70,1 1,90 89,6 1,88 88,6 solution 1%

73,6

1,92

90,6

1,91

90,1

Possibilities of extracting the anthocyanic substance remained from the fermented grape pomace (the average of the years 2011 – 2012)

Table 4

1,48

Alcoholic solution

50% + citric acid 1,5%

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THE INFLUENCE OF REDUCING THE NUMBER OF FRUITS PER PLANT TO PEPPERS GROWN IN FIELD FOR FRESH CONSUMPTION

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Keywords: pepper, number of fruits per plant

ABSTRACT

In recent years, domestic consumers options of fresh pepper fruits are heading higher, preferably at physiological maturity similar to those imported from southern Europe and Minor Asia. To cope with the fierce market requirements, pepper Romanian producers are increasingly using more and more cultivars with medium and late growing seasons, with large fruit that can be eaten at technical or physiological maturity, in order to emphasize the earliness of the first harvest and the growth of medium weight. In late cultivars with large fruits are possible interventions on plants, which consist of removing from the upper part of shrub the last buds and shoots, thus limiting the number of high quality fruit harvested from plant.

Full paper made within the doctoral program POSDRU/107/1.5/S/76888, shows the influence of the number of fruits per plant on the earliness and quality in some cultivars such as Yellow Superior, Atris F1, California Wonder.

INTRODUCTION

Ecological product peppers in field crops from Romania, is competing in the domestic market the fruits of this species imported from geographic areas with favourable climatic conditions than specific to the southern Romania. With a favourable season of vegetation, longer than in Romania, peppers imported from southern Europe and the Near East is present in the domestic market with high or very high fruit harvested at physiological maturity.

Pepper of similar cultures produced in Romania, offers cultivars in the first part of the harvest season fruit production lower late at technical maturity. Towards the end of the normal crop of sweet fruits of these crops can be harvested only small at the consumer maturity.

Especially after the normal harvest period in Romania, due to the low commercial quality of sweet fruit are needed and also possible special character works applied to the plants, which directly influences the development of high quality crops.

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The primary purpose of this paper work is to establish the effect of limiting vegetative growth and fructification of the pepper production and quality for fresh consumption by working protocol we established experimental variations necessary. Since the plant manual interventions are costly experimental grid was applied on a set of ecological cultivation of peppers, which, thanks to capitalize on higher prices, allocated additional production costs can be recovered.

MATERIAL AND METHODS

The biological material used was composed of the following experience cultivars:

Yellow superior: semi-early variety of sweet pepper, by weight of 110-120 g per fruit. Eaten at the maturity the color is light yellow, and at physiological maturity the colour is red. The shrub is vigorous, has a height of 55-60 cm. Fruits with 3-4 lobes are pointed, conical, 8-10 cm long and 6.5-7 cm in diameter. Pulp thickness is 7.5-8 cm. Production potential is 35-40 t / ha.

Atris F1: is a hybrid of pimiento type, early and highly productive fruit conical, elongated and rapid ripening from green to red. Fruits long (15-20 cm), straight, thick and extremely uniform pulp. The plant is vigorous, with no need of trellising to field crops. Culture in greenhouses: trellising and weeding are required. The foliage is rich, balanced fruit, with a length of 19 to 22 cm and a maximum diameter of 5-6 cm. Is a suitable material for paprika processing but also for fresh consumption.

California Wonder: pepper, late growing determined.

Plant height of 50-80 cm, is very vigorous and fast growing.

The fruit is with four lobes, blocky type, with sweet and firm flesh. Growing season: 75 - 80 days.

Fruit weight: 130-150 g fruit color: dark green at maturity of consumption red at the physiological maturity. Way of culture: through seedling. Culture type: in field, protected.

Experimental variants are shown in Table 1.

Table 1

Var.no.	Cultivars (A)	Interventions to limit plant fructification (B1)	
1 (wit)	Superior Yellow (a1)	Growth and fruiting free (b1)	
2		Limiting the number of fruits (6-7 on the plant) (b2)	
3	Atris F1 (a2)	Growth and fruiting free (b1)	
4		Limiting the number of fruits (6-7 on the plant) (b2)	
5	California Wonder (a3)	Growth and fruiting free (b1)	
6		Limiting the number of fruits (6-7 per plant) (b2)	

The experimental variants- Fruits for fresh consumption

Bi-factorial experience, type 3x2, 6 variants was mounted in four repetitions without randomization.

The main elements of culture applied technology experience were:

Experimental organic culture of peppers for fresh consumption was achieved by normal calendar with planting seedlings on May 10, aged 51 days (Gedda Agnes, 2007).

Planting distances of 70 cm between rows and 20 cm between plants in the row cm provide a density of 71400 plants / ha. Interleaved, 5 plants per row were planted peppers, sage seedlings, species with a strong repellent effect on aphids, the main pest of pepper (Otto Schmid & Henggeler, 2000).

Modeled and mulched plot was irrigated by drip lines using nozzles at 20 cm, with water flow 21 / hour.

In addition to other specific organic crop care works peppers on plants versions 2.4 and 6 were applied to limit the fructification works, for the following reasons:

In temperate areas between latitudes 40 o and 45 o C in the Mediterranean basin and in confined areas thereof on pepper plants in field crops occur up to 25 points of fructification, of which only about 6-7 have favourable conditions to develop forming fruit harvest (Foury C., 1964).

In southern Romania, on similar cultivars, the number of fruits harvested at technical maturity is only 3-5 fruits/plant.

In the case of production of seed of a plant can be harvested in late cultivars 2-3 fruit up to physiological maturity. Natural consequence of the aforementioned seed crops is reducing the number of fruits up to a maximum of 5-6 per plant, as done by manual removal of buds and small fruit by pinching the growing tips. The work done in 2-3 innings is performed, for the last time, 40-45 days before harvest the last fully ripened fruit at physiological maturity (ICLF Vidra, 1972).

The plants were not carried out the elimination of fruit made only after the third stage of fructification. In this way, seven fruits per plant is formed from the one to the first branching point, two (or three) in the second level of branching and four in the third level of fructification.

Harvesting was done manually, by installments, at times when fruit left on the plant reached maximum size as possible and desired doneness.

In greenhouse conditions at similar latitudes in Japan on pepper plants grown in the greenhouse to 400 fruits are harvested at maturity technique with low weights .

Based on these considerations, the removal of the plant sprouts peaks, the buds, flowers and small fruit crops for fresh consumption can provide the conditions for the collection of at least 5-6 fruits at physiological maturity, the required market to consumers.

RESULTS AND DISCUSSIONS

In the experiments were performed observations and measurements after which were obtained the following results.

Following the observations and measurements were recorded, with priority on the main components of output data: the number of marketable fruit harvested per plant for each variant, average fruit weight, yield per plant and total production per square meter. Primary data on total production were statistically interpreted by the analysis of variance method.

Observations followed the physiological state of the plant and phytosanitary situation experience.

The data presented in Table 2 regarding the production and some of its components highlight the following.

The application works by limiting the number of fruits per plant, average weight has increased in all 3 cultivars, as follows:

- The Yellow Superior cultivar, from 86.5 g (without limitation fructification) to 105.2 g (with limiting the number of fruits);

- The cultivation Atris F1 similar difference increased from 148.3 g at V3 to 164.3 g, at V4, and the cultivar California Wonder 140.7 g, at V5 to 168.1 g at V6.

The number of fruit harvested from plants varied within relatively small, very little influencing the production level shown in Table 2.

Production data, per square meter, were interpreted statistically by analysis of variance method, specific to the bi-factorial experiences.

The data in Table 3 highlight that the experimental factor "cultivar" significantly influence the production.

Table 2

Var.no.	Cultivars (A)	Interventions to limit plant	Average fruit	No. STAS fruit	Produc	ction
		fructification (B)	weight (g)	on the plant	The plant (kg)	On m2 (Kg)
1	Superior Yellow (a1)	Without limiting the number of fruits (b1)	86,5	5,69	0,493	3,520
2		By limiting the number of fruit 6-7 fruits / plant (b2)	105,2	5,89	0,620	4,426
3	Atris (a2)	Without limiting the number of fruits (b1)	148,3	4,37	0,649	4,640
4		By limiting the number of fruit 6-7 fruits / plant (b2)	164,3	5,30	0,872	6,225
5	California Wonder (a3)	Without limiting the number of fruits (b1)	140,7	4,93	0,695	4,962
6		By limiting the number of fruit 6-7 fruits / plant (b2)	168,1	4,17	0,702	5,012

Results on total production Green pepper in field crops in 2012

Table 3

The influence of production on average assortment of the variants with, and without limiting the number of fruits per plant Green pepper in field crops in 2012

no. nem	Cultivars	Production kg / m2	Differences Production kg / m2	Production%	Output gap	Meaning
1	Superior Yellow(mt)	3970	-	100.00%	-	-
2	Atris F1	5430	+1,460	136.77	36.77	XXX
3	California Wonder	4980	+1,010	125.44	25.44	XX

DL - 5% - 0.670 kg / m²

DL - 1% - 1.240 kg / m $^{\rm 2}$ DL - 0.1% - 1.620 kg / m $^{\rm 2}$

Relation to the production control (Yellow Superior) of 3,970 kg/m² at Atris F1 was a highly significant difference of + 1.460 kg /m², and the California Wonder, the difference from the control of 1.010 kg /m² is only distinctly significant.

The results of the plant production were interpreted statistically by two-way variance analysis method specific experiences.

Summary of experimental results on the influence of limiting the number of fruits per plant, show that by performing this work, the average yield of the three cultivars grow + 0.844 kg/m^2 (distinct significant positive difference), the average production of the same cultivars at not limit the number of fruits per plant.

Table 4

	Green pepper in field crops in 2012									
Nr.crt	Interventions to limit the number of fruits per plant	Production Kg /m2	Differences Production kg / m2	Production%	Output gap%	Significance				
1	Growth and fruiting free	4,374	-	100,00%	-	-				
2	By limiting the number of fruit 6-7 fruits / plant	5,221	+0,844	119,36	+19,36	XX				

Influence of limiting the number of fruits per plant on average production of the three cultivars Green pepper in field crops in 2012

DL - 5% - 0.670 kg/m2

DL - 1% - 1,240 kg/m²

DL - 0.1% - 1,620 kg/m²

The data in Table 4, the synthesis of experimental results highlight differences in plant production by removing the tip shoots, the buds, flowers and small fruit crops for fresh consumption. The Differences are apparent and cultivars California Wonder and Atris F. Thus the plant production in kg is significantly higher than the witness Yellow Superior making a difference size and average fruit weight (table 3).

CONCLUSIONS

Limiting the number of fruits harvested per plant positively influence the increasing number of fruits and their average weight at the three cultivars that worked in experience.

The largest increases were recorded at a decisive parameter for grown - average fruit weight (Table2).

The statistical interpretation of the results of production (table 3) shows that, compared to witness cultivar (Superior Yellow 3,920 kg/m²) at Atris F1 cultivars California Wonder were recorded positive difference of +1.460 kg/m² (very significant) that +1.101 kg/m² (distinct significant).

By applying works of limiting the number of fruits per plant, average production of three cultivars increased from 4.374 to 5.221 kg/m², the difference of 0.844 / m^2 is significant.

By ranking and comparing the results, is found that limiting the number of fruits per plant increases production at Atris F1 1585 kg/m², compared to the same cultivar grown production without limiting the number of fruits.

Based on the results, we recommend that in the south of the country to promote the green culture, Atris F1 cultivar, with the restriction to 6-7, the number of fruits per plant.

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NEW TECHNOLOGICAL SOLUTIONS IN THE PRODUCTION OF MATERIAL FOR VINE PROPAGATION

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Keywords: vine, propagation material, technology

ABSTRACT

The investigation work was carried out at the vine nursery of the Institute of Viticulture and Enology – Pleven in the period 2007-2010.

The results of study the affinity in the nursery of newly-selected varieties at IVE-Pleven with used rootstocks in the world vine-growing practice are presented. Comparative study of paraffin waxes Rebwax PRO, Rebwax VF, Aktigref (2010) and PP 140 was conducted. Mathematical model of the root system growth of the planted cuttings was developed for the irrigation regime control. The efficiency and selectivity of some herbicides with soil activity were studied for weeds control in the vine nursery.

INTRODUCTION

The production of material for vine propagation is topical in nature and it is directly related to the biological potential of vine growth and development, grape yield and longevity of the plantation. One of the main technological problems was the incompatibility between the rootstock and the grafting that necessitated the investigation of affinity for defining the interrelated effect of both components during stratification and in the vine nursery (Lilov, 1977; Mamarov, 1989; Zuluaga et all., 1959). With the application of the technology for outdoor cultivation of rooted cuttings, the different types of waxes and their effects on the regeneration processes in stratification and subsequently preventing the tissues in the nursery from dehydration, had been an object of investigation (Todorov, 2005; Dimitrova et al. 2007, 2009). Ensuring the needed moisture for complete coalescence at the site of grafting and young vines growth had raised the question of irrigation regime control (Mishurenko, 1964; Magriso, Georgiev, 1968; Radulov, 1979). Another factor influencing the vine growth and development are weeds that imposed the need of investigating the effect of various types of herbicides on weeds and rooted cuttings (Chelebiev, Katerova, 1988; Todorov, 2005; Moretti, Borgo, 1992).

The objective of the study was to offer new technological solutions for improvement the production technology of material for vine propagation.

MATERIAL AND METHODS

The investigations were carried out at the vine nursery in the experimental field of the Institute of Viticulture and Enology – Pleven in the period 2007-2010. The study of affinity in the nursery was carried out with Rubin and Storgozia varieties (red wine

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varieties, selection of IVE Pleven), grafted on rootstocks Berlandieri x Rupestris 110 Richter and Riparia x (Kordifeliya x Rupestris) 44-53 Maleg. The experimental work for the rest of the technical studies for waxing, irrigation regime and the efficiency and selectivity of herbicides was conducted with Muscat Kailashki variety (white wine variety, selection of IVE - Pleven) grafted on rootstock Berlandieri x Riparia CO4. The paraffin waxes Rebwax PRO, Rebwax VF, Aktigref (2010) and PP 140 were investigatied for waxing in the following variants: reference (no waxing of the grafted cuttings), waxing with Rebwax Pro, Rebwax VF and Aktigref applied after grafting and subsequent waxing with PP 140 after the stratification of the grafted cuttings. A system of drip irrigation with one irrigation wing per row, positioned between the two rows of the transplanted cuttings was made for determination the vine water consumption. The irrigation wings had built-in drippers at 15 cm distance at a rate of 1.0 L h⁻¹. Under atmospheric drought - high temperature and low humidity irrigation refreshing was carried out by micro spraying systems at the rate of 3-6 mm. Micro spraying devices with flow rate of 156 L h⁻¹ at 0.2 MPa and a range of 5.0 m were used. For following the dynamics of the roots growth, 5 vines of each variant were dug out of which the number, the average and maximum length of the roots were determined. The soil herbicides Goal 2E (240 g / l oxyfluorfen), Dual Gold 960 EK (960 g/l s-metolachlor), Stomp 33 EK (330 g/l pendimethalin) were studied. The studied variants were: V1 - Stomp 33EK - 0.6 l/da; V2 - Stomp 33EK - 0.8 l/da; V3 -Dual Gold 960 EK - 0.3 l/da; V4 - Goal 2E - 0.2 l/da; V5 - Goal 2E - 0.3 l/da; K1 -Untreated reference variant, thricely weeded. The effect of herbicides on weed density was tracked in dynamics (on the 30th, 60th, 90th day) in permanently marked parcels, as the manifestation of phytotoxicity (visually) and the obtained rate of the rooted grafted vines (% compared to the transplanted cuttings) were recorded. The herbicides were applied immediately after the cuttings were transplanted in the nursery, just before spraying. The data were processed by analysis of variance (Dimova, D., E. Marinkov, 1999). The grafted and waxed cuttings were rooted in the vine nursery and raised in line with the technology for production of vine propagation material applied at IVE-Pleven, in raised beds, 120 cm wide, in two rows at 40 cm distance. The soil is leached chernozem.

RESULTS AND DISCUSSIONS

Affinity study in vine nursery ._The data on shoots germination of the planted cuttings for the period 2008-2010 were similar for both varieties Rubin and Storgozia (fig. 1). However better rates of germination were found in the case of grafting to 44-53 Maleg rootstock. The percentage of germinated shoots during the last reading was 88.9% for Rubin variety and 96.5% - for Storgozia variety. The rate of first-class vines corresponded to the obtained results for shoots germination in the nursery as it was higher for 44-53 Maleg rootstock (fig. 2). The percentage of first-class vines was 53.5% and 65.1% for Rubin and Storgozia varieties, respectively. The rate of first-class vines corresponded to the obtained results for shoots germination in the nursery as it was higher for 44-53 Maleg rootstock (fig. 2). The percentage of first-class vines was 53.5% and 65.1% for Rubin and Storgozia varieties, respectively. The rate of 53.5% and 65.1% for Rubin and Storgozia varieties, respectively.

Wax types testing – Rebwax Pro, Rebwax VF and Aktigref, applied after the cuttings were grafted

Figure 3 shows the data of the 4-year testing of callus formation between the grafted components during stratification. The percentage of cuttings with a full circular callus was 56% when waxed with Rebwax Pro and 79.2% with Rebwax VF. For the not-waxed cuttings (the reference), the percentage of cuttings with a full circular callus was



45.8% or 10.2% less compared to the cuttings waxed with Rebwax PRO and 33.4% less than with Rebwax VF.

Figure 1. Germination dynamics



The results of the grading after the grafted rooted vines were removed from the nursery were indicative of the wax effect. With Rebwax VF - 44.2% were obtained to 47.5% with Rebwax PRO (Fig. 4). Approximately the same percentage of first-class rooted grafted vines was obtained in the one-year study in 2010 of Aktigref – 42.1%. The lowest rate of first-class grafted rooted vines was achieved in the reference (without waxing) - 30.1%.



Figure 3. Results of the regeneration processes during stratification



Figure 4. Rate of obtained first-class grafted rooted vines

Determination of the active soil layer depending on the root system growth.

The dynamics of increase in the average length of the roots in the three experimental years is shown in fig. 5. A regression equation is also presented describing with high degree of confidence ($R^2 = 0.998$) the correlation between the average length of the roots and the number of days after planting of the grafted cuttings.



Figure 5. Dynamics of increase in the average length of the roots

It is known that that with Berlandieri x Riparia SO4 stock the roots grow at 45° angel towards the soil surface. Considering the planting depth of the cuttings, the increasing in the depth of the active soil layer could be described by the following equation:

$$H = (0.001T^2 + 0.053T + 0.034)\sin\alpha + z;$$

where: H – active soil surface layer (cm); T – days after transplanting of the grafted cuttings in the nursery; z – depth of planting of the cuttings in the soil (cm), here z = 15 cm; α – angel at which the roots grow in the soil towards the soil surface, here $\alpha = 45^{\circ}$.

The width of the active soil layer also increases with the roots development as it depends also on the pattern of planting (the number of rows in the bed and the distance between them). In general, the increase in the width of the active soil layer would be:

$$B = 2(0.001T^2 + 0.053T + 0.034)\cos\alpha + b;$$

where: B – active surface layer width (cm); T – days after transplanting of the grafted cuttings in the nursery; b – distance between both rows cuttings in the bed (cm), here b = 50 cm; α - angel at which the roots grow in the soil towards the soil surface, here α = 45°.

The data reveal that irrigation has to ensure wet soil layer of about 55 cm at the time of planting of the grafted cuttings to approximately 80 cm at the end of the irrigation season and a depth in the range of 15 cm to 30 cm (fig. 6).



Figure 6. Increase of active soil layer depth and width depending on the number of days after planting of the cuttings in the nursery (the number of days is displayed next to each mark).

Effect on some soil herbicides in the nursery

Stomp 33 EK and Dual Gold 960 EK in the applied doses exhibited good herbicidal effects against the annual weeds until the sixtieth day after treatment (Fig. 7). Weak sensitivity was recorded for Xanthium strumarium, Solanum nigrum L. and all root sprouting weeds. Better weed control was achieved with higher doses (V2 and V5) however they did not affect those species. The herbicide Goal 2E (V4 and V5) controlled in full the annual and to a significant degree the perennial weeds found in the nursery. When applied at a dose of 0.3 l/da (V5) it maintained the lowest level of weeding throughout the vegetation period. Ninety days after the treatment, it was still 1.9 pc. /m².



Figure 7. Dynamics of the average density of weeds for the period 2007 - 2009

The grafted cuttings showed some sensitivity to Stomp 33 EK and Goal 2E. The direct contact of the herbicide solution with developing buds caused different manifestations of phytotoxic response. Stomp 33 EK caused yellow spots on leaves and distortion of the leaf blade. Brown necrotic spots with irregular shape were clearly outlined after Goal 2E fell on them. The plants overcame the phytotoxic response to the sixtieth day and at the end of vegetation no traces of the herbicide were found. The damages to the leaves, observed during the first days after the treatment did not significantly affect the life processes of the grafted cuttings and the majority of them grew and developed as standard

rooted vines -54.0% for Stomp 33 EK -0.8 l/da (V2), 53.7% for Goal 2E -0.3 l/da (V5), 52.5% for Goal 2E -0.2 l/da (V4) and 51.7% for Stomp 33 EK -0.6 l/da (V1).

The highest average yield for the period of the study showed V5 Dual Gold 960 EK – 0.3 l/da (54.4%). Despite the lack of proof of the differences (at *GD* 5% = 13.581; *GD* 1% = 13.627; *GD* 0.1% = 19.724), the following variants significantly exceeded the reference (47.4%) in obtained rate of rooted vines.

CONCLUSIONS

The variants of grafting to 44-53 Maleg rootstock showed better affinity compared to 110 Richter. The percentage of first-class vines for Rubin variety was 53.5%, and for Storgozia variety - 65.1%.

From the studied waxes, 44.2% first-class grafted vines were obtained with Rebwax VF, 47.5% with Rebwax PRO, while the reference (without waxing) - 30.1%.

Corresponding to the root system growth of the planted cuttings, the irrigation in the vine nursery should ensure wet soil layer with a width of 55 cm at the beginning when the grafted cuttings were planted to 80 cm at the end of the irrigation season and depth in the range from 15 cm to 30 cm.

It was found different duration of action of the tested soil herbicides depending on the active ingredient. With Stomp 33 EK and Dual Gold 960 EK, it became weaker after the sixtieth day, with Goal 2E it continued throughout the whole growing period. Despite the signs of phytotoxicity caused by Stomp 33 EK and Goal 2E it was not found a negative impact on yield.

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STATE, TRENDS AND PROBLEMS IN THE DEVELOPMENT OF VITICULTURE IN BULGARIA

Daniela Dimitrova¹, Valeriy Peykov¹, Vladimir Dimitrov¹, Violeta Dimitrova¹

Key Words: viticulture, state, trends, organic production

ABSTRACT

The purpose of this article is to perform an assessment of the state of viticulture before and after the accession of Bulgaria to the European Union. It analyzes the dynamics of harvested areas, average yields and produced output of wine and table grapes during the period of 2001-2012. The work follows up the structural changes in the sector under the influence of the Common Agricultural Policy of the European Union. It examines the development of organic viticulture. The article specifies the main problems of the conventional and organic production of grapes in the context of overall state and prospects of development of viticulture in our country.

INTRODUCTION

Viticulture is a traditional for Bulgaria subsector of agriculture, providing employment for the majority of the rural population of the country (Borisov and Radev, 2011). However, as a result of many organizational and economic problems in the sector, unresolved already for a second decade, its socio-economic importance continues to decline. According to the official information disclosed by National Statistical Institute, the share of the produce of the subsector in the total plant-growing production has dropped down from 7.49% in 2001 to 2.57% in 2011. For the same period it was reported decrease in the percentage share of the area of vineyards towards the used agricultural area, respectively from 2.5% in 2001 to 1.5% in 2011. With the accession of Bulgaria to the EU-27 the Bulgarian vine-growers had the opportunity for financial support under the Common Agricultural Policy, but at this stage the allocations had not yet stimulated adequately the economic activity in the sector (Slavova et al., 2011; Radev and Borisov, 2012).

The objective of the study is to assess the state of viticulture before and after the accession of our country to the EU-27 and to specify the main problems of conventional and organic grapes production in the context of prospects of development in the next programming period 2014-2020.

MATERIALS AND METHODS

The analysis is based on official statistical data from the Ministry of Agriculture and Food (MAF), Department "Agrostatistics", National Statistical Institute (NSI) and Eurostat. The average values of the main indicators characterizing viticulture development

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in the period before (2001-2006) and after the accession of Bulgaria to the European Union (2007-2012) have been compared, and thus the annual impact of weather conditions on the results of the economic activity in the sector have been neutralized.

The research methods of comparative and dynamic analysis, a method of statistical groupings and expertise assessment have been used in the study.

RESULTS AND DISCUSSIONS

Analysis of the state and trends in vine-growing development before and after the accession of Bulgaria to the European Union

> Dynamics of the harvested areas, grapes production and average yields of wine and table grapes varieties

In 2012 the total area of vines in the country was 77 341 hectares of which 62 701 hectares were cultivated vineyards and 14 640 hectares were not maintained plantations, not included in farms (MAF, 2013). The dynamics of the harvested area for the period 2001-2012, showed a steady decrease, which in 2012 compared to 2001 amounted to 53.0% (Fig. 1). The largest share in the production structure of the sector belonged to red wine grape varieties (56% of the harvested area), followed by white wine grape varieties (41%) and table grapes (4%). The weak entrepreneurial interest determined the negative development of production potential in its three main areas, as it was particularly noticeable the reduction of the vineyards for table grapes - a total of 6750 ha for the period 2001-2012.



Source: MAF, Department "Agrostatistics" Figure 1. Dynamics of vineyards area and grapes production in the period 2001-2012

Despite the fluctuations in the volume of production per years in the long term plan the grapes production continues to decline. The obtained total wine and table grapes in 2012 represents 60.1% of the volume of the production for 2001. The comparison between the average values of the indicator for the six consecutive years before the full membership of Bulgaria in the EU-27 (2001-2006) and the last six after that (2007-2012) confirmed the outlined negative tendency. Logically, the greater rate of decrease belonged to the table grapes production as a result of drastically reduced areas as the rate of decrease amounted to 30.5% in 2007-2012 compared to 2001-2006. For the same period, the quantity of wine grapes decreased by 19.2% (Table 1).



Source: MAF, Department "Agrostatistics"



The dynamics of the long-term rate of development of the productivity per unit area showed growth both for wine and table grapes varieties (Fig. 2). The obtained average yields of the vineyards for table grapes have grown by 45.8% averagely for the period 2007-2012 compared to 2001-2006. In wine varieties the increase amounted to 24.2%. It should be noted, however, that the level of the obtained average yields for the entire period of the study remained one of the lowest in the Community and it was significantly below the potential of the grown wine and table grape varieties in the country.

Table 1

No.	Indicators	2001-2006	2007-2012	Rate of change
		average	Average	2007-12/2001-06, %
1	Total area of the harvested	95552	60834	-36,3
	vineyards, ha, incl.:			
1.1.	Red wine varieties, ha	54373	37011	-31,9
1.2.	White wine varieties, ha	35707	21247	-40,5
1.3.	Table grapes varieties, ha	5472	2576	-52,9
2	Grapes production ² , t	355152	284952	-19,8
2.1.	Wine grapes, t	336542	272018	-19,2
2.2.	Table grapes, t	18610	12934	-30,5
3	Average yield, kg/ha	Х	х	Х
3.1.	Wine varieties, kg/ha	3785	4700	+24,2
3.2.	Table grapes varieties, kg/ha	3436	5011	+45,8

Areas, production and average yields from vineyards in Bulgaria

Source: MAF, Department "Agrostatistics" and own calculations

The results of the analysis of these tendencies in viticulture in the last twelve years had shown that in the years after the accession of Bulgaria to the European Union it was observed a delay in the negative rate of change of cultivated wine and table grapes area.

² Not included grapes from trellis vines

However, the reduction in production potential of viticulture continues and the process of establishing new vineyards is very slow and at this stage it does not provide sufficient reproduction of cultivated areas. The marked increase in the average productivity per unit area could be interpreted as an indication of the ongoing processes for stabilization of the sector, but taking into consideration that almost half of the vineyards in the farms are over 29 years it is expected that the reduction of plantations would continue in the coming years.

Structural changes in the sector

The ongoing structural changes in the sector during the period as a result of implementation of the national policy and the Common Agricultural Policy concern primarily the reduction of the total number of farms growing vines (by 38.4% in 2010 compared to 2005) and an increase in the concentration of production. The average size of areas with vineyards has increased about 1.6 times for the period 2005-2010 (Table 2).

Table 2

No	Indicators	2005	2007	2010	Change
					2010/2005,
					%
1	Farms, growing grapes, number	141 330	98 260	87 000	-38,4
2	Area, ha	52 670	51540	52340	-0,6
3	3 Average size, ha/farm		0,525	0,602	+61,4
4	Specialized vine-growing farms, number	15380	14420	18120	+17,8
5	Area, ha	19660	25880	27780	+41,3
6	Average size, ha/farm	0,782	1,795	1,533	+96,0
7	Regular labour force in the specialized vine-	28050	28750	35010	+24,8
	growing farms, pers.				
8	Family labour force, pers.	26770	26340	32900	+22,9

Number and size of farms growing grapes

Source: Eurostat and own calculations.

A positive tendency has been observed regarding the degree of specialization of production in the subsector. The percentage of vineyards grown in specialized vine farms has increased from 37.3% in 2005 to 53.1% in 2010. The number of newly created production units with main activity in viticulture for the three years following the adoption of our country as a member of EU was 3700. Although the average size of the area of vineyards in the specialized farms has decreased by 14.6% in 2010 compared to 2007, the rise of this indicator was almost double compared to the data for 2005.

The number of permanent employees in viticulture has also gone up. In 2010, 35 thousand people earned their living by grapes production, with 6260 people more than the registered in 2007. Almost 94% was the share of the family labor force from the total employment in the sector, compared to 91.6% in 2007.

Undoubtedly the above tendencies indicate a higher motivation of the owners of vineyards, aimed at consolidating the production under the influence of the financial support granted by the EU funds and the national budget. However, the organizational and economic structure of vine-growing continues to be irrational from the point of view of the economic conditions under which the farms are run.

> Development of organic grapes production

Unlike the continuous reduction in harvested area of vines grown by the conventional method, the areas for organic grapes production grew steadily over the last seven years (Fig. 3). In 2012 the total area of vineyards cultivated according to the requirements of organic farming was 2 058 ha. From those grown by the organic method, 1512 are in a period of transition, of which 1404 ha are wine and 107 ha are table grapes, as 546 ha of the vineyards have passed the transition period. The growing demand for organic products worldwide, combined with the financial incentives has determined the recorded rise in the size of organic vineyards in the country, as the growth rate was 140.8% in 2009 compared to 2007 and 185.8% in 2012 compared to 2009.





The main conclusion of the analysis is that the expected positive impact on the sector as a consequence of the country's accession to the EU is very weak and does not provide the necessary conditions for the overall implementation of the existing potential for the production of table and wine grapes.

Problems in conventional and organic grapes production

The reasons for the outlined negative tendencies in the sector should be looked in the whole chain of production, processing and marketing of wine and table grapes. The unsatisfactory profitability of viticulture and the lack of strong enough economic incentives define the problematic status of the production potential mostly in terms of age and varietal structure as well as agricultural and health status of cultivated plants. The unequal rise of the producer prices compared to the rate of increase of production costs does not create conditions for expanded reproduction, just the opposite it results in restricting the amount of current inputs at risk for the quantity and quality of the final production. In addition, the fact that the majority of vine-growers have small vineyards without pronounced desire for association for processing and marketing of products, defines significant variations in the profitability of production, depending on the climatic conditions of the year, the market conditions, unfair commercial practices in the purchase of grapes and unregulated imports. The growing competitive pressure on the domestic market by similar goods produced in EU Member States with higher levels of subsidies and the introduction of regulations governing quality and safety of food and the environment, results in reduced competitiveness of the sector.

The positive trend in the development of organic viticulture is encouraging, although here there are a number of restrictive conditions too. From agro-technical aspect, the main problem remains the protection of the harvest and the pest control (Kostadinova et al., 2012). In terms of the economic interests of the vine-growers there is the issue with the marketing of the products and setting a rational organization of distribution channels at the domestic and international markets, providing the desired high profitability.

CONCLUSION

Viticulture in Bulgaria in the context of the strategic objectives for the new CAP 2014-2020 (viable food production, sustainable management of natural resources and balanced territorial development) requires timely overcoming of the existing problems. The biggest challenge to the conventional viticulture in the medium term plan is stabilization of the overall production potential in the meaning of increasing the competitiveness of the sector.

With a view of the priorities the role of organic viticulture is becoming more important, both as a way to diversify the production and the income of farmers as well as an opportunity to satisfy the growing consumers' demands.

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Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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EFFECT OF THE HUMIC ACIDS AND THEIR COMBINATION WITH BORON AND POLYPHENOLS EXTRACTED FROM THE SEEDS OF *VITIS VINIFERA* TO CULTURE OF TOMATOES IN SOLAR

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Keywords: tomatoes, humic acids, poliphenols, boron

ABSTRACT

This study shows the effect of foliar fertilization with three fertilizers consisting on: humic acids (HA); humic acids + polyphenolic extract of Vitis vinifera seed (ESVv); humic acids + polyphenolic extract of Vitis vinifera seed + boron (B); on tomato cultivar Antalya. Biometric and biochemistry measurements were made of plants and tomato fruits. Bio-fertilisers were applied to foliage at a concentration of 10 mL / L humic acid and 15 mL / L for variants with polyphenolic extract and boron. Fertilization was carried out at 3 weeks after planting in three stages with an interval of 10 days. HA treatment had a beneficial effect on plant growth both in height and in diameter of base stem and also on the leaf growth. The ESVv application determine a retardant character of tomato plants and HA+ESVv+B determine very good vegetative growth compared with the control. The foliar application with HA+ESVv has positively influence the average fruit weight. The study shows that HA mixed with extract of Vitis vinifera and boron can be successfully used to achieve significant increases in crop production of tomatoes in solar.

INTRODUCTION

Increased interest in the use of natural growth regulators in the vegetables crop and especially tomatoes helped to develop a wide range of fertilizers based on natural components.

Humic acids in combination with other products is an important source of natural organic matter used for the agricultural fertilization.

Humic acids are compounds that contribute to increased permeability of cell membranes in plants (Kaya et al., 2005) and recent studies show their role on the process of seed germination, seedling raising growth, development and weight gain of the root system and the development as a whole plant (Karakurt et al., 2007; Katkat et al., 2009).

The polyphenols action in plants is seen through their role in the formation of pigments, increasing the resistance to pathogens and UV protection (Latanzzio et al., 2006).

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The literature report a variety of effects at simple application of humic acids or combine with other stimulator on plants. An important role is played by the boron compounds in the plant development. Boron favor flowering, fertilization and seed development, stimulates root growth, activate some enzymes (pectinase, tyrosinase, sucrase, dehydrogenase); acts indirectly on the photosynthesis by accelerating the transport of carbohydrates, especially sucrose from leaves. Boron has a favorable effect on flowering and fruiting of plants because it stimulates the rapid germination of pollen.

Also, boron influence the synthesis of aromatic compounds in plants; protoplasmic membrane permeability; carbohydrate translocation; cell division and extension; cell differentiation; maturation of fruits; accumulation of free auxin and nucleic acid biosynthesis (Waquar et al., 2012; Blevins et al. 1998).

A deficiency of boron in plants and soil lead to the decline in vascular plant, root elongation, a slowdown of carbohydrates metabolism, reducing nuclei acid synthesis. (Blevins et al., 1998). In his absence leaves become vulnerable to their growth point blank, begin to roll inward. Flowers usually spades (abort). Fruits begin to stain (start tissue damage).

The aim of this study was to evaluate the effect of humic acids in combination with polyphenols extracted from the seeds of Vitis vinifera and boron on tomato culture in solar.

MATERIAL AND METHODS

The research was conducted in 2012, in terms of solar, unheated, placed in the Mărăcine Banu field of teaching discipline gardening.

It was studied tomato cultivar Antalya. Experience has been placed in randomized blocks with five variants of 4 repetitions each variant. The variants was: V1-control (Mt.) V2 - Vitis vinifera seed extract (ESVv.) V3 humic acids (HA.) V4 - humic acids plus Vitis vinifera seed extract (HA + ESVv.) V5 - humic acids + Vitis vinifera seed extract + boron (HA + ESVv + B).

Have been performed observations and measurements, in vegetation, and crop was recorded by stages of harvest.

Determination of chlorophyll a+b and total carotenoid

The weighed samples, having been put separately in 95% in acetone (50ml for each gram), were homogenized with Braun MR 404 Plus for one minute. The homogenate was filtered and was centrifuged using the Hettich Universal 320/320R centrifuge at 2500 rpm for ten minutes. The supernatant was separated and the absorbance was read at 400-700 nm on Cary 50 spectrophotometer. It was recorded that Chlorophyll a showed the maximum absorbance at 662 nm, chlorophyll b at 646 nm and total carotene at 470 nm and the amount of these pigments was calculated according to the formulas (Dinu et all., 2013; Nagata M., 1992):

Ca = 11.75 A662 - 2.350 A645

Cb = 18.61 A645 - 3.960 A662

Cx+c = 1000 A470 - 2.270 Ca - 81.4 Cb/227

Ca = Chlorophyll a, Cb = Chlorophyll b, Cx+c = Total carotene.

For statistical interpretation of the data, the ANOVA method has been used.

RESULTS AND DISCUSSIONS

In order to ascertain the effect of foliar application of humic acid (HA) in combination with the extract from the seeds of Vitis vinifera (ESVv) and boron (B) on tomato crop in solar have made a series of observations and measurements.

To begin with, observations were made as to how to increase the plants after each treatment.

Biometric measurements have focused on: plant height, diameter of the base stem and the number of leaves per plant. As can be seen from Table 1, the best results on plant height were recorded at V2 (HA) variant were we obtained increases of 25.4 cm compared to the control. Humic acids determine the vigor of tomato plants (average 21.6 leaves per plant). This effect is also found to V5 when we observed the combined effect of humic acids, the polyphenols from *Vitis vinifera* seed and boron. We recorded positive differences in all three variants studied relative to unfertilized control. These differences were maintained after each treatment (data not shown) and will be reflected in biochemical determinations performed on leaves and fruit.

The leaves were harvested and the biochemical measurements have been performed in order to establish the effect of increase of bio-fertilizer content in the most important biochemical constituents (Carotenoids and chlorophyll). As shown in Table 2 the best results were observed in the V5 where chlorophyll "a" has a SP value of 174.6 mg/100 chlorophyll 'b' sp 79.7 mg/100g, while the total Carotenoids 2 62 mg/100g fresh matter. Presented data certifies the biostimulator role played in fertilization of tomato plants.

The elevated chlorophyll concentration are due to the effect of humic acids in combination with polyphenolic extract and boron to enhance the process of photosynthesis in leaves with a synthesis of sugars that are rapidly transported and released into the rhizosphere root them. These substances removed by root ensures soil micro nutrients, microorganisms that synthesize substances needed when growing plants and fruiting. There is a higher mineral nutrition on the growth effect of chlorophyll concentration, concentration is related to higher nitrogen assimilation in plants (Haboudane, 2002 Amaliotis et al. 2004 Lelyveld et al. 2004 Cabrera, 2004).

The green fruits of tomato, Table 3, there is an evident increase in total chlorophyll and carotene in variants 2 and 5 compared to the other due to rises in grape seed polyphenols and boron determining photosynthetic acceleration. It is noted that the samples treated with humic acids and humic acids in combination with polyphenolic extract the values of chlorophyll and Carotenoids lower than the other samples. Determinations have highlighted the role of boron in fertilizer combination on the parameters studied.

In firstfruits of tomato fruit, Table 3, there is the same effect as putting green fruit work out an optimal combination.

As regards the factors of production, average fruit weight of tomato as observed, not recorded positive difference to the unfertilized control (Table 4).

For the culture of solar tomatoes considered to be early culture it is preferred that the average weight of the fruit does not exceed 80-100 g is consumed as fresh fruit. Cultivar fruit Antalya has an average weight of 170-190 g, and from our results it appears that all variants had values below the above.

The HA fertilized variants (V3) and ESVv AH + + B (V5) to obtain fruit caused an average weight of 108 g, fruit valued by consumers. Although fruits were lower than the control average production of fruits produced per plant was higher, 11.07 kg / plant at V2 and 12.26 kg / plant at V5. Increase production of unfertilized and fertilized variants ranged from 35.83 to 51.54% the highest yield obtained for V5 (12.26 kg / plant) and lowest in V2 (11.07 kg / plant). Note that all fertilized variants exceeded unfertilized.

These production increases were preserved and reflected very well in tomato fruit production obtained/ m^2 .

Height Diameter Leaf (±) dif. (±) dif. (±) dif. Variant relative to relative to relative to Var. % % % cm mm nr. control control control (cm) (nr) (mm) 147,0 V1. Unfertilized control 100,0 control 8,66 100,0 control 19,2 100,0 control 157,2 102,7 20,1 V2. ESVv 106,9 +10,28,89 +0,23104,7 +0,9+ 25,4 21,6 + 2,4 V3. AH 172,4 117,3 8,55 98,7 - 0,11 112,5

+ 21,7

+25,1

Biometric measurements in the tomatoes	grown in solar under the influence of treatments applied (a	verage)

\mathbf{B} 1/\/\ \mathbf{F}	
\mathbf{D}	
Dioenenneur unurysis or tomato rea	

7,28

8,30

84,1

95,8

- 1,38

- 0,36

20,6

23,0

107,3

119,8

+ 1,4

+ 3,8

Var.	Variant	Clorophyll "a" mg/100g f.m.	Clorophyll "b" mg/100g	Total carotene mg/100g f.m.
V1.	Unfertilized control	147.8	70.2	2.44
V2.	ESVv	149.8	71.4	2.52
V3.	AH	154.5	74.2	2.54
V4.	AH + ESVv	170.2	79.6	2.60
V5.	AH+ ESVv + B	174.6	79.7	2.62

168,7

172,1

114,8

117,1

V4.

V5.

AH + ESVv

AH+ ESVv + B

Table 1

Table 2
	G	breen tomatoes		Tomato firstfruits			
	Clorophyll	Clorophyll	Total	Clorophyll	Clorophyll	Total	
Variant	''a''	"b"	carotene	''a''	"b"	carotene	
	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	
	f.m.		f.m.	f.m.		f.m.	
Unfertilized	51.0	24.7	6.5	26.2	11.3	8.5	
control							
(V1)							
ESVv	54.0	23.0	7.5	28.9	14.2	9.4	
(V2)							
AH (V3)	50.2	22.9	4.2	26.2	10.8	6.4	
AH +							
ESVv	47.0	24.2	6.1	25.5	13.4	8.3	
(V4)							
AH+							
ESVv + B	75.3	34.5	8.1	37.9	15.4	10.1	
(V5)							

Biochemical determinations carried out on tomato fruits at different stages of ripeness

Table 4

Elements of the tomato crop production in solar

	We avera	eight ge/fruit		Weight average/fr	uit	Crop/m ²		
Variant	g	%	kg	%	(±) dif. relative to	Kg/m ²	%	(±) dif. relative to
					control (kg)			control (kg)
Unfertilized control (V1)	115,5	100,0	8,09	100,0	Mt.	25,07	100,0	Mt.
ESVv (V2)	114,6	99,22	11,07	135,83	+ 2,98	34,31	136,85	+ 9,24
AH (V3)	108,3	93,76	11,70	144,62	+ 3,61	36,27	144,67	+ 11,20
AH + ESVv (V4)	123,8	107,18	10,97	145,24	+ 2,88	34,00	135,62	+ 8,93
AH+ ESVv + B (V5)	108,4	93,8	12,26	151,54	+4,17	38,00	151,57	+12,93

CONCLUSIONS

Results of the tests performed reveal the influence of fertilizer on tomato crop by both biometric and biochemical changes.

Plant height and their vigor is seen positively in plants treated with humic acids and their vigor is influenced by the combined treatment between humic acids and polyphenolic extract. Biochemical changes (containing chlorophyll "a" and "b" and carotene) control samples have higher values. Fruit production per m2 increased in fertilized samples.

We notice the effect of boron in combination with polyphenolic extract and humic acids.

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RESEARCHES CONCERNING THE QUALITY OF MULTIVARIETAL WINES OBTAINED IN GALICEA MARE VITICULTURAL AREA

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Key words: multivarietal wines, composition, tasting, quality

ABSTRACT

Multivarieties wines obtained by combinations in different proportions of the three varieties grown in the viticultural area of Galicea Mare offers an interesting perspective on improving their sensory characteristics and typicity. This study, conducted on wines produced in specific climatic conditions of year 2011, highlights in particular the combination of international varieties Cabernet Sauvignon and Merlot and indigenous varieties Fetească Neagră, the proportion of Fetească Neagră being 25% or 33%.

INTRODUCTION

Phenolic compounds in foods cause variation in various organoleptic qualities like taste, bitterness and sourness; and color properties are also ltered with phenolic compounds and anthocyanins (Nizamlioglu N. M. and Nas S., 2010). Phenolic compounds are essential for the quality of plant-derived food products through their contribution to oxidative stability and organoleptic characteristics (Peleg H. et al. 1999). Indeed, wine organoleptic properties are largely related to phenolic compounds extracted from the grape during the winemaking process (Beleniuc G. et al. 2013). Astringency and bitterness are two major characteristics in grape and wine quality definition. Astringency is a tactile sensation, whereas bitterness is a taste. The molecular size of proanthocyanidins affects their relative bitterness and astringency level (Cheynier V. et al. 2006). Overall, monomers are more bitter than astringent, whereas the reverse is true in the case of large molecular weight derivatives. For grape seed tannin, reducing the degree of galloylation only decreases astringency (Vidal S. et al. 2003). Wine phenolic composition depends on the grape used and on winemaking processes that determine their extraction into the must and subsequent reactions (Lorrain B. et al. 2013). Both the quantity and the extractability of anthocyanins and tannins increase throughout the grape ripening (Fragoso S. et al. 2011).

MATERIAL AND METHODS

The Galicea Mare viticultural area of Dolj district enjoy of generous climate resource, characterized by a great wealth of warmth and sunlight. Accordingly, basic

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assortment of the viticultural area consists of 3 main varieties for quality red wines in Romania - Cabernet Sauvignon, Merlot, Fetească Neagră. Of the three species are obtained quality red wines as a single kind but also various combinations of two or three species. A basis study for this paper represent part of a research program initiated in 2010 that aims to diversify the range of quality red wines produced from Galicea Mare. This study was conducted on wines produced in 2011, of 3 varieties produced individually. After alcoholic fermentation and malolactic fermentation, the wines were stored for 1 year in oak barrels. After this phase were made combinations of two and three varieties, resulting 10 wines studied: 3 samples obtained from the pure species, 3 samples resulting from the combination of 2 wines and 4 wine varieties resulting from the combination of all three species, including a wine in which all varieties are in equal proportion and 3 samples in which two varieties are in equal proportion. The wines were followed by regular tastings to control the development of sensory characteristics. The data presented in this paper refers to the wine tasting at 6 months after the combination was done.

RESULTS AND DISCUSSIONS

The quality of wines produced in 2011 from Galicea Mare, in one or more varieties, was strongly influenced by the specific climatic conditions of extremely warm and dry year. Under these conditions, the grapes have reached very high content in sugars and wines reached unusually high alcoholic strengths of over 15% volume. Also sensory characteristics of the wines were marked by the less common chemical composition (Table 1). The data of chemical composition show that Fetească Neagră wine, even if it had the alcoholic strength of 15.8 % vol., lower than Cabernet Sauvignon (16.4% vol.) and Merlot (16.0 % vol.), had over 18 g/L residual sugar, while Cabernet Sauvignon and Merlot wines were dry wine. This means that the harvest of Fetească Neagră had higher sugar content than the other two varieties.

Table 1

Wine	Alcohol, % vol.	Glycerol, g/L	Total acidity, g/LH ₂ SO ₄	Volatile acidity, g/L acetic acid	Residual sugar, g/L	Dry extract unreduced, g/
Cabernet Sauvignon	16,4	14,2	3,72	0,42	3,2	28,2
Merlot	16,0	14,0	3,80	0,52	3,5	27,6
Fetească neagră	15,8	14,0	3,36	0,48	18,4	27,9

The chemical composition of wines Galicea Mare, 2011

Due to high alcohol content wines showed unusually high content in glycerol, at least 14 g/L, which is very important for the balance of taste and smoothness in taste sensation. Also with regard to the chemical composition of the wine higher values are observed for volatile acidity, of 0.42 to 0.52 g/L CH₃COOH that are determined primarily by the long duration of the alcoholic fermentation, with over 10 days in each variety, due to a very high sugar gravy.

The results of wine tasting (Figure 1) show that the combination of two or three species were obtained wines at least the same level or even higher of those from pure specie. The three wines produced from pure varieties were very well appreciated at tasting,

scoring high marks. The best score from the Fetească Neagră wine, which slightly exceeded the Cabernet Sauvignon and Merlot wines due to its residual sugar content, that alleviated much of the harshness and bitterness of tannins. However, tasters felt that the balance of this wine taste was a little bit affected by the sweet character, which prevented him from obtaining a higher score.

Wines made from combining two varieties in equal proportions, led to better appreciation of the tasters, translated by the slightly increase in scores. The two combinations that contain Fetească Neagră, both with Cabernet Sauvignon and with Merlot were better appreciated from the combination of the two varieties. The residual sugar from the Fetească Neagră variety covered well the harsh of tannins from the wine partner.

Wines made by combining all the 3 varieties have been better appreciated , obtaining the highest scores in tasting, passing 90 points. Of the four wines by combining different proportions of the three varieties , the best two alternatives were considered those in which Fetească Neagră was 25 %, while Cabernet Sauvignon and Merlot are 50 % and 25 % of assemblage. These variants have received slightly higher scores compared to the variant in which all 3 varieties were assembled in equal proportions, succeeding better to attenuate the sweetness and softness of Fetească Neagră wine.



Figure 1 – Wines tasting resultats

On visual examination all were highly appreciated wines with intense and very beautiful color, a good clarity. On olfactory examination the Fetească Neagră wine impressed by the richness and complexity of flavors, which noted in particular a very pleasant smell of dried prunes, very well supported by notes of chocolate and coffee acquired during maturation. For this reason, the tasting, all three olfactory characteristics of the wine was appreciated as excellent. Cabernet Sauvignon and Merlot wines showed also intense and pleasant aroma with dominant notes of berries - especially blueberries and vanilla and cocoa but, unfortunately, the higher alcohol content was underlined by its burning character which reduced the enjoyable character of primary and tertiary aromas. Instead, at the tasting exam this high alcohol content assisted by the glycerol was

the one who gave the wines the fat character, fleshy, full-bodied, well balancing the firm and astringent structure of the tannins. Under these conditions Cabernet Sauvignon and Merlot wines were highly rated at tasting examination by Fetească Neagră wine, where the residual sugar present affected a little bit the balance taste. In these circumstances, in multivarieties wines, the combining of Fetească Neagră variety with any kind of wine gave an improving of taste by reducing sweet and soft character. This improvement was more obvious where the Fetească Neagră wine proportion was lower (25 %).

CONCLUSIONS

Multivarieties wines obtained by assembling various proportions of pure varieties wines is a very interesting and promising solution for the diversification and increasing quality of red wines produced in the viticultural area of Galicea Mare. This study conducted on wines produced in the particular climatic conditions of the year 2011 shows that the best results are obtained by combining the three varieties, where the proportion of Fetească Neagră is 25% or 33% at most. A higher proportion of Fetească Neagră is favorable to reduce the fragrance but the taste balance is decrease because of the high residual sugar content. In terms of wines produced in different climatic years, it remains to verify which combinations of varieties give the best results and also need to continue research on the optimal time to achieve assembly.

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HARNESSING SUSTAINABLE AGRO - ECOLOGICAL POTENTIAL OF SANDY SOILS BY GROWING OF FRUIT TO OBTAIN QUALITY FRUIT PRODUCTION COMPETITIVE MARKET

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Key Words: sandy soils, fruit, quality

ABSTRACT

In order to efficiently concerns sandy soils in the field have objective behavior of fruit tree species with a high potential for adaptability and establishing the main technological links and hence the technology culture of fruit trees under ecopedological low fertile sandy soils to obtain fruit quality competitive on the market. Promote the most valuable crop varieties and hybrids of fruit trees.Research conducted on fruit trees on sandy soils revealed high adaptability and production capacity of wild peach and apricot stone .A core factor for the growth and fruiting of trees grown on sandy soils is the concern to preserve and increase the fertility of these soils poorly fertile.Choosing the range of tree species with high suitability ecopedological conditions of sandy soils , is essential in getting fruit production , safe and stable to better use of these soils .

INTRODUCTION

Recovery sandy soils involves achieving a specific agricultural system, soil fertility caused by the choice of a range of tree species with increased adaptability to climate and soil conditions, resistance to pathogens and pests, efficient use of irrigation water and fertilizer. Research has shown great versatility and production capacity of tree species such as peach and apricot (Cociu et. al., 1981). A core factor for the growth and fructification of these tree species is a concern to preserve and increase the fertility of fertile poor sandy soils (Durău, 2003a and 2003b). The choice of tree species, the establishment of appropriate technological links is essential in getting fruit production, secure and stable (Ivaşcu , 2003).

MATERIAL AND METHODS

The research was conducted at S.C.D.C.P.N. Dabuleni the experimental polygon.

Agrotechnics applied in the experimental plots was developed by SCDCPN Dabuleni for each species. The soil on which were placed protosol experience is a typical anthropogenic erodisol covering a clay soil is poor iluviar lameler freatic. Fertility, humus content ranging from 0.18 to 0.58 %, the total nitrogen 0.021 to 0.032 %, P 12-32 ppm, K -AL 27 to 31.9 ppm.

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RESULTS AND DISCUSSIONS

From the assortment of peach varieties and hybrids in experimental field study on sandy soils in the , a total of six varieties of fruit obtained , where the values were different depending on the variety. The most productive variety has proved Redhaven cultivar with 12.7 t / ha, yields other varieties obtaining statistical uninsured (Table 1).

In order to know the prospects for extending the crown forms used in the culture of peach and nectarine varieties of behavior, driven crown - shaped low volume at SCDCPN Dăbuleni was organized experience with three varieties of nectarine Cora, Delta, Romamer, and 5 forms the crown on five planting distances as follows:

- palmetto distance 5.0 to 3.5 m = 571 trees / ha
- vas Verona distance pot 5.0 / 3.0 = 666 trees / ha
- palprinder distance from 5.0 to 3.0 m = 666 trees / ha
- tatura trellis distance 5.0 to 2.0 = 1000 trees / ha
- vertical cord distance belt 5.0 / 1.5 m =1333 trees / ha .

For a better assessment of cumulative production was calculated for 2001-2004 as certain the following: all varieties led tatura as treellis give best production results or 6.15 t / ha variety Cora, 5.85 t / ha Delta variety, 5, 55 t / ha variety Romamer (Table 2).

Vertical cord is best for providing a variety Delta cumulative production of 4.1 t / ha.

Among the technological factors which contribute to achieving high yields and quality peaches are grown on sandy soils: the soil maintenance and fertilization. The results obtained on sandy soils production was found that the yields of the fruits were obtained in the dark field maintenance. The analysis of the statistical calculation fertilization variants within black field maintenance system found that only unfertilized to fertilized with N100 P80 K100 achieved a production difference 6.26 t / ha provided statistically significantly positive (Table 3).

Table 1

Variety	Production, t / ha	Production relative, %	The difference compared to the control, t / ha	Significance
Redhaven	12,7	100,00	-	mt
Harvester	4,1	32,67	- 8,5	
Harbinger	4,7	37,40	- 7,9	
N. J. 265	2,3	18,50	- 10,3	00
Springold	4,9	38,58	- 7,8	
Harken	6,0	47,63	- 6,6	
DL 5%			9,59	
DL 1%			15,04	
DL 0,1%			25,6	

Fruit production of peach varieties grown on sandy soils in Dabuleni

Using technology foliar peach crop on sandy soils is a very important operation leading to improved processes of growth and fructification. After applying foliar fertilization was found that the highest yields of fruit were obtained in variants fertilized with Azofil 411 Fertiltel, Agroleaf where differences in production to control are statistically very significant and positive significantly distinct (Table 4).

The most productive apricot variety has proved CR2 - 82 with 6.3 t/ha where production growth to control variety was provided statistically significantly positive (Table 5).

Variety	Crown shape and distance of planting	Production, t / ha	Production relative, %	The difference compared to the control t / ha	Significance
	Palmetto - 5/3,5	3,05	100,00	-	mt
	Vas verona - 5/3	4,25	139,34	+ 1,20	
	Palprinder - 5/3	3,05	100,00	+0,00	
Cora	Tatura trellis - 5/2	6,15	201,63	+3,10	*
Cola	Vertical cord 5/1,5	4,00	131,14	+0,95	
	DL 5%			2,51	
	DL 1%			4,15	
	DL 0,1 %			7,77	
	Palmetto - 5/3,5	2,90	100,00	-	
	Vas –veronez - 5/3	3,70	129,31	+0,85	
	Palprinder - 5/3	3,50	120,69	+0,60	
Delta	Tatura trellis - 5/2	5,85	201,72	+ 2,95	**
	Vertical cord - 5/1,5	4,10	141,37	+ 1,20	*
	DL5%			1,05	
	DL1%			1,74	
	DL0,1 %			3,26	
	Palmetto - 5/3,5	2,65	100,00	-	mt
	Vas- veronez - 5/3	3.55	133,96	+0,90	
	Palprinder - 5/3	3,80	143,96	+ 1,15	
Domemor	Tatura trellis 5/2	5,55	209,43	+2.90	*
Romamer	Vertical cord - 5/1,5	4,70	177,35	+ 2,05	
	DL 5%			2,30	
	DL 1%			3,83	
	DL 0,1 %			7,18	

Nectarines fruit production according to the shape of the crown and planting distance

Table 3

Fruit production of peach variety jerseyland according to the system for maintenance and fertilization

The soil' maintenance	The dose of chemical fertilizer	Production, t / ha	Production relative, %	The difference compared to the control, t / ha	Significance
	N0 P0 K0	5,76	100,00	-	mt
Dlook field	N50 P0 K0	6,63	115,02	+0,86	
Diack field	N50 P40K50	8,56	148,55	+ 2.80	**
	N100 P80 K100	12,03	208,67	+ 6,26	***
	N0 P0 K0	5,60	100,00	-	mt
Natural	N50 P0 K0	6,33	113,09	+ 0,73	
revegetation	N50 P40 K50	7,16	127,97	+ 1,56	
	N100 P80 K100	9,70	173,21	+4,10	**
	DL 5%			1,9	
	DL 1%			2,8	
	DL 0,1%			4,5	

Fruit production of peach variety jerseyland depending different versions of foliar fertilization

Foliar fertilization	ertilization Production, Prod		The difference compared	Significan
variants	t / ha	relative, %	to the control, t / ha	ce
Mt.(sprayed with water)	5,76	100,00	-	mt
Azofil 411	10,00	162,16	+ 3,83	***
Fertiltel	9,00	145,94	+ 2,83	**
Agroleaf	8,60	139,45	+ 2,43	**
Universol violet	7,96	129,18	+ 1,80	*
DL 5%			1,3	
DL 1%			2,3	
DL 0,1%			2,9	

Table 5

Fruit production apricot varieties on sandy soils									
Variety or	Production,	Production	The difference compared to	Significance					
hybrid	t / ha	relative, %	the control, t / ha						
N.J.A. 42	4,5	100.0	-	MT					
TUDOR	1,9	42,6	- 2,6	00					
N.J.A. 32	4,4	97,5	- 1,1						
C.R 2 - 62	6,3	140,2	+ 1,8						
N.J.A. 19	5,2	115,8	+0,7						
DL 5%			1,4						
DL 1%			2,0						
DL 0,1%			3,1						

CONCLUSIONS

It can be concluded that under the peach and apricot sandy soils give the best fruit production .The best way to crown the nectarine is tatura trellis .

The best system is field soil maintenance and fertilization black N100 P80 K 100 kg S.A./ha. The best variant of foliar fertilization is with a production Azofil 411 10.0 t/ha.

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THE EVALUATION OF THE CHEMICAL COMPOSITION OF THE JUGLANS REGIA FRUIT WITH THE SELECTIONS FROM THE NORTHERN AREA OF OLTENIA

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Keywords: walnut, populations, selections, Juglans regia.

ABSTRACT

The Walnut is a pomological plant known from back in the old times, is a traditional fruit, important from an economical and social point of view.

In Romania, the walnut has been cultivated from back in the old times, its fruit being complete food. The studied selections are part of the Walnut populations in North Oltenia.

Besides other reseaches, analyses were also made in what concerns the chemical composition of the walnut fruits of these selections.

The studies were carried out under natural environmental conditions. The Walnuts form individual populations which are, from a genetic point of view, natural hybrids grown on their own roots and with an irregular fruit production.

INTRODUCTION

Romania is one of the European countries with temperate climate which has relevant walnut production. The region of Oltenia is located in the SW of Romania and has 15% of the walnut trees. The walnut trees are distributed all over the country being located into organized orchards or as individual trees. Important differences were observed regarding the behaviour to bacterial blight attack Xanhmonas campestris pv. Juglandis. Besides the phenotypical characterisation of the walnut, the evaluation of the biochemical composition of the Juglans regia fruit was also realised. In this period, observations have been carried out in the walnut populations from the Northern area of Oltenia. This area of Oltenia contains 3 counties: Vâlcea, Gorj and Mehedinți; there, the walnut trees are widely spread (over 250 thousands plants). The growth vigor of the walnut trees is large or very large in all the populations studied. Low vigorous walnut trees were not found in the area.

MATERIALS AND METHODS

The researches that were made in North Oltenia, were following up the behavior of 56 selections. The selections are hybrids cultivated on their own roots. The trees have

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between 30 and 50 years old. The investigation was carried out in the Northern area of Oltenia, in different localities from the following counties: Gorj, Vâlcea and Mehedinți.

The measurements, observation and determination were referring to the bearing characteristics (type of bearing yield, fruit characteristics), growth (habitus), and low temperatures during winter and behavior to diseases.

The observations were made under the conditions of natural infection in field and followed the evolution of the pathogen (XC) agent on leaves and fruit.

The Evaluation of the Biochemical Composition of the Fruit:

- dry substance (%);
- extractible protein (%);
- raw fat (%);
- reducer carbohydrates (%);
- total carbohydrates (%);
- humidity (%).

The climatic conditions of the area are generally favorable to the growth and bearing of the walnut. The average annual temperature ranges from 10,2°C to 10.4°C; the lowest temperature was -30°C, but normally the temperature ranges from -20°C to -22°C; the average sum of rainfall is of 700-750 mm; the relative humidity is of 64-85% and the duration of sunshine is of 1900 hours per year.

RESULTS AND DISCUSSIONS

The trees used in the research are generatively obtained and planted in familial gardens and orchards, near footways (road plantations) and isolated. A strict genetic evidentiation and delimitation with the maintaining of integrity between some limits of entire population is difficult. The characteristic of this population is represented by the large variability of the growth characteristics, disease tolerance or resistance, resistance to low temperatures, which are present at the individuals that compose the population. The studied biological material is made of 56 representative selections, selected from over 31 localities in North Oltenia

Analyzing the biochemical components of the kernel of the walnut selections in North Oltenia, a variability for the main components was observed: dry substance (%), extractible protein (%), raw fat (%), reducer carbohydrates (%), humidity (%) (Analyses carried out by the Alimentary Research Institute in Bucharest).

Following the biochemical analyses carried out on the kernel of the walnut selections in Gorj, the below values were obtained(table no 1):

- dry substance (%): 70,5% (Bumbeşti-Pițic S17) - 81,7% (Baia de Fier S13);

- extractible protein (%): 5,0% (Novaci S35) - 9,45% (Baia de Fier S11);

- raw fat (%): 40,2% (Bălănești S20) - 57,5% (Alimpești S3);

- reducer carbohydrates (%): 0,6% (Polovragi S39) - 1,2% (Alimpești S3, Novaci S33, Novaci S35, Scoarța S50);

- total carbohydrates (%): 1,5% (Alimpeşti S3, Bălăneşti S20, Leleşti S29, Polovragi S43, Schela S47, Scoarța S50) - 2,2% (Runcu S45);

- humidity (%): 18,5% (Baia de Fier S13) - 29,5% (Bumbeşti-Pițic S17)

Population	Selection	Dry substance	Extractible protein	Raw fat	Reducer carbohydrates	Total carbohydrates	Humidit
		%	%	%	%	%	У
							%
Alimpești	S3	73.7	6.65	57.5	1.2	1.5	26.3
	S7	74.8	5.07	46.43	1.1	1.6	25.2
	S9	75.8	5.33	42.28	0.96	1.9	24.2
Baia de Fier	S11	80.6	9.45	51.4	08	2.1	19.6
	S13	81.5	7.40	54.1	0.7	1.9	18.5
	S15	80.5	9.43	51.3	0.8	1.8	19.5
Bumbești Pițic	S17	70.5	7.28	53.0	1.02	2.0	29.5
Bumbești Jiu	S18	73.5	6.3	46.4	1.1	1.7	26.5
Balanesti	S20	76.1	5.97	40.2	0.84	1.5	23.8
Bengești	S21	71.8	5.8	45.7	0.86	1.7	28.2
Ciocadia							
Crasna	S23	72.6	6.6	51.2	0.7	1.9	27.4
	S25	77.3	6.38	50.7	0.9	2.1	22.7
	S27	74.8	6.8	48.1	1.1	1.7	25.2
Lelești	S29	73.6	6.7	47.5	0.7	1.5	26.4
Novaci	S31	75.5	6.4	48.1	0.7	1.7	24.5
	S33	76.1	6.2	51.2	1.2	1.8	23.8
	S35	74.8	5.0	46.5	1.2	1.9	25.2
Muşeteşti	S37	75.7	7.2	47.5	0.9	2.1	24.3
Polovragi	S39	80.1	9.3	50.5	0.5	2.1	19.2
	S41	80.5	9.3	50.5	0.7	1.7	19.5
	S43	80.3	9.1	53.1	0.8	1.5	19.7
Runcu	S45	72.5	6.6	47.2	1.1	2.2	27.5
Schela	S47	74.6	5.1	45.8	1.1	1.5	25.4
Săcelu	S49	71.7	5.8	45.9	0.9	1.6	28.3
Scoarța	S50	76.4	6.7	47.7	1.2	1.5	27.6

Biochemical Analyses of the Kernel of the Walnut Selections (Gorj)

In Vâlcea, following the biochemical analyses carried out on the kernel of the walnut selections, the below values were obtained(table no 2):

- dry substance: 72,9% (Măldărești S61, Horezu S73) - 81,5% (Stroiești S57);

- extractible protein : 5,7% (Costești S67) - 9,5% (Costești S69, Mateești S83);

- raw fat (%): 47,0% (Slătioara S80) - 55,5% (Stroiești S55, Măldărești S61, Horezu S73);

- reducer carbohydrates (%): 0,72% (Mateești S83) - 1,2% (Stroiești S55, Măldărești S51, Horezu S73);

- total carbohydrates (%): 1,1% (Măldăreşti S63) - 2,4% (Vaideeni S65);

- humidity (%): 18,5% (Stroiești S57) - 26,2% (Costești S57).

The biochemical analyses carried out on the kernel of the walnut selections in Mehedinți(table no 3):

- dry substance: 72,3% (Ilovița S113) - 81,5% (Ponoarele S95);

- extractible protein: 5,4% (Florești S111, Bala S101) - 9,4% (Bala S105);

- raw fat (%): 42,6% (Podeni S97) - 55,4% (Ilovița S113);

reducer carbohydrates (%): 0,6% (Bala S105) - 1,2% (Ponoarele S90, Ponoarele S93, Butoiești S119);

- total carbohydrates (%): 1,5% (Butoiești S109) - 2,5% (Isverna S89);

- umidity (%): 18,5% (Ponoarele S95) - 27,2% (Isverna S85).

Table 2

Biochemical Analyses of the Kernel of the Walnut Selections (Vâlcea)

Population	Selection	Dry	Extractible	Raw	Reducer	Total	Humidity
_		substance	protein	fat	carbohydrates	carbohydrates	%
		%	%	%	%	%	
Stroiești	S53	76.1	8.1	51.6	0.8	1.8	23.9
	S55	72.9	7.3	55.5	1.2	2.1	27.1
	S57	81.5	7.5	55.2	0.73	1.9	18.5
Berbești	S59	77.1	6.2	51.7	1.1	1.7	22.9
Măldărești	S61	72.9	7.3	55.5	1.2	2.2	27.1
	S63	73.8	6.7	53.1	0.9	1.1	26.2
Vaideeni	S65	80.1	7.8	47.9	1.1	2.4	19.9
Costești	S67	73.8	5.7	47.8	0.9	2.1	26.2
, í	S69	80.7	9.5	51.4	0.8	2.1	19.3
	S70	76.2	5.9	46.1	0.8	1.5	23.8
Horezu	S71	75.4	6.6	47.8	0.9	2.1	24.6
	S73	72.9	7.3	55.5	1.2	2.2	27.1
	S75	81.4	7.4	55.2	0.74	2.0	18.6
Slătioara	S77	77.1	6.6	51.8	1.2	1.8	22.9
	S 80	80.1	7.8	47.	1.1	2.4	19.9
Mateești	S83	80.1	9.5	51.4	0.2	2.1	19.9

Population	Selection	Dry	Extractible	Raw	Reducer	Total	Humidity
1		substance	protein	fat	carbohydrates	carbohydrates	%
		%	%	%	%	%	
Isverna	S85	72.8	7.2	55.3	0.84	1.6	27.2
	S87	75.4	5.7	43.5	1.1	2.1	24.6
	S89	80.1	7.3	47.8	1.1	2.5	19.9
	6 00						
Ponoarele	S90	76.1	5.5	51.2	1.2	1.6	23.9
	S93	73.8	6.7	54.5	1.2	1.5	26.2
	\$95	81.5	7.4	53.8	0.75	1.9	18.5
Podeni	S97	75.8	5.3	4.1	0.84	1.8	24.2
Bala	\$101	74.8	5.1	47.8	11	17	25.2
Dala	S101	75.4	5.2	44.7	0.9	2.1	24.6
	S105	80.6	9.4	51.3	0.6	2.1	19.4
	~~~~						
Butoiești	S107	76.1	5.8	51.3	0.8	1.7	23.9
,	S109	73.8	6.7	54.5	1.2	1.5	26.2
Florești	S111	74.8	5.1	46.5	1.1	1.8	25.2
Ilovița	S113	72.3	7.3	55.4	0.8	1.6	27.1
Husnicioara	S115	72.4	6.8	50.1	0.9	1.9	19.3

Biochemical Analyses of the Kernel of the Walnut Selections (Mehedinți)

# CONCLUSIONS

The whole population has individuals (natural hybrids) which are characterized by a large genetic variability and this aspect has a great importance in the breeding programs.

The North of Oltenia has favorable climatic conditions and permits the forming and evolution, in time, of a natural population of Juglans regia (more than 250.000 plants).

In what concerns the chemical composition of the walnut selections, they had the following values:

- dry substance : 70,5% (Bumbești-Pițic S17) and 81,7% (Baia de Fier S13);

- extractible protein: 5,05% (Novaci S35) and 9,5% (Costești S69 and Mușetești

- raw fat : 40,2% (Bălănești S20) and 57,5% (Alimpești S3);

S83);

- reducer carbohydrates: 0,6% (Polovragi S39, Bala S105) and 1,2% (Alimpeşti S3, Stroieşti S65, Ponoarele S93, Butoieşti S119);

- total carbohydrates: 1,1% (Măldărești S63) and 2,5% (Isverna S89);

-umidity: 8,5% (Baia de Fier S13, Stroiești S57, Ponoarele S95) and 29,55% (Bumbești-Pițic S17).

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# STUDY REGARDING THE INFLUENCE OF IMPROVERS ON QUALITY OF BREAD OBTAINED THROUGH MODERN METHODS

Gabriela Constantinescu (Pop)¹

Keywords: Alveograph curves, technological properties, bakery products

### ABSTRACT

The use of frozen dough's allows easier and more profitable baking and bread can be made available around the clock, reducing labor and production costs while facilitating transportation. However, the quality of bread prepared from frozen dough is often inferior to freshly baked breads. The specific volume of the final bread is reduced, the texture of the bread deteriorates, and starch retrogradation proceeds faster, causing an increase in bread firmness. To eliminate these problems, several additives have been used to improve the baking quality and extend the shelf life of bakery products made from frozen dough. This study reveals the major importance of using an improver for the quality of finished products, bread made from frozen dough respectively. The advantages of using this improver have been identified through various analyses both qualitative and sensory and physico-chemical ones made in the laboratory of the Faculty of Food Engineering.

# INTRODUCTION

Bread is one of the most widely consumed food products in the world and bread making technology is probably one of the oldest technologies known. This technology has evolved continuously over the years as new materials, ingredients and equipments have been introduced to produce better quality bread while research has generated steady and impressive progress in bread making.

Fresh bakery products, however, have a relatively short shelf life since during their storage, a number of physical and chemical changes occur, a process known as staling. These preservation problems coupled with the complex processes involved in conventional bread making and increasing market demands, have led to the continuous search for efficient methods to produce superior bakery products while preventing undesirable changes and extending the shelf life.

The quality of bread made from frozen dough is influenced by dough formulation, as well as by the process parameters such as dough mixing time (Rouille et. al., 2000), freezing rate, storage duration, and thawing rate (Inoue &.Bushuk, 1991; Lu &.Grant, 1999). These factors may either act independently or synergistically to reduce yeast

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activity which results in reduced carbon dioxide (CO₂) production or to damage the gluten network which in turn results in poor CO₂ retention and poor baking performance (Keny et al., 1999).

The inclusion of improvers in bread formulations may overcome these problems associated with frozen dough. Numerous researches have been focused on the development and application of different additives for improving the baking quality and extending the shelf life of bread products produced from frozen dough by retarding the staling process in stored bread. Different emulsifiers have been tested as anti-staling agents and bread improvers in wheat bread. When improvers/dough strengthening agents are used, the dough matrix is strengthened and hence a higher gas pressure (gassing power of yeast) is necessary to produce an increment in the loaf volume.

This paper presents research on the effects of freezing technology on the microstructure and baking performance of frozen dough's, and provides an overview of the activities of dough improvers used in frozen dough applications.

### MATERIALS AND METHODS

In order to obtain some available experimental data, wheat flour obtained from DROPIA wheat variety ground in Chopin Laboratory Mill was used as control sample.

The analytical flours' quality obtained (table 1) was determined according with the international standard methods (ash content - ICC104/1, wet gluten - ICC105/2, protein content - ICC106/2, hydration capacity with Pharinograph - ICC115/1). The moisture content of the wheat flour and bran were determined by oven drying at  $130^{\circ}$ C for 1 hour.

Table 1

Analytical parameters of Control flour					
Moisture, % Ash, % Wet gluten, % Protein, % Hydration capacity, 9					
12.96	1.2274	27.2	12,3	78.3	

. . . .

Compacted fresh yeast (*Saccharomyces cerevisiae*) provided by S.C. ROMPAK, Pascani, with 32.5% dry matter and 46.54% protein content (N x 6.25), was used as raw material.

A Chopin Alveoconsistograph was used to determine the deformation resistance (tenacity) P, dough extensibility, L, the value of P/L, and the mixing energy W according with the international standard SR ISO 5530 - 4.

An improver for frozen dough, "*Techni cru surgel*", provided by Enzymes & Derivates S.A. Romania, containing a mixture of ascorbic acid, emulsifier and vital gluten, was also used.

Dough was prepared using a double phase dough method with liquid sponge.

In order to be frozen, after kneading, dough was divided into smaller pieces, which were slightly round shaped by hands. All the samples were placed in pans, wrapped with plastic and placed in the freezer at  $-25\pm3^{\circ}$ C. Optimum thawing-proofing conditions were established based on other studies (Takano, Naito, Ishida, Koizumi & Kano, 2002).

After baking, the samples were cooled for 6-8 hours in controlled atmosphere (UV lamps). Then, in order to establish the specific volume, the samples were weighed and estimated with rape seeds displacement and expressed as the ratio of bread volume to dough weight.

The experiments were made in the research laboratory of the Faculty of Food Engineering, "Stefan cel Mare" University of Suceava.

# **RESULTS AND DISCUSSION**

In order to evaluate the influence of the various amounts of improvers on the technological characteristics of flour the following samples were made:

Witness sample - only control flour;

P1 control flour with 0,4 % "techni cru surgel";

P2 control flour with 0,6 % "techni cru surgel"

P3 control flour with 0,8 % "techni cru surgel"

After establishing the flour mixtures, they were subjected to laboratory analyses: hydration capacity, wet gluten content and acidity; the last two were studied comparatively to see the difference in the behavior of flour mixtures (table 2).

# Table 2

The technological properties of flour mixing samples

Characteristic	Flour mixing samples					
	Control	P1 (0.4%)	P2 (0.6%)	P3 (0.8%)		
Acidity, acidity grades/100 g	1.3	1.4	1,5	1,68		
Wet Gluten, [%]	28	28.15	28.29	28.41		
Hydration Capacity, [%]	62.6	63.11	63.79	64.25		

The results of these tests led to the following conclusions:

- Samples' acidity increases with increasing the addition of improver in flour;

- When the content is high the flour hydration capacity is greater;

- Wet gluten content increase depending on the proportion of exogenous amount of improver in the sample.

In order to make possible correlations between the physical-chemical characteristics and the technological behavior of the flours obtained, we studied the rheological parameters of the four samples (figure 1).

Research showed that a number of significant correlations, between physicochemical parameters of flour and dough alveographic parameters were established, suggesting that it is possible to achieve a predictive model of alveographic characteristics based on the physico-chemical properties of flour.

The control sample and P1 (0,4%) samples had very good rheological properties: a W (kneading energy) between 123-241 E -4J, which results in obtaining high quality flour, with very good tenacity and extensibility and could be used to manufacture a quality bread.

P2 (0,6%) had good rheological properties (with a W like118-136 E -4J) which led to the obtaining of good bakery products.

P3 (0,8%) had weaker rheological properties and could be used only for simple biscuits or cakes and cake tops (fluid doughs).

Three bread samples were obtained in the laboratory, namely 0,4%, 0,6%, 0,8% improvers were added in relation to wheat flour used in the formulation.

The finished product was analyzed in terms of sensory (figure 2) and physicochemical properties (table 3).



Figure 1 Alveograph curves of flour mixture samples

The results of these tests are shown in the table above where one can notice that the acidity is low enough , the core porosity ranges between 25% and 35%.

Ta	ble	3

The physico-enemical analyses of the packed bread samples							
		Bread samples					
Characteristic	Control	P1 (0,4%)	P2 (0,6%)	P1 (0,8%)			
Height/ Dimension ratio	0,64	0,68	0,65	0,60			
Volume, cm ³ /100g	295	307	300	297			
Porosity, [%]	79	83	82	81			
Elasticity, [%]	79.3	92.2	91.6	91.8			
Acidity, acidity grades/100 g	0,6	0,9	1,4	1,6			
Humidity, [%]	69,46	70,73	78,78	79,02			

The physico-chemical analyses of the packed bread samples

Sample P1, bread made with 0,4% improver is the bread with the best organoleptic and physico-chemical properties. This bread sample has a normal appearance, glossy shell, corresponding smell and sweet taste as well. The core is uniform with a very good

elasticity, optimal porosity and acidity , a normal bread which can be considered a very good one.



Figure 2 Bread samples

The general conclusions which can be drawn from the experimental data obtained are the following: the frozen dough samples obtained whith exogenous addition of *techni cru surgel* showed an increase in quality, in terms of volume, porosity and elasticity.

**CONCLUSIONS** 

Several samples with different proportions of improvers added in frozen doughs were made and these samples were compared with a blank one (a sample made from wheat flour provided by DROPIA wheat variety ground in Chopin Laboratory Mill).

The results show that the improver addition leads to increase in acidity and flour hydration capacity.

Wet gluten content increased depending on the proportion of *techni cru surgel* improver added in the sample, the lowest value being registered by the control sample.

The bread samples obtained from frozen dough were studied from the point of view of the core porosity and elasticity, and the acidity occuring in the technological process was checked as well.

The results show that the bread made with 0,4% improver (*,,techni cru surgel* ,,) is the one having the best organoleptic and physico-chemical properties. It had normal appearance, glossy shell, corresponding smell and sweet taste.

The core is uniform with a very good elasticity, optimal porosity and acidity, the same as normal bread, therefore it can be considered a very high quality one.

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# DESCRIPTION OF SOME OLD VARIETIES OF GRAPE VINES FROM DRĂGĂȘANI VINEYARD- ROMANIA

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Keywords: descriptors, old varieties, germplasm, vineyard, Vitis vinifera

### ABSTRACT

The study was conducted between 2010 - 2012 at the grape vine varieties (Vitis vinifera ssp. sativa), 'Cârlogancă', 'Gordan', 'Braghină albă' and 'Berbecel' through OIV, UPOV, Bioversity – IBPGR type descriptors, for a better knowledge and a wider germplasm, the purpose being their promotion and conservation on farm, in situ and ex situ. In the next step, valuable clones can be selected from the studied varieties. This study was aimed on four varieties which are in decline or endangered, prephylloxera varieties such as 'Cârlogancă', 'Gordan', 'Braghină albă, (basic varieties of the old traditional assortment of Drăgăşani vineyard), and 'Berbecel variety. The conservation of these old varieties of vines, on farm , in situ and ex situ are important measures in the international viticulture context and current approaches (Gorjan, 2012).

### **INTRODUCTION**

In the current viticulture conditions, when the number of current varieties is situated around 5.000, regardless the interspecific hybrids and the synonymies (at least 40.000), the necessity of this concern is justifiable even more. The large number of varieties, the polymorphism shown by the vines in culture, the number of used characters, their complexity and non taking into consideration of the agrobiological, agrophytotechnical and technological characteristics, determined in practice to acknowledging varieties, the numerous confusions and errors. Therefore, in describing varieties, et becomes necessary the establishment of a common global language of a description code, which would act, defining the used botanical descriptors for a better international understanding (Olteanu et al., 2002). The description of vine varieties was and will be a priority in international viticulture. After the complete description of the existing genetically resources on farm, it can be taken into consideration their preservation methods both in situ, and also ex situ, in ampelographic collections. The advantage offered by these methods is that it can continue the evolution processes through specific forms of genetically diversities, and the populations' adaptability (Frankel & Soule, 1981). The efforts of measuring the diversities of local varieties within the European production systems have shown that local varieties in culture are not only complex but also extremely diversified in their genetic structure, dynamic and in a continuous evolution, features which are now recognized in developing the politics in order to support their maintenance (Devra Jarvis, 2009).

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### MATERIAL AND METHODS

The observations and measurements were made in the private plantations at the ancient vine varieties, 'Cârlogancă', 'Gordan', 'Braghină albă' and 'Berbecel' variety. For this study, it has been used OIV, UPOV, Bioversity– IBPGR ampelographic descriptors from the list of descriptors *varieties* and *Vitis* species, 2009 which comes with a wider description of these four varieties' characters, assuming a better knowledge of germplasm. It has been analyzed a number of 27 typical characters of *Vitis vinifera* ssp. *sativa* specie.

The studied ampelographic descriptors are morphological descriptors (the young shoot, the shoot, mature leaf, the woody shoot, the flower, the bunch, the berry); phenological descriptors (time of bud burst-stage C, time of full bloom, time of beginning of berry ripening (veraison), time of full physiological maturity of the berry, time of beginning of wood maturity, the autumn coloration of leaves); agrobiological descriptors (the vigor of shoot growth, growth of lateral shoots); physiological descriptors (resistance to diseases); technological descriptors (the sugar content of must, the total acidity of the must).

### **RESULTS AND DISCUSSIONS**

The locations of the varieties studied in Drăgăşani vineyard are: 'Cârlogancă' variety, marked vineyard Guşoieni-Spârleni 44°42'18 North, 24°07'56 East, 224 m altitude. 'Gordan' variety, marked vineyard Suteşti-Pietroasa 44°40'49 North, 24°12'09 East, 218 m alt. 'Braghină albă' variety, marked vineyard Suteşti-Pietroasa 44°40'47 North, 24°12'06 East, 217 m alt. 'Berbecel' variety, marked vineyard Drăgăşani-Dealul Olt 44°38'50 North, 24°15'50 East, 161 m alt. In tables 1 and 2 we have presented all four varieties, according to the descriptors accredited by the international agencies in the domain. These varieties belong to *Vitis vinifera* ssp. *sativa* specie. It has also been established that certain hubs from 'Cârlogancă' and 'Braghina albă' varieties accumulate a whole lot of sugar towards previous years due to global warming, from these being able to select new clones. 'Gordan', 'Berbecel' varieties have presented resistance to diseases and pests in the Drăgăşani vineyard conditions.

From this study we observe that "Cârlogancă' variety is highlighted at the morphological descriptors, opening of the young shoot tip is half open, attitude (before tying) of the shoot is semi-erect, the number of consecutive tendrils are 2 or less. The mature leaf is large, pentagonal with 3-5 lobes. The cross section of the woody shoot is eliptic and the main colour is yellow-brownish. Flower is fully developed stamens and fully developed gynoecium. The shape of the bunch is cylindrical-conical, and the berry's shape is globose. The phenological characteristics are medium, the autumn coloration of leaves being yellow. The agrobiological characteristics are strong, in terms of the vigor of shoot growth and growth of lateral shoots. Is resistant to diseases and pests. The technological characteristics are generally medium, from the study shown it has been pointed out that 'Cârlogancă' variety accumulates at full maturity 205 g / 1 sugar (Table 1).

'Gordan' variety presents distinctive characters regarding resistance to diseases, the morphological characters are obvious for *Vitis vinifera* ssp. *sativa*, concerning the young shoot, mature leaf, woody shoot. The flower is fully developed stamens and fully developed gynoecium. The agrobiological and phenological characteristics are specific to the variety. The technological characters are generally low (Table 1).

'Braghina albă' variety presents distinctive characters regarding the morphological, agrobiological, phenological, physiological and technological qualities (Table 2).

'Berbecel' variety presents the following morphologically important characteristics: the opening of the young shoot tip is fully open, attitude (before tying) of

the shoot is semi-erect. The size of blade of mature leaf is large, pentagonal with 3-5 lobes. The cross section of the woody shoot is circular and its main colour is brownish. The flower is fully developed stamens and fully developed gynoecium. The bunch's shape is cylindrical-conical and the berry's shape is globose. The phenological and agrobiological characteristics are medium. The physiological characteristics are very good, being a variety resistant to diseases and pests in Drăgășani vineyard conditions (Table 2). We generally observe similar characteristics to the other studied varieties, characters of *Vitis vinifera* ssp. *sativa* specie.

# CONCLUSIONS

Romania offers very good conditions for cultivating these old, also new varieties and it can provide genetically material for international viticulture. In the future, preserving these *on farm*, *in situ* and *ex situ* varieties it follows, in ampelographic collections, with the purpose of using them in programs of genetically improvement, and in commerce. Based on the descriptions accredited by the international agencies in the domain, these *Vitis vinifera* ssp. *sativa* specie could be better known, and through the selection of valuable elites, new clones could be promoted.

### ACKNOWLEDGMENT

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*** Code des caractères descriptifs de *variétés* et *espéces* de *Vitis*, 2009, 2 – eme édition de la liste des descripteurs OIV Paris, Franța.

# The main characters of the varieties studied

Table 1

					10010 1	
	Cârlogancă		Gordan		Codes	
	Location Drăgășani vineyard-viticultural		Location Drăgășani vineyard	OIV,UPOV,		
Verieter	roads smothered Guşoieni-Spârleni -		smothered	smothered		
variety	Romania		Sutești-Pietroasa – I	Romania	IBPGR	
	44°42'18 N, 24°07'56 E, 22	24Alt)	(44°40'49 N, 24°12'09	E, 218 Alt)		
Characteristics	Expression	Notes	Expression	Notes		
	character		character			
0	1	2	3	4	5	
Morphological descriptors:						
Young shoot:						
Young shoot: opening of the shoot tip	fully open	5	fully open	5	001; 2; 6.1.1	
Shoot:						
Shoot: attitude (before tying)	semi-erect	3	semi-erect	3	006; 9; 6.1.5	
Shoot: number of consecutive tendrils	2 or less	1	2 or less	1	016, -; 6.1.14	
Mature leaf:						
Mature leaf: size of blade	large	7	large	7	065;17;6.1.21	
Mature leaf: shape of blade	pentagonal	3	pentagonal	3	067;18;6.1.22	
Mature leaf: number of lobes	3-5	2/3	one (entire leaf)-3	1/2	068; 20; 6.1.23	
Woody shoot:						
Woody shoot: cross section	eliptic	2	circular	1	101, -, -	
Woody shoot: main color	yellow- brownish	1/2	yellow- brownish	1/2	103, 44, 6.1.42	
Flower:						
Flower: sexual organs	fully developed stamens and	3	fully developed stamens	3	151: 16: 6.2.1	
č	fully developed gynoecium		and fully developed			
			gynoecium			
Bunch:						
Bunch: shape	cylindrical -conical	1/2	cylindrical -conical	1/2	208; -; -	
Berry:						
Berry: shape	globose	2	globose	2	223; 36; 6.2.6	
Phenological characteristics:			Ť			
Time of bud burst	medium	5	medium	5	301; 1; 7.1.1	
Time of full bloom	medium	5	medium	5	302; -; 6.2.21	
Time of beginning of berry ripening (veraison)	medium	5	medium	5	303; 31; 7.1.4	
	1.	_	1.		204 7110	
11me of full physiological maturity of the berry	medium	5	medium	5	504, -; 7.1.10	
	1	1		1	1	

0	1	2	3	4	5
Time of beginning of wood maturity	medium	5	medium	5	305; -; -
Autumn coloration of leaves	yellow	1	yellow reddish	1/2	306; -; -
Agrobiological characteristics:					
Vigor of shoot growth	strong	7	strong	7	351; -; -
Growth of lateral shoots	strong	7	medium	5	352; -; 7.1.11
Physiological characteristics:					
Leaf:degree of resistance to Plasmopara	high	7	high	7	452; -; 9.2.3
Cluster: degree of resistance to Plasmopara	high	7	high	7	453; -; 9.2.4
Leaf: degree of resistance to Oidium	high	7	high	7	455; -; 9.2.5
Cluster: degree of resistance to Oidium	high	7	high	7	456; -; 9.2.6
Leaf: degree of resistance to Botrytis	high	7	high	7	458; -; 9.2.1
Cluster: degree of resistance to Botrytis	high	7	high	7	459; -; -9.2.2
Technological characteristics:					
Sugar content of must	medium	5	low	3	505; -; 7.1.17
Total acidity of must	low	3	low	3	506, -; 7.1.18



Figure 1 'Cârlogangă' Bunch



Figure 4 'Gordan' Bunch



Figure 7 'Braghină albă' Bunch



Figure 10 'Berbecel' Bunch



Figure 2 'Cârloangă' Leaf the upper part



Figure 5 'Gordan' Leaf the upper part



Figure 8 'Braghină albă' Leaf the upper part



Figure 11 'Berbecel' Leaf the upper part



Figure 3 'Cârloangă' Leaf the lower part



Figure 6 'Gordan' Leaf the lower part



Figure 9 'Braghină albă' Leaf the lower part



Figure 12 'Berbecel' Leaf the lower part

					Table 2
Variety	Braghină albă Location Drăgășani vineyard- viticultural roads smothered Sutești-Pietroasa – Romania (44°40'47 N. 24°12'06 F. 217 Alt)		Berbecel Location Drăgășani vineyard-viticultural roads smothered Dealul Olt-Drăgășani – Romania (44°38'50 N, 24°15'50 E, 161 Alt)		Codes OIV,UPOV, Bioversity- IBPGR
Characteristics	Expression character	Notes	Expression character	Notes	
0	1	2	3	4	5
Morphological descriptors: Young shoot:					
Young shoot: opening of the shoot tip	fully open	5	fully open	5	001; 2; 6.1.1
Shoot:					
Shoot: attitude (before tying)	semi-erect	3	semi-erect	3	006; 9; 6.1.5
Shoot: number of consecutive tendrils	2 or less	1	2 or less	1	016, -; 6.1.14
Mature leaf:					
Mature leaf: size of blade	large	7	large	7	065;17;6.1.21
Mature leaf: shape of blade	wedge-shaped	2	pentagonal	3	067;18;6.1.22
Mature leaf: number of lobes	3-5	2/3	3-5	2/3	068; 20; 6.1.23
Woody shoot:					
Woody shoot: cross section	circular	1	circular	1	101, -, -
Woody shoot: main color	yellow- brownish	1/2	brownish	2	103;44; 6.1.42
Flower:					
Flower: sexual organs	reflexed stamens and fully developed gynoecium	4	fully developed stamens and fully developed gynoecium	3	151; 16; 6.2.1
Bunch:					
Bunch: shape	conical	2	cylindrical -conical	1/2	208; -; -
Berry:					
Berry: shape	globose	2	globose	2	223; 36; 6.2.6
Phenological characteristics:					
Time of bud burst	medium	5	medium	5	301; 1; 7.1.1
Time of full bloom	medium	5	medium	5	302; -; 6.2.21
Time of beginning of berry ripening (veraison)	medium	5	medium	5	303; 31; 7.1.4

# The main characters of the varieties studied

0	1	2	3	4	5
Time of full physiological maturity of the berry	very late	9	medium	5	304, -; 7.1.10
Time of beginning of wood maturity	medium	5	medium	5	305; -; -
Autumn coloration of leaves	yellow	1	yellow	1	306; -; -
Agrobiological characteristics:					
Vigor of shoot growth	medium	5	medium	5	351; -; -
Growth of lateral shoots	medium	5	medium	5	352; -; 7.1.11
Physiological characteristics:					
Leaf:degree of resistance to Plasmopara	medium	5	high	7	452; -; 9.2.3
Cluster: degree of resistance to Plasmopara	high	7	high	7	453; -; 9.2.4
Leaf: degree of resistance to Oidium	high	7	high	7	455; -; 9.2.5
Cluster: degree of resistance to Oidium	medium	5	high	7	456; -; 9.2.6
Leaf: degree of resistance to <i>Botrytis</i>	high	7	high	7	458; -; 9.2.1
Cluster: degree of resistance to Botrytis	high	7	high	7	459; -; -9.2.2
Technological characteristics:					
Sugar content of must	medium	5	medium	5	505; -; 7.1.17
Total acidity of must	low	3	low	3	506, -; 7.1.18

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# SLAVIŢĂ- THE VARIETY TRADITIONAL OF ROMANIA VINEYARDS

Gorjan Sergiu Ștefan¹, Botu Mihai²

Keywords: Vitis vinifera, grape vine, descriptors, preservation

# ABSTRACT

The study aimed at identification, locate and characterization of the 'Slăviță' variety, which the endangered in Drăgăşani vineyard in order to promote, rescuing and conservation on farm, in situ and ex situ. The study was performed in the years 2010 and 2011 and followed technology on farm of private plantations vineyard. The characterization of the variety was performed using modern descriptors accredited by international organizations in the area.. The study carried out has demonstrated that this variety is value certain, the direction of production is of quality white wines.

### INTRODUCTION

"Just as other countries under vines of outstanding the tradition with Romania, as well as France, Spain, Portugal, Italy and other, they knew to retain and to promote varieties own, by requiring them on the international market, just as ought to made and our country. We lost the traditional varieties, we lost the good position in the market. And guilt is not only history" (Isărescu cited by Puşcă, 2006).

The Drăgășani vineyard is considered to be great grandmother of Romanian viticulture, being one of the oldest vineyards from Romania.

Aside from Station of Researchers and Development for Viticulture and Vinification Drăgășani, where there is into a ampelographic collection, 'Slaviță' variety arrived to be found very hard being the endangered. The origin of this variety is difficult to specify, because it was spread in the vineyards of Romania long before the phylloxera attack. Can also be found in the other the Romania vineyards in ampelographic collections of research stations and also in very low number in private plantations.

# MATERIAL AND METHODS

In year 2010 in Drăgășani vineyard, viticultural roads smothered Sutești-Pietroasa, have been identified and localized an number of 6 hubs from this variety, in a old plantation of over 90 years, planting distance between rows of 1,6 m and 1,0 m between the hubs. These hubs are found in the biologically mixture with other old varieties of grape vines. We studied the phenology variety and agroproductive characteristics analyzed were: fertile

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shoots, hub vigor, the grapes production at hub, grape weight, grapes quality. Technological elements studied were: weight of 100 berries, sugar content in must, must acidity (Gorjan, 2012). Analyses were made on must using Carl Zeiss hand refractometer method and titrimetric method for acidity ( $H_2SO_4$ ). Description of the variety was performed through observations and determinations on the basis descriptors OIV; UPOV; Bioversity-IBPGR from the descriptors list *varieties* and *species Vitis*, 2009. Were analyzed an number of 122 characters.

# **RESULTS AND DISCUSSIONS**

Location where they identified hubs of the variety 'Slaviţă' is Drăgăşani vineyard - viticultural roads smothered Suteşti-Pietroasa with the following coordinates 44°40'46 N latitude; 24°12'02 E longitude; 198 m altitude.

The variety 'Slaviță' is a autochthonous local romanian variety, known before the of phylloxera being known as the the 'Slavitza (Munteanu & Roman, 1900). In Croatia it is cultivated an variety with the same name 'Slaviță (Pejic, 2012) which could be identical to this one.

The phenology studied in years 2010-201 shows us fact that 'Slaviță' is a normal variety presenting average phenophases (Table 1).

The data average phenological 2010-2011

Table 1

Variety	Bud burst	Full bloom	Beginning of	Full maturity	Beginning of wood
			berry ripening	of the berry	maturity
					maturity
'Slaviță'	14.04	31.05 - 10.06	05.08	16.09	09.08

The main characteristics agroproductive and technological (Varga et al., 2005) studies show that the variety 'Slaviță' is a very valuable variety that must be promoted and used in viticulture in terms of fertile shoots, vigor, production, quality, sugar content in must, acidity (H₂SO₄). (Table 2).

The characters studied based on OIV, UPOV, Bioversity-IBPGR descriptors point out that the variety 'Slaviţă' is a variety intended for quality white wines. Presents the characteristics agrobiological, physiological and technological distinct. Regarding the morphological characters, the opening of the young shoot tip is half open, attitude (before tying) of the shoot is semi-erect. The mature leaf is medium, pentagonal with five lobes, color of the upper side of blade is medium green. The cross section of the woody shoot is circular while structure of surface is striate. Woody shoot has a yellow-brownish color. The flower is hermaphrodite normal. The length of bunch is short-medium, cylindrically shaped, the density being medium. The berry is short, globose, the color of skin is greenyellow. The length of seeds is medium while weight of seeds is low.

The phenological characteristics are medium, the autumn coloration of leaves being yellow. The characters agrobiological is outstanding with a vigor of shoot growth strong. Linked to physiological characteristics themselves highlights that have a good resistance to drought, *oidium*, *plasmopara* and *botrytis*. The characters technological are medium in terms of production, sugar content in must being high. The total acidity of must is low-medium (Table 3). In the figures below are presented images of the variety 'Slaviță'.

Specification	U.M.	Variety
		'Slaviță'
Fertile shoots	%	80
Vigor hub	-	high
Production grapes/ hub	kg	3,500
The average weight of	g	165
grapes		
Quality of grapes	%	85
Weight of 100 berries	g	175
Sugar content in must	g/l	216
Acidity (H ₂ SO ₄ )	g/l	5,2

# The main characteristics agroproductive and technological 2010-2011



Figure 1. 'Slaviță' Bunch and Berry



Figure 3. 'Slaviță' Leaf the upper part



Figure 2. 'Slaviță' Hub



Figure 4. 'Slaviță' Leaf the lower part

# Description of the variety 'Slaviță' using modern descriptors

'Slav	'Slaviță'					
Location Drăgășani	vineyard - Romania					
viticultural roads smothered Sutești-Pietro	basa (44°40'46 N 24°12	'02 E 198	m Alt.)			
Characteristics	Expression	Notes	Codes			
	character		OIV,UPOV,			
			Bioversity-			
			IBPGR			
Morphological descriptors:						
Young shoot:		-				
Young shoot: opening of the shoot tip	fully open	5	001; 2; 6.1.1			
Young shoot: distribution of anthocyanin	absent	1	002;-;-			
coloration on prostrate hairs of the shoot tip						
Young shoot: intensity of anthocyanin coloration	none	1	003; 4; 6.1.2			
on prostrate hairs of the shoot tip						
Young shoot: density of prostrate hairs on the	low	3	004; 3; 6.1.3			
shoot tip						
Young shoot: density of erect hairs on the shoot	none	1	005; 5; 6.1.4			
tip						
Shoot:						
Shoot: attitude (before tying)	semi-erect	3	006; 9; 6.1.5			
Shoot: color of the dorsal side of internodes	green	1	007; 10; 6.1.6			
Shoot: color of the ventral side of internodes	green	1	008; 11; 6.1.7			
Shoot: color of the dorsal side of nodes	green	1	009; 12; 6.1.8			
Shoot: color of the ventral side of nodes	green	1	010; 13; 6.1.9			
Shoot: density of erect hairs on nodes	none	1	011: - :			
			6.1.10			
Shoot: density of erect hairs on internodes	none	1	012: 14:			
			6.1.11			
Shoot:density of prostrate hairs on nodes	none	1	013; - ; 6.1.12			
Shoot: density of prostrate hairs on internodes	none	1	014; -;			
5 1			6.1.13			
Shoot: distribution of anthocyanin coloration on	up to 3/4 of bud	3	015-1; -; -			
the bud scales	scale		, ,			
Shoot: intensity of anthocyanin coloration on the	very weak	1	015-2; -; -			
bud scales	5		, ,			
Shoot: number of consecutive tendrils	2 or less	1	016, -; 6.1.14			
Shoot: length of tendrils	short	3	017: 15.			
		_	6.1.15			
Young leaf:						
Young leaf: color of upper side of blade (4th	green/bronze	1/3	051: 6. 6.1.16			
leaf)	0		, -,			
Young leaf: density of prostrate hairs between	none	1	053: 7:			
main veins on lower side of blade (4th leaf)			6.1.17			
Young leaf: density of erect hairs between main	none	1	054: - :			
veins on lower side of blade (4th leaf)			6.1.18			
Young leaf: density of prostrate hairs on main	low	3	055: -: 6.1.19			
veins on lower side of blade (4th leaf)			, ,			
Young leaf: density of erect hairs on main veins	none	1	056; 8: 6.1.20			
on lower side of blade (4th leaf)		-	, .,			

Mature leaf:			
Mature leaf: size of blade	medium	5	065; 17: 6.1.21
Mature leaf: shape of blade	pentagonal	3	067; 18; 6.1.22
Mature leaf: number of lobes	five	3	068; 20; 6.1.23
Mature leaf:color of the upper side of blade	medium green	5	069; -; -
Mature leaf: area of anthocyanin coloration of	absent	1	070; -; -
main veins on upper side of blade			
Mature leaf: area of anthocyanin coloration of main veins on lower side of blade	absent	1	071; -; -
Mature leaf: goffering of blade	absent	1	072; -; -
Mature leaf: undulation of blade between main or lateral veins	absent	1	073; -; -
Mature leaf: profile of blade in cross section	involute	3	074: -: 6.1.25
Mature leaf: blistering of upper side of blade	absent	1	075; 19;
			6.1.26
Mature leaf: shape of teeth	both sides straight	2	076; 26; 6.1.27
Mature leaf: size of teeth in relation to blade size	medium	5	077; -; -
Mature leaf: length of teeth compared with their	long	7	078; 25;
width	8		6.1.29
Mature leaf: degree of opening / overlapping of neticle sinus	open	3	079; 23; 6 1 30
Mature leaf: shape of base of petiole sinus	U-shaped	1	080: -: -
Mature leaf: teeth in the petiole sinus	none	1	081-1; -:
Mature leaf: petiole sinus base limited by vein	not limited	1	081-2; -;
Mature leaf: degree of opening / overlapping of upper lateral sinuses	open	1	082; 22;
Mature leaf: teeth in the upper lateral sinuses	none	1	083-2: -: -
Mature leaf: shape of the base of upper lateral sinuses	U-shaped	1	083-1; -; -
Mature leaf: density of prostrate hairs between main veins on lower side of blade	none	1	084; 28;
Mature leaf: density of erect hairs between main veins on lower side of blade	none	1	085; -, 6.1.36
Mature leaf: density of prostrate hairs on main veins on lower side of blade	none	1	086; -; 6.1.37
Mature leaf: density of erect hairs on main veins on lower side of blade	none	1	087; 29; 6.1.38
Mature leaf: prostrate hairs on main veins on upper side of blade	absent	1	088; -; 6.1.39
Mature leaf: erect hairs on main veins on upper side of blade	absent	1	089; -; -
Mature leaf: density of prostrate hairs on petiole	none	1	090; -: -
Mature leaf: density of erect hairs on petiole	none	1	091; -: -
Mature leaf: length of petiole compared to length	slightly longer	7	093; 30;
of middle vein			6.1.40
Mature leaf: depth of upper lateral sinuses	medium	5	094; 21;

			6.1.34
Woody shoot:			
Woody shoot: cross section	circular	1	101, -, -
Woody shoot: structure of surface	striate	3	102 - 6.1.41
Woody shoot: main color	brownish-vellow	2/1	103, 44
	orowinshi yenow	-/ -	6.1.42
Woody shoot: lenticels	absent	1	104, -, -
Woody shoot: erect hairs on nodes	absent	1	105, -, -
Woody shoot: erect hairs on internodes	absent	1	106, -, -
Flower:			
Flower: sexual organs	fully developed	3	151; 16;
	stamens and fully		6.2.1
	developed		
	gynoecium		
Inflorescence: insertion of 1stinflorescence	3/4	2	152; -, -
Inflorescence: number of inflorescences per	2,1-3 inflorescences	3	153; -; 7.1.3
shoot	1.	~	1.5.5
Shoot: fertility of basal buds (buds 1-3)	medium	5	155; -; -
Bunch:		2/5	
Bunch: length (peduncle excluded)	short/medium	3/5	202; -; 7.1.5
Bunch: width	narrow	3	203; -; -
Bunch: densit	medium	5	204; 33; 6.2.3
Bunch: length of peduncle of primary bunch	short	3	206; 34; 6.2.4
Bunch: lignification of peduncle	up to about the	5	207; -; -
D 1 1	middle	1	200
Bunch: snape	cynndrical	1	208; -; -
Bunch: number of wings of the primary bunch	absent	1	209; - ;-
Berry:	short	2	220
Derry, leligui	short	3	220; -; -
Berry: uniformity of size	not uniform	3	221, -, -
Berry: shape	debose	2	222, -, -
Berry: color of skin	groon vellow	1	225, 30, 0.2.0
Berry: uniformity of skin color	uniform	2	225, 57, 0.2.8
Berry: bloom	high	7	220, -, -
Berry: thickness of skin	medium	5	227, -, -
Berry: hilum	visible	2	220, 35, 7.1.0
Berry: intensity of flesh anthocyanin coloration	none	1	231:40:629
Berry: iniciness of flesh	very inicy	3	232: -: 6 2 10
Berry: must vield	hjøh	7	233 719
Berry: firmness of flesh	slightly firm	2	235. 45-41 -
Berry: particular flavor	none	1	236. 42.
berry: particular navor	none	1	6.2.12
Berry: length of pedicel	short	3	238; -; 7.1.7
Berry: ease of detachment from pedicel	easy	2	240, 38;
	-		6.2.13
Berry: formation of seeds	complete	3	241; 43; 6.2.7
Berry: length of seeds	medium	5	242; -; 6.2.14
Berry: weight of seeds	low	3	243; -; 6.2.15
Berry: transversal ridges on dorsal side of seeds	absent	1	244; -; 6.2.16
Phenological characteristics:			
Time of bud burst	medium	5	301; 1; 7.1.1
------------------------------------------------------------	---------------	-----	----------------
Time of full bloom	medium	5	302; -; 6.2.21
Time of beginning of berry ripening (veraison)	medium	5	303; 31; 7.1.4
Time of full physiological maturity of the berry	medium	5	304, -; 7.1.10
Time of beginning of wood maturity	medium	5	305; -; -
Autumn coloration of leaves	yellow	1	306; -; -
Agrobiological characteristics:			
Vigor of shoot growth	strong	7	351; -; -
Growth of lateral shoots	medium	5	352; -; 7.1.11
Length of internodes	medium	5	353; -; 7.1.12
Diameter of internodes	small	3	354; -; -
Physiological characteristics:			
Resistance to iron chlorosis	high	7	401; -; 8.5
Resistance to chlorides (salt)	high	7	402; -; 8.6
Resistance to drought	high	7	403; -; 8.3
Leaf:degree of resistance to <i>Plasmopara</i>	high	7	452; -; 9.2.3
Leaf: degree of resistance to Plasmopara (leaf	medium	5	452-1; -; -
disc test)			
Cluster: degree of resistance to Plasmopara	high	7	453; -; 9.2.4
Leaf: degree of resistance to Oidium	high	7	455; -; 9.2.5
Leaf: degree of resistance to Oidium (leaf disc	medium	5	455-1; -; -
test)			
Cluster: degree of resistance to Oidium	high	7	456; -; 9.2.6
Leaf: degree of resistance to <i>Botrytis</i>	high	7	458; -; 9.2.1
Leaf: degree of resistance to <i>Botrytis</i> (laboratory	medium	5	458-1; -, -
analysis)			
Cluster: degree of resistance to <i>Botrytis</i>	high	7	459; -; -
			9.2.2
Degree of resistance to Eutypa dieback	high	7	460; -; 9.2.8
(laboratory analysis)			
Leaf: degree of tolerance to <i>Phylloxera</i> (leaf gall)	high	7	461; -; 9.1.1
Root: degree of tolerance to <i>Phylloxera</i> (root	very low	1	462; -, 9.1.2
gall)			
Technological characteristics:			
Percentage of berry set	medium	5	501; -; 7.1.13
Bunch: single bunch weight	very low/ low	1/3	502; -; 7.1.14
Berry: single berry weight	low	3	503, -; 7.1.15
Yield per m2	medium	5	504; -, 7.1.16
Sugar content of must	high	7	505; -; 7.1.17
Total acidity of must	low/medium	3/5	506, -; 7.1.18
Must specific pH	low	3	508; -; -

# CONCLUSIONS

The variety 'Slaviță' is a variety with special quality agroproductive and technological.

This variety should be promoted and brought in actuality wine growing romanian and international, being a variety with very good potential viticultural.

The future will continue identification of other hubs of this variety and other varieties lost by over time in view conservation *on farm*, *in situ* and *ex* situ of germplasm wine growing existing in vineyard.

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## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# VARIABILITY OF ELECTRICAL CONDUCTIVITY AND pH VALUES, OF THE NUTRIENT SOLUTION, IN GREENHOUSE TOMATO CULTIVATION, WITH COIR AS SUBSTRATE

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Keywords: soilless cultivation, nutrient solution, coir substrate, tomato.

#### ABSTRACT

In Soilless tomato cultivation, with coir substrate, the variability of electrical conductivity and pH of the used nutrient solution, during the influx and efflux (open system), was studied, in the greenhouse (TEI of Larissa). During fertigation, the electrical conductivity (EC) of the inflow of the solution fluctuated from 1.80 to 2.37 dS.m⁻¹ and pH from 5.5 to 6.8, while for the runoff solution the electrical conductivity fluctuated from 2.0 to 3.33 dS.m⁻¹ and pH from 5.5 to 6.9.

With regard to the substrate of coir, the electrical conductivity throughout the duration of the crop ranged from 0.3 to 1.13 dS.m⁻¹ (water extract of 1part of coir: 5 parts of  $H_2O$ ) and pH from 5.87 to 6.83. According to the study, the coir is a good choice as substrate, for tomato soilless cultivation, with satisfactory return.

#### **INTRODUCTION**

The development of the hydroponics growing (Manios V.I. *et al.*1995, Inden H. *et al.*2004), is based on both in the modern facilities of greenhouses, as well as on modern production systems, concerning the very efficient preparation and distribution of nutrient solution (Sonneveld C. et al. 1999, Zekki H. et al. 1996).

#### MATERIALS AND METHODS

In the glass greenhouse of TEI of Larissa, an open hydroponics system, with a unit of automatic production and control of nutrient solutions has been established. The unit contains three stock solutions, that are in three differently barrels, (figure1), and tomato plants were cultivated in an area of  $100 \text{ m}^2$ , from 14-10-2010 to 11-04-2011. The substrate used for the cultivation of plants, was a pressurized packages of coir, (table 1). According to the directives of the supplier of the central unity, it is recommended for tomato plants a nutrient solution with pH:6 and electrical conductivity (EC) 2 dS.m⁻¹.

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The content of the three parent nutrient solutions were:

A' Barrel : (Ca⁺⁺ = 507, NO₃⁻ = 680.6, K⁺= 116.8 , NH₄⁺= 55, Fe⁺⁺ = 1.8) me/Lt H₂O.

B' Barrel: (Mg⁺⁺=195, K⁺= 644.2, H⁺= 286, SO₄⁼ = 580,NO₃⁻= 115.2, PO₄⁻⁻⁻ = 430) me/LtH₂O.

C: Barrel: 6.6 ml concentrated HNO₃ / Lt H₂O.

The necessary amounts of solution from each barrel come out automatically, by creating the final nutrient solution, while the central unit regulates the EC and pH values by the convenient program.

Each watering had duration 3 min and the inflow of the nutrient solution was 58.9 ml/min. The first 60 days of the establishment of the crop three watering were applied per day, the next 50 days 4 watering, and for following last days 5 times per day.

The purpose of the experiment is to investigate the effectiveness of the system that ensures the desired pH values and electrical conductivity, and study the eventual deviations of this adjustment.

Coir was also studied concerning his affectation by the solution applied. Then, ammonium and nitrate nitrogen, available P and exchangeable K were measured, following Page et al. 1982 and Hesse P.R. et al.1972, methods. Organic matter content of the substrate was measured by chemical oxidation of soil with 1 mol/l  $K_2Cr_2O_7$  and titration of the remaining reagent with 0.5 mol/l FeSO₄. Both ammonium and nitrate nitrogen were extracted with 0.5 mol/l CaCl₂ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P (Olsen-P) was extracted with 0.5 mol/l NaHCO₃ and measured by spectroscopy. Finally, exchangeable K was extracted with 1 mol/l CH₃COONH₄ and measured by Flame Photometry.



Figure 1. System preparation of the nutrient solution of the experiment RESULTS AND DISCUSSIONS

**Nutrient solution/Substrate:** In Figure 2, the values of electrical conductivity and pH during the influx of nutrient solution of the culture were recorded, and their respective values of the same parameters of the efflux solution from the substrate (open system) were measured. It was observed that during the inflow of the solution in the crop, with regard to the electrical conductivity, it was produced a solution with slightly higher values of electrical conductivity, while for pH it was produced also a solution with slightly higher values. Moreover, the runoff solution showed values of electrical conductivity and pH, higher than the respective values of the influx solution.

In Figure (2), the values of electrical conductivity and pH were also recorded concerning the substrate, during the cultivation, as they were affected by the nutrient solution. It was observed that the first days of cultivation the electrical conductivity of the substrate was fluctuated, thereafter, it was reduced and stabilized at the value of 0.6 dS.m⁻¹, ninety (90) days after the starting of the cultivation. That remark, in relation to N, P, K contents found at the end of the cultivation period (Table1), showed that low amounts of elements were accumulated to substrate used. Also the pH of the substrate during the cultivation was fluctuated from 5.87 to 6.83. Moreover, a remark to quote is the lower content in organic substances of the substrate found at the end of the cultivation period, 8,39% from 11,71 % in the starting (table:1); it is attributed to biodegradation of the organic material during the cultivation period, and it could be an instability index of the substrate.

Despite the variations of pH and electrical conductivity of the nutritive solution influxes, the plants showed a good evolution. The foliar analysis of plants, 120 days after the starting of the cultivation, showed that they were in satisfactory nutritional conditions. The annual crop production was 100 tn/ha (3.5 kg/plant) with mean fruit weight 0.25 kg.



Figure 2. Fluctuations of electrical conductivity and pH values, of the nutrient solution (influx, efflux) and of the coir substrate, of soilless tomato cultivation, in relation to the stage of cultivation

Every value of the electrical conductivity and pH is the mean of three replicates.

Chemical properties of the coir subst	rate used, before and a	tter utilization
Chemical Property	Coir initial	Coir final
	(wet m	aterial)
pH, (1part substrate:5parts H ₂ O)	6.7	6.1
CaCO ₃ (%)	0.13	0.13
Organic matter (%)	11.71	8.39
CEC (cmol.kg ⁻¹ substrate)	49.5	75.2
Electrical conductivity, water extract	0.68	0.65
(1part of substrate:5parts H ₂ O), (dS.m ⁻¹ )		
$N-NH_4^+$ (mg.kg ⁻¹ )	214	22.6
$N-NO_3^{-}(mg.kg^{-1})$	119	167.6
Exchangeable-Na (mg.kg ⁻¹ )	402.5	69.0
P-Olsen (mg.kg ⁻¹ )	14.73	80.36
Exchangeable-K (mg.kg ⁻¹ )	2072.1	733.2
Zn-Total (mg.kg ⁻¹ )	6.03	24.59
Cu-Total (mg.kg ⁻¹ )	-	11.11
$Zn - DTPA (mg.kg^{-1})$	1.66	17.53
Cu - DTPA (mg.kg ⁻¹ )	0.56	4.60
Mn - DTPA (mg.kg ⁻¹ )	1.7	2.85
Moisture (%)	79.65	87.21

C.1 . 1 . . . . . . . . .

Table 1

#### CONCLUSIONS

The effectiveness of a soilless cultivation system in greenhouse conditions, was evaluated, concerning, the fluctuations of the nutrient solution, in comparison to the indicated values of pH and Electrical Conductivity. A small divergence upwardly was observed of the electrical conductivity value, while as it concerns pH, the produced nutrient solution showed higher values. With regard to the runoff solution, which seeps of the substrate, also higher values of electrical conductivity and pH were found. The coir substrate stabilized its salinity in the middle of cultivation period. Finally the yield of cultivation was satisfactory, but the obtained remarks should be taken into account, in order to be respected for the next cultivation period, and the necessary settings to the system irrigation-fertilization should be restored.

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# Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# RELATIONSHIP BETWEEN FERTIGATION AND QUALITY CHARACTERISTICS OF INDUSTRIAL TOMATO

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Keywords: industrial tomato, fertilization, fruit quality characteristics, soluble solids.

#### ABSTRACT

Some of the most essential factors, that must be met to achieve optimal production of the industrial tomato and ensure exceptional product quality, are the adequacy of water and nutrients and sunshine. The present study explores the relations between fertilization and production of the outdoor industrial tomato. The experimental data show a positive correlation of fertilization with some of the quantitative and qualitative characteristics examined. The results can be utilized to the optimum use of the fertilization treatment of the crop with minimal effect on the soil and aqueous environment.

#### INTRODUCTION

Farming of industrial tomato is considered as one of the most intensive agricultural land use, in terms of irrigation and use of chemicals (Rinaldi et al., 2003). According to the literature (Siomos and al, 2009) in the region of Thrace annual applications of fertilizer per acre sown area of industrial tomatoes include the application of 1650 kg/ha fertilizer (1000 kg/ha 11-15-15 + 300 kg/ha 0-0-50 + 350 kg/ha 33.5-0-50). Aggidis (1995) reports to produce 80 tons of tomatoes per ha, a field with 3% organic matter requires total fertilization points of the essential elements, nitrogen (N) 20-25 phosphate ( $P_2O_5$ ) 10-12, Potassium (K₂O) 30-35.

Consequently optimizing irrigation and fertilization conditions of industrial tomato farming will not cease to employ researchers since the global production is increasing. Specifically in 2009 it increased by 7% compared to 2008 and by 15% compared to 2007. The same happens in the production of EU member states where in recent years has been steadily increasing. Dominant position in the production held by Italy with 5.0  $10^6$  Mg (2009), followed by Spain with 2.3  $10^6$  Mg, Portugal with 1.1  $10^6$  Mg and Greece with 0.8  $10^6$  Mg.

Therefore it is appropriate that Greece to optimize the production conditions of this crop, in order to become competitive in a product whose demand is growing globally.

In terms the quality of industrial tomato, among the main desirable characteristics which should characterize the production, is the high content of soluble solids, low acidity,

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transfer resistance, high productivity and high rate of first fruits class (Goose and Binsted, 1964).

The factors affecting the quality of industrial tomato are endogenous (variety, etc.) and exogenous (environment management). In the environment, are the soil and climatic conditions. In the management is the way of cultivation, harvest season, the irrigation depth and frequency of irrigation etc, in other words techniques that without particularly burdening the inputs of cultivation, contributes to high yields.

Harvesting (manual or mechanized) of industrial tomato in Greece, takes place in the last ten days of July (early varieties), in August-September (mid-early varieties), and October (late varieties), with slight variations depending on the soil conditions (Boulekou S., 2010).

The present work investigates the influence of fertigation on the quality of industrial tomato in Larissa. It examines the levels of implementation fertilization and application of organic farming to industrial tomato. Additionally, reuse of urban wastewater took place in the municipality of Larissa. The application of irrigation water was the method of drip irrigation and the climate data was taken from the installed automatic telemetric meteorological station of Larissa, with the objective of optimum utilization of fertilize produced for quality industrial tomato.

## MATERIAL AND METHODS

a) **Description of installation.** The irrigation network consisted of the main pipeline of PE Ø32 /6 Atm. and secondary pipelines of PE Ø20/6 Atm. The Drip lines of surface and subsurface drip irrigation were spacing of row was 1.5 m, 5 m long and were of type NAANPC/ Ø16 with integrated drippers. Drippers were self-regulating and self-cleaning spacing on the line 0.70 m, dripper discharge 2.2 lph at an operating pressure of 0.5 to 3.5 atm., Double flushing mechanism through two water channels. Four procedures of Fertilization were implemented:

Two lines were with surface drip irrigation, with applied common fertilization (A), organic cultivation without fertilization (B) and two with subsurface drip irrigation and 10% of water with sewage effluent (C), with 15% of water with wastewater (D). Procedures in the subsurface drip line were installed at 15 cm depth from the ground surface. The fertilizer was with fertigation using mixing tang. The experimental pieces shown in Figure 1 are characterized by two letters and a number. The first letter designates the system (S for surface, SS for the subsurface), a second level of each treatment and the number represents the number of each replication. The dimensions of each experimental unit were 5 x 3 = 15 m². The experimental design consisted of randomized groups with four replications.

## b) Description of the experiment

For the implementation of the research program installed experimental fields of industrial tomato in Larissa, irrigated with drip irrigation and applied various fertilization levels in experimental units. Analyzing of soil samples and its classification showed that it is sandyclayloamy and belongs to the subgroup of Vertic Xerochrept of Inceptisols, in the area TEI of Thessalia.

Plants of industrial tomato (hybrid Heinz S3402) were transplanted at the stage of 6-7 true leaves on the ground, on 3 May. Each experimental unit includes four rows of industrial tomatoes at distances 0.45 m between the twin rows and 1.0 m between them.

In terms of crop protection, an application of fungicide Ridomil Cold MZ 68 VG was made (s.c. metalaxyl-M and S-isomer 64%), at the rate of 200 g/100 Liters of water on

03-07-2011 and an application of the insecticide Decis 2.5EC, (s.c. deltamethrin 2.5% SC), at a dosage of 30 cc/100 Liters of water on 11-05-2011.

•	<b>₄</b> 3m →							
<b>↑</b> 5 m	SSC3	SA3	SSD3	SA3	SB4	SB3	SSC4	SSG4
	SSC1	SA2	SSD1	SA1	SB2	SB1	ssc2	SSD2

Figure 1. Schematic of experimental units of industrial tomato farm in Larissa.

In organic crop, nettle extract on was sprayed on 07-03-2011 and fungicide theiochalkini (40% S, 4% Cu) was sprayed on 11-05-2011.

The harvest took place on the red ripening stage according to the classification of the USDA (1997), on 25 August by hand. From each repeat of the experiment was collected and weighed separately for each line the produce of 10 plants. Each data item for each repeat was examined for the qualitative characteristics of industrial tomato in Agricultural Products Quality Laboratory of the Department of Biosystems Engineering, TEI of Larissa.

For finding of marketable and non-(green-rotten) production per experimental unit and treatment only the production of 10 medium-sized plants of the two middle rows planting was harvested, while the analysis of data concerning the establishment of fruit (organoleptic properties) the completely randomized design was used, with four repeats per treatment and five similar fruits per repeat. The comparison of averages was done with the multiple range test Duncan, at a significance level of 0.05 (statistical package SPSS 10).

## c) Water consumption

The amount of water applied at each irrigation was calculated from climatic data of the automatic telemetric meteorological station, that was at the location of the experiment, with the method of Penman-Mothieth FAO 56. For the determination of the evapotranspiration control culture used the modified Penman-Monteith method as described by FAO-56 (Allen et al., 1998).

The measurements refer to the period May - August 2011. After the transplantation of industrial tomato, irrigation took place for the germination of the crop with single sprinklers and irrigation depth of 15 mm. The total rainfall during the growing season amounted to 33.9 mm. The total depths irrigation and total water (irrigation + rain +

irrigation germination) in each treatment is 602 mm for A, 560.6 mm for B, 467.8 mm for C and 498.7 mm for C.

## d) Fertilization

The nutrition included the surface application of 53 Kg of nitrogen per ha on the line on 5/27/2011 at 3 interventions and 6660 Kg/ha mold (polyhum) and 25000 Kg/ha manure in organic farming. The mold contained organic matter 90% by weight, pH (H₂O) 5.5 and nutritious 14-16-18 (N-P₂O₅-K₂O). Manure contained organic matter 16.39% by weight, 441 mg/kg (NH₄⁺ + NO₃⁻), 418.6 mg/kg (P-Olsen) and 25.49 me/100 g K Exchangeable. The water in the wastewater of Larissa used in both treatments contained NH₄⁺ 0,9 mg/Lt, NO₃⁻ 7.5 mg/Lt, P 4.5 mg/Lt, EC 0.8 dS/m and pH (H₂O) 7.47.

On 6-18-2011 fertigation took place with fertilizer 24-4-4 (N-P₂O₅-K₂O) in treatment A (3-0.5-0.5) to C (2.4-0.4-0.4) in D (3.6-0.6-0.6). On 6-28-2011 second application in A (5.18-0-0) to C (3.45-0-0) to D (2.58-0-0). On 07-07-2011 third application in A (5.18-0-0) in C (3.45-0-0) in D (2.58-0-0). On 7-19-2011 in A (5.18-0-0). Table 1 shows the nutrient balances in different treatments.

Table 1

	Treatment	N (kg/ha) Inorganic	P ₂ O ₅ (kg/ha) P-Olsen	K2O (kg/ha) Exchangeable
	А	26.30	166.2	1740.7
Start growing	В	263.0	166.2	17.0.7
period	C	263.0	166.2	1740.7
	D	263.0	1.6.2	1740.7
	А	238.0	5.0	0.5.0
Add	В			
fertilization	C	146.0	4.0	4.0
	D	140.7	6.0	6.0
	Α	366.7	96.0	14.4.5
End of growing		501-366.7=134.3	171.2-96=72.5	1745.7-1494.5=251.2
period	В	138.8	145.6	621.2
penou	С	130.9	73.7	921.9
	D	87.3	41.1	931.2

Inorganic elements availability in soil

#### **RESULTS AND DISCUSSIONS**

The treatment with the highest rate of fertilizer (E) had the highest marketable production (7.87 kg m⁻²) and the largest fruit weight (45.95 g). The fact that the same treatment gave higher production of waste (green = 1.57 kg m⁻² and decaying = 2.37 kg m⁻²) in relation to the biological crop which had the less rotten does not result ultimately against the treatment, because the ratio of total marketed production to the green did not differ statistically significantly among the treatments (p = 0.077 Table 2). Besides, the same treatment gave greater plant mass (Table 3).

The price of industrial tomatoes depends on the rate of the red production namely the ratio of red/green, a statistical processing of samples using this parameter was made (Table 2) from which there were no differences among the treatments (p = 0.07). Therefore different rates of fertilization do not optimize the output for this crop in this qualitative aspect of production. Production another qualitative parameter evaluated as soluble solids (brix), was found to differ statistically significantly treatments A and B compared among treatments C and D (Table 3). The highest price presented by the fruits of treatment D at (5.28%) and G (5.20%) The price of this qualitative parameter also affects the price of industrial tomatoes. When ranges are between 4.0 and 4.6% the price for the producer is 0.04  $\epsilon$ /kg, between 4.7 and 5.0% the price increases to 0.06  $\epsilon$ /kg but where SAP is higher than 5.1% the price is 0.07  $\epsilon$ /kg (Table 3).

# Table 2

Treatment	Number Treatment (# fruit)	Total commercial production ( kg m ⁻² )	wasted production (kg m ⁻² ) green decaying		Ratio red /green	Fruit weight (g)			
А	4 (181)	7.87a	1.57ab		5.01	45.95a			
В	4 (176)	6.53b	1.64a	1.75b	3.98	43.07b			
С	4 (174)	6.79ab	1.38b	2.4a	4.92	45.59ab			
D	4 (196)	6.15b	1.36b	2.93a	4.52	44.68ab			
Sig.		< 0.05	< 0.056	< 0.001	0.077	< 0.05			
	Statistical analysis was performed with the criterion Duncan (p <0,05). Different letters indicate that there are statistically significant differences.								

Total marketable and waste production of tomato (kg m⁻²), ratio red / green and fruit weight

Table 3

Fresh weight of plant crown per plant (kg), values of soluble solids of fruit (%) and sale of industrial tomato (€ kg⁻¹)

	Number	Fresh weight	Number	55	Price				
Treatment	Treatment (# fruit)	of plant mass (kg plant ⁻¹ )	Treatment (# fruit)	(%)	of sale (€ kg ⁻¹ )				
Е	4 (181)	0.63a	4 (239)	5.06b	0.06				
F	4 (176)	0.62ab	4 (239)	5.07b	0.06				
G	4 (174)	0.62ab	4 (236)	5.20a	0.07				
Н	4 (196)	0.58c	4 (219)	5.28a	0.07				
Sig.		0.000		< 0.000					
	Statistical analysis was performed with the criterion Duncan (p <0,05).								
	Different letters	indicate that there are	statistically significa	ant differences.					

In this paper, the smallest price (0.06 €/kg, Table 3) for a producer who might follow organic farming will result in a financial loss due to this parameter, because the same producer had less marketable production compared to the common fertilization (Table 2). This would apply if the producer gave his biological production to a tomato paste factory which it wouldn't be in his interest. Other quality parameters of fruits such as pH and hydrolytic acidity values, in the bibliography are reported for the pH to range from 4.26 to 4.82 and acidity from 0.40 to 0.91% (Stevens et al., 1986). These values are affected by the genotype, solar radiation, potassium fertilization and temperature during maturation. It has also been expressed that every factor that contributes to the increase of K content in the fruit at the same time causes a corresponding increase in organic acids resulting in the pH of the fruit to remain stable (Karaoulanis, 2003). In this paper were not found statistically significant differences in hydrolytic acidity. The pH values ranged from 4.96 to 5.06 and hydrolytic acidity from 0.46 to 0.48%.

#### CONCLUSIONS

Although the results of this study relate to measurements of a growing period, the application of different levels of fertilization seems to have affected the quality and quantity properties of industrial tomato. Specifically, it affected the total marketable production, average fruit weight and soluble solids, while not affecting other sensory characteristics examined. Organic cultivation of industrial tomatoes can be spread without major problems.

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# EFFECTS OF IRRIGATION AND FERTILIZATION ON QUALITY CHARACTERISTICS OF INDUSTRIAL TOMATO FRUIT, IN CENTRAL GREECE

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Keywords: industrial tomato, fruit quality, total soluble solids, polyphenols.

#### ABSTRACT

The reduction of available water for irrigation in the Mediterranean region and especially in Thessaly which suffers particularly from reduced rainfall in recent years imposes the proper use of irrigation; certainly it is the key for the sustainable management of water.

In a field experiment, established on the farm of the Technological Educational Institute of Larissa in 2012, the effects of irrigation and fertilization on the production and the basic characteristics of industrial tomatoes were evaluated. Four irrigation and four fertilization levels were applied. In the present work the relationships between irrigation, fertilization, production and essential qualities of the outdoor industrial tomato in central Greece were studied. The experimental data show a positive correlation of irrigation water to some of the quantity and quality characteristics examined. The results are in favour for an optimum use of irrigation and fertilization. Moreover, according to obtained data, the organic cultivation of the industrial tomato can be spread without major problems in the area of central Greece.

## INTRODUCTION

The cultivation of industrial tomato is considered as one of the most intensive agricultural land use, in terms of irrigation and chemical usage (Rinaldi et al., 2003).

The production field of tomatoes for processing, had a dominating position among the branches of rural economy of Greece, but after year 2004, it started its continuous downward path. The fact that the industrial tomato works under the contract farming, the complete decoupling of aid and its integration in the single payment scheme, unlike other competing products such as cotton and corn, could stimulate the interest of producers to increase the cultivated land.

The industrial tomato is a crop, very demanding in water, as it requires about 600 mm per year. But in terms of fertilizer uptake, as mentioned by Agidis (1995), to produce 80 tons of tomatoes per ha in a field with 3% organic matter, the total units of fertilization for the major elements, are required as followed: (N) 20-25, (P₂O₅) 10-12, (K₂O) 30-35. Therefore it is appropriate for Greece to optimize the production conditions of this crop, which become competitive in a product whose demand is growing globally.

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Then this paper, investigates the effect of the amount of irrigation water and fertilization rates applied, on quantity and quality of industrial tomato production in Central Greece.

#### MATERIALS AND METHODS

The irrigation network consisted of the main pipeline of PE Ø32/6 Atm. and secondary pipelines of PE Ø 20/6 Atm. The Drip lines of surface irrigation were spacing of row 1.5 m, 5 m long and were of type NAANPC²/Ø16 type with integrated drippers. Drippers were self-regulating spacing on the line 0.70 m, discharge 2.2 Lph at an operating pressure of 0.5 to 3.5 atm. Double flushing mechanism through two water channels. Four treatment irrigation and four fertigation were applied: The application of irrigation treatment is included of 120% (A), 100 % (B), 80% (C) and 60% (D) of evapotranspiration of the crop The application of fertilization included of common fertilization (E) as stated in the report a fertilization of Rural Development, Larissa Prefecture, organic farming without pesticides and fertilization (F), fertilization with 80% of the of common fertilization (G) and fertilization with 60% (H). The surface fertilizer was fertigation using mixing tang. The experimental pieces shown in Figure 1 are characterized by a letter and a number. The first letter designates the irrigation or fertilization level and the number represents the number of each replication. The dimensions of each experimental plot were 5 x 3 = 15 m². The experimental design consisted of randomized groups with four replications.



Figure 1. Schematic of experimental plots, of industrial tomato farm in Larissa

For the implementation of the research, experimental fields of industrial tomato program in Larissa were installed, irrigated with drip irrigation and with application of various fertilization levels in each experimental unit. Analyses of soil samples (Tbl 1) and its classification, showed that it is sandy clay loamy, and it belongs to the subgroup of Vertic Xerochrept of Inceptisols, situated in the area of TEI of Thessalia.

Plants of industrial tomato (hybrid Heinz S3402) were transplanted at the stage of 6-7 true leaves on the ground, on 3 May. Each experimental unit includes four rows of industrial tomatoes at distances 0.45 m between the twin rows and 1.0 m between them.

In terms of plant protection two applications were made, one of the fungicide eco Vacciplant at the dosage of 50 ml/100 liters of water and one of the ecological insecticide Belthirul (3200 wp) at a dosage of 50 g/100 liters of water. The harvest took place on the red ripening stage according to the classification of the USDA (1997), on 25 August manually. From each repetition of the experiment, the production of 10 plants was collected and weighed separately from each line. For each experimental unit and each repetition, the qualitative characteristics of industrial tomato were examined at the Agricultural Products Quality Laboratory of the Department of Biosystems Engineering, TEI of Thessalia.

Table 1

		Tex	ture					EG			ĸ	
Depth (0-30) (cm)	Sand (%)	Silt (%)	Clay (%)	Prof.	Organic mater (%)	CaCO3 (%)	pH H ₂ O (1:5)	EC (1:5 H2O) (dS/m)	N- Inorganic (mg/kg)	P- Olsen (mg/kg)	Excha ngeable (cmol/kg)	CEC (cmol/kg)
	52,5	22,7	24,7	SCL	1,80	1,30	7,6	0,121	93,31	20,83	1,1	35,87

Chemical properties of the soil

For the finding of marketable and non-(green-rotten) production per experimental unit and treatment, only the output of 10 medium-sized plants of the two middle rows planting was harvested, while the analysis of the data, concerning the establishment of fruit (organoleptic properties), the completely randomized design was used, with four repetitions per treatment and five similar fruits per repetition. The comparison of averages was done by the multiple range test Duncan, at a significance level of 0.05 (statistical package SPSS 10).

The amount of water applied, at each irrigation was calculated from climatic data derived from the automatic telemetric meteorological station that was installed at the test site, using the method of Penman-Mothieth FAO 56. For the determination of the evaporation for the cultivation reference, the modified Penman-Monteith method was used as described by FAO-56 (Allen et al., 1998).

The measurements refer to the period May - August 2012. After the transplantation of the industrial tomato, irrigation took place for germination of the crop with single dose irrigation sprinklers and irrigation depth of 15 mm. The total rainfall during the growing season amounted to 110.4 mm.

Table 2 shows the total irrigation water (rain irrigation+ irrigation germination) and total applied water depths for each system and treatment.

The total water depth of all treatments of fertilization amounts to 508,48 mm. The nutrient alimentation included the basic application of 41 kg / ha 12-12-17 (2 Mg+8 S) on the line on 8-6-2012, and four applications of 60 kg/ha (34.5-0-0), two applications of 15 kg/ha (13.5-0-46.2) using fertigation in all irrigation treatments. In Fertilization

treatments, E) 4.92-4.92-6.97, G) 4.92-3.94-6.97, H) 4.92-2.95-6.97 (N- P₂O₅ -K₂O) on the line on 8-6-2012 and 5300 kg manure to the organic cultivation (F). The manure contained organic matter of 16.39% by weight, 541 ppm inorganic N, 418.6 ppm P and 9941.0 ppm K..

Table 2

Total irrigation water for each treatment, percentage of irrigation compared to treatment B and total water depths

Treatment	Irrigation	%	Total	
	mm	of B	Water mm	
А	704.61	120	814.61	
В	587.18	100	697.58	
С	469.74	80	580.14	
D	352.31	60	462.71	

Three interventions, using fertigation, were made applying fertilizer 34.5-0-0 and three interventions, using fertigation, applying fertilizer 13.5-0-46.2. In total, in treatment E were added 24.49-4.92-20.83 units, in treatment G 19.59-3.94-16.66 and in H 14.69-2.5-12.50.

Associately Elements

Table 3

	Treatment	N(kg/ha.) Inorganic	P2O5 (kg/ha.) P-Olsen	K2O (kg/ha.) Exchangeable
Start of growing period	A,B,C,D, E,G,H	39.19	20.04	218.39
Fertilizer	A,B,C,D, F	25.27 24.49	4.92 4.92	16.21 20.83
Added	F	2.87	5.08	63.49
	G	19.59	3.94	16.66
	Н	14.69	2.95	12.50
	А	24.38	19.80	160.75
End of more in a	В	37.38	18.43	192.15
period	С	48.38	19.32	201.01
P	D	42.25	12.69	210.55
	E	33.10	20.31	209.37
	F	29.63	21.78	209.40
	G	40.94	10.90	203.18
	Н	46.34	63.80	216.41

Soil organic matter, ammonium and nitrate nitrogen, available P and exchangeable K were measured following the Page et al. (1982) methods. Organic matter content was calculated by chemical oxidation of soil with 1 mol/l  $K_2Cr_2O_7$  and titration of the remaining reagent with 0.5 mol/l FeSO₄. Soil organic matter was estimated by multiplying soil organic carbon content by the factor 1.724 as reported by Hesse (1972). Both ammonium and nitrate nitrogen were extracted with 0.5 mol/l CaCl₂ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P (P-Olsen) was extracted

with 0.5 mol/l NaHCO₃ and measured by spectroscopy. Finally, exchangeable K was extracted with  $1 \text{ mol/l CH}_3\text{COONH}_4$  and measured by Flame Photometry.

Extract preparation: For phenols determination, 80% of ethanol was added in test tubes which contained 10g of fruit sample. Test tubes were kept for 1 h in dark at room temperature. After centrifugation the extracts were brought to 20 ml with aqueous ethanol and used for further chemical analysis Kanner et al., (1994).

Determination of total phenols content:. Total phenols (TP) content were determined with the Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965) and were expressed as gallic acid equivalent (GAE).

## **RESULTS AND DISCUSSIONS**

Table 3 presents the balances of nutrients in different treatments. Since the industrial tomato has high demands in potassium, each ton of total produced tomato removing from the soil 4 Kg K₂O, addition of all the necessary elements, was insured with the appropriate nutrient fertilization practices. With treatment A, the larger amount of nitrogen and potassium was removed from the ground, as shown in Table 3; nevertheless, the production data, does not justify such a great up taking, so, the excess of water having moved to deeper layers, part of these elements may have been forced to. On the contrary, treatment D with less water use and lower production, removed large amounts of nitrogen, phosphorus and potassium, from soil sampling taken near the dripper.

Table 4.

Total mark	Total marketable production, discarded tomato production (kg $m^{-2}$ ) red / green ratio and									
fruit weight										
Number Total Discarded Production										

	Number	Total	Discarded	l Production		
Traatmont	of Repeats	Marketable	( kg m ⁻² )		Red/Green	Fruit
Treatment	(# of	Production	green	rotten	Ratio	Weight
	fruits)	( kg m ⁻² )				(g)
Α	4 (160)	5.70a	1.82a	2.31a	3.13	51.00a
В	4 (160)	4,37b	1,20b	2,57a	3,64	42,80b
С	4 (140)	3.13c	0.66c	2.35a	4.74	41.60b
D	4 (160)	2.08d	0.84c	2.44a	2.47	43.80b
Sig.		< 0.05	< 0.05	< 0.05	0.077	0.000
E	4 (161)	4.71ab	1.11a	1.60cb	4.24	50.10a
F	4 (160)	4.09b	1.20a	1.28c	3.40	49.80a
G	4 (140)	4.57ab	1.20a	2.22a	3.80	47.00b
Н	4 (160)	5.55b	1.15a	2.04ab	4.82	49.50ab
Sig.		< 0.267	< 0.573	< 0.219		< 0.063
The	Statistical ana	lysis was perform	ed with the c	riterion Dunca	n (p <0.05).	
The Differ	ent letters ind	icate that there are	e statistically	significant dif	ferences.	

The treatment with the larger water percentage (A), resulted to the greater marketable production  $(5.70 \text{ kg/m}^2)$  and the heavier fruit weight (51.00 g). The fact that the same treatment resulted also to the greater discarded production (green=1.82 kg m⁻² and rotten=2.31 kg m⁻²) does not cause finally a loss for the treatment, because the ratio of total marketable production and green, did not differ statistically significantly among the treatments (p=0.077) (Table 4), then, the same treatment gave the greater plant mass (Table 5).

The treatment with the minimal rate of fertilizer (H), exhibited the greatest

marketable production (5.55 kg m⁻²), which means that fertilizer was saved. The organic cultivation showed the lowest production (4.09 kg m⁻²), but did not differ statistically significantly compared to the other treatments. The organic cultivation of industrial tomato can be spread without major problems in Central Greece. The heaviest fruit weight (50.10 g) was exhibited by treatment E but did not differ statistically significantly among treatments F and H. The fact that treatment F showed the least discarded production (green = 1.20 kg m⁻² and rotten = 1.28 kg m⁻²) resulted in the end in favor to the organic cultivation.

The percentage, in other words the red/green ratio, a statistical processing of samples with this parameter was adopted (Table 4); from that, no differences resulted among the treatments (p=0.077). Thus, the different treatments of irrigation and fertilization do not optimize the output of this particular crop, as far as this quality parameter of production is concerned.

The soluble solids (S.S) differ statistically significantly among the treatments; the highest value (5.35%) exhibited the fruits of the treatment with the least quantity of irrigation and the lowest (4.31%) the fruits with the highest quantity of irrigation. The value of this quality parameter also affects the price of the industrial tomato. When it's ranged between 4.0 and 4.6% the price for the producer is  $0.04 \notin kg^{-1}$ , between 4.7 and 5.0% the price rises to  $0.06 \notin kg^{-1}$  and when the S.S are more than 5.1% the price reaches to  $0.07 \notin kg^{-1}$  (Table 5).

Table 5

	Number of	Wet	Number of	6 6	Market				
Treatment	Repeats	weight of plant	Repeats	5.5	Price				
	(# of Fruits)	mass(kg φυτό ⁻¹ )	(# of Fruits )	(%)	(€ kg-1)				
Α	4 (323)	1.91a	4 (120)	4.31c	0.04				
В	4 (352)	1.77a	4 (120)	4.77bc	0.06				
С	4 (252)	1.24b	4 (117)	5.19ab	0.07				
D	4 (289)	1.28b	4 (120)	5.35a	0.07				
Sig.		0.000		< 0.000					
E	4 (16)	1.47a	4 (20)	4.88a	0.06				
F	4 (16)	1.55a	4 (20)	4.86a	0.06				
G	4 (16)	1.55a	4 (20)	4.78a	0.06				
Н	4 (16)	1.64.	4 (20)	4.52a	0,04				
Sig.		0,300		<0,061					
The S	The Statistical analysis was performed with the criterion Duncan ( $p < 0.000$ ). The Different								
letters indic	ate that there are	e statistically significat	nt differences.	- /					

Wet weight of plant mass (kg), percentages of the Soluble Solids of fruits (%) and market price of the industrial tomato ( $\notin$  kg⁻¹)

Due to the fact of the price of the industrial tomato depending from the red production.

In the current study, the lowest price  $(0.04 \in \text{kg}^{-1}, \text{Table 5})$  obtained by a producer where the applied irrigation was at 120% of the evapotranspiration, it would cause not only environmental pollution but also his own financial downfall due to this parameter; this producer had the greatest marketable production but he will obtain the lowest price (Table 5).

Table 6

Treatment	Number of Repeats (# of Fruits)	pH	Acidity (%)			
А	4 (8)	4.65	0.48			
В	4 (8)	4.58	0.48			
С	4 (7)	4.72	0.47			
D	4 (8)	4.64	0.44			
Sig.		=0.163	=0.590			
Е	4 (8)	4.58	0.64			
F	4 (8)	4.57	0.55			
G	4 (7)	4.58	0.77			
Н	4 (8)	4.58	0.58			
Sig.		=0.78	=0.173			
The Statistical analysis was performed with the criterion Duncan (p <0.05). The different letters indicate that there are statistically significant differences						

Values of pH and acidity of fruits (% content of fruit in citric acid)

In fertilization treatments, the S.S did not differ statistically significantly (Table 5). The highest values were exhibited the fruits of treatment E with (4.88%) and F (4.86%). In the current study, the lowest price ( $0.04 \in kg^{-1}$ , Table 4) was exhibited by treatment H which it may have given the highest production but did not cover the benefit accruing from reduced fertilization. Thus, treatment G accomplishes fertilization saving by 20%.

In relation with other quality parameters of the fruits such as pH and acidity, references are mentioning values for pH from 4.26 to 4.82 and for acidity from 0.40 to 0.91% (Stevens et al., 1986). Using these parameters, no statistically significantly differences were found (Table 6). Table 7

sample (whole tomatoes)	Moisture (%) on fresh	Total phenols (mg GAE/kg fresh matter)	sample (cortex)	Moisture (%)	Total phenols (mg GAE /kg fresh matter
А	94.5	220	А	77.5	552
В	94.5	248	В	77.0	564
С	94.0	292	С	76.5	581
D	93.5	302	D	76.0	589
Е	94.0	253	Е	77.0	570
F	94.0	272	F	77.0	573
G	93.5	304	G	77	592
Н	93.0	315	Н	76	620

Total phenols in the whole tomato and cortex

The total polyphenols (Table 7) corresponds to higher values on the cortex compared to the whole tomato. As to the irrigation, the more water is applied the lesser polyphenols are concentrated. The common fertilization exhibited the lowest value, and the 60% of the common fertilization the highest value.

## CONCLUSIONS

This study which was realized for a second successive year, on a neighboring field and a similar soil, showed that the application of different treatments of irrigation, seems to have affected the quality and quantity of the industrial tomato. In particular, it affected the total marketable production. The treatment with the irrigation rate at 120% of the evapotranspiration, gave the highest production but did not resulted to a financial benefit for the producer; the producer has a lower financial result, compared to the 100%, due to the reduced market price of the product, because that treatment, affected negatively the percentage of soluble solids.

Application of different levels of fertilization, affects both the quantitative and qualitative characteristics of the industrial tomato cultivation. The organic cultivation, gave the lowest production (4,09 kg m⁻²), having saved fertilizers by 20%, but did not differ statistically significantly concerning the producer profit, compared to other treatments. The organic cultivation of the industrial tomato can be spread without major problems in the area of central Greece. That treatment concerning a different irrigation and fertilization, didn't affect the rest of the organoleptic characteristics examined with that study, like the average fruit weight, pH and acidity.

The total polyphenol concentrations exhibited the highest values on the cortex compared to the whole tomato (Gougoulias et al., 2012). As to the irrigation, the more water is applied the less polyphenols are concentrated. The common fertilization (E) exhibited the lowest value and the 60% of the common fertilization (H) the highest value.

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# Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# THE INFLUENCE OF HUMIC SUBSTANCES ON GREEN PEPPER CULTURE

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Keywords: humic substances, potassium lignohumate, germination, pepper, yield

## ABSTRACT

Seeds of pepper (Capsicum annuum L.,cultivar Cila, F1 hybrid) after soaked 3 hours in water (V1) and in potassium humate solution 0.01% (V2) were planted in the wafers with peat for germination in greenhouse. The seeds soaked with potassium humate 0.01% sprang in 7-8 days and germination rate was 94.7% while water soaked seeds sprang after 8-12 days and germination rate was 34.6%. Plants derived from soaked seeds were planted in high tunnel and there were sprayed twice with water (V1-Control) and with potassium humate the plants derived from seeds soaked in potassium Humate. Potassium Humate has lead to more rapid flowering by five days, it increased the number of flowers and flowers fertility by 19.4%. The results have shown that Potassium humate recorded a statistically significant increase of fruit yield per plant by 35.3% and the total yield on experimental plot by 64.1% and it stimulated the accumulation of more dry soluble matter in fruits.

## INTRODUCTION

In the present circumstances when the inputs for vegetable production increase substantially more quickly than do the price of vegetables, it is necessary to use all methods which can contribute to effective growing of vegetable: stimulation of seeds germination, increase nutrient uptake from the soil and improve the utilization of nutrients taken up by plants.

Humic substances are the subject of many studies in various areas of soil types, fertility, plant physiology or environment conditions, because of the multiple roles played by this material that the plant growth can benefit (Tan, 1998, quoted by Nardi et al. 2002). On the higher plants beneficial effect may be related to their indirect (increase of fertilizer efficiency or reducing soil compaction), or direct (improvement of the overall plant biomass) effects. Physiological effect of humic substances on higher plants depends on de source, concentration and molecular weight of humic fraction. The effects on intermediary metabolism are less understood, albeit it seems that humic substances may influence both respiration and photosynthesis. Humic matter appears also to display hormone-like activity and exhibit stimulatory effect on plant cell growth and development (Nardi et al. 2002).

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Humic acid (HA) applications increased permeability of cellular membranes in plants and significantly increase seed germination energy and play a significant role in improving pepper plant tolerance to drought stress, the growth and growth parameter of plants and significantly increased N, P, K, S,Fe,Zn,Ca, Mg, S, Cu and Mn contents of shoot and of roots of pepper seedling, while Na contents of shoot and root of pepper decreased with increased humic acid doses (Çimrin et al. 2010).

The results of many studies showed that applied humic substances had a stronger effect on biological characters in early growth stage.

Soaking of seeds by potassium humate promoted germination by high percentage emergence at shorter period because the application of humic acids increase the water absorption and germination rate in wheat (Laila Ali & Elbordiny, 2009).

This research aimed to examine the effect of soaking with potassium humate on germination of pepper seeds and foliar spray with potassium humate on pepper growth and yield.

#### MATERIAL AND METHODS

The study was conducted in greenhouse and high tunnel from SC Serele Adrian SRL Rusanesti in the year 2013. Pepper seeds from red pepper, *Capsicum annuum.L.*,cv Cila F1 hybrid were previously soaked for three hours in water (V1-Control) and in 0.01% Potassium humate (K-Humate) solutions, (V2-KH).

For seeds soaked and for second foliar treatament we used commercial Potassium humate called Potassium Lignohumate AM. Chemical composition of the Potassium Lignohumate AM (made by Amagro s.r.o., Czech Republic) is shown in the table 1.

For first foliar treatement we used Vitalic, a complex soluble water-miscible mixture of potassium substances (Lignohumate, two nitrophenolates and nitroguayacolate) (table 2).

Table 1

SOM	K-humic	K-Fulvic	V	ç	Ц.О			Μ	licroele	ments		
*	Total	Acids	к	5 H ₂ O	Mg	Fe	Cu	Co	B	Mo	Zn	
900	800-850 150	800-850 150-200 80	20	120	0.1	0.1	0.1	0.1	0.15	0.05	0.1	
			00	80 50	30 120	2.0	2.0	1.2	1.2	1.15	1.15	1.2

Chemical characterization of Potassium Lignohumate AM, g/kg

SOM^{*}=Solubile Organic Matter

Table 2

					10
C	Chemical composition and dosage of	Vitalic (b	y Amagro	s.r.o., Czech Republic	2)
	Substance	0/2	a/litro	doso / hostoro —	1

Substance	%	g/ntre	dose / nectare $=$
			0.41
4 – potassium nitro-phenolate	0.225	2.25	0.9 g/ha
2 – potassium nitro-phenolate	0.150	1.50	0.6 g/ha
5 - potassium nitro-guayacolate	0.075	0.75	0.3 g/ha
Potassium lignohumate, AM	15.000	150.00	60.0 g/ha

After three hours soaked seeds were seeded in greenhouse in the peat substrate wafers. The percentage of the sown seeds emerging to the surface was observed and recorded daily and germination percentage was calculated after 14 days.

Two leaf developing stage plants were transplanted in bigger wafers and after another 21 days they were transplanted in high tunnel. Experimental area was  $350 \text{ m}^2$  and divided in two equal plots:

- V1-Control: 650 plants from water soaked seeds were planted and plot area was split in five rows-replicates.

- V2-KH treatment: 650 plants derived from soaked seeds in potassium lignohumate were applied two foliar treatments: first with Vitalic and second with Potassium Lignohumate AM. There was split in five rows-replicates.

Before seedlings planting all experimental plot was fertilized with 2 tones compost and 15 kg complex fertilizers (NPK:15,15,15) and six fertilizations with 1.0 kg NPK (12,61,0) and two fertilizations with 1.0 kg NPK(19,19,19) were carried out by drip irrigation after plants transplanting (Table 3).

For first foliar treatment with K-Humate has been applied Vitalic, 10ml/10 l dose and 14 days after were applied potassium lignohumate AM, 2 g/10 l water.V1-Control plants were sprayed with water to exclude the effect of spraying.

To estimate the effect of potassium humate there were analyzed ten plants for each replicate and there was calculated the total yield which was extrapolated for all survival plants, relate to V1-control and V2-KH. The chlorophyll content determined by Chlorophyll Metter SPAD 502 By Minolta. Taste properties of the fruits were evaluated by measuring total soluble solids (TSS) with refractometter. Also, there was measured the thickness of the pericarp.

#### Table 3

The rates of nutrient in the experiment									
Variant	Base fertilization Dri Before planting, Pla kg/175 m ²		zation Drip irrigation after nting, m ² Planting, kg/175m ²		Seed soaked	Foliar app KF	lication I		
	Compost	NPK 15,15,15	6xNPK 12,61,0	2xNPK 19,19,19		Vitalic	KL**		
V1-Control	1000	7.5	0.5	0.5	Water	Water	Water		
V2-KH	1000	7.5	0.5	0.5	0.01%KL*	1ml/l	0.02%		
TTTT: D				**					

KH*- Potassium humate ;

KL^{**}- Potassium Lignohumate AM

## **RESULTS AND DISCUSSIONS**

Potassium humate promoted a faster germination of soaked seeds and enhanced germination percentage. We used in this experiment a very low percentage of germination seeds produced in bad weather conditions in autumn 2012. Soaked seeds in distillated water started to germinate after seven days and finished after twelve days, total germination percentage was 34.6%, close to certificate germination value (35%). Results showed that the diluted solution of 0.01% potassium Lignohumate AM promoted shorter period of germination by 2-6 days, seeds germinated after 5-6 days in uniform seedlings and final germination percentage was of 94.7% (Table 4).

Table 4

Effect of 0.01% Potassium Lignohumate AM solution on germination of seeds soaked and seedlings plants

Variants	Germination rate,%	Germination time, days	Number of survived plants	Survival Percentage %
V1-Control	34.6	7-12	525	80.8
V2-K-H	94.7	5-6	637	98.0

Application of potassium humate to soak seeds has increased the seeds germination, energy of germination and also stimulated the growth and development of sprouts. Our results are in agreement with results of Laila Ali & Elbordiny, 2009, who reported that soaking treatment enhanced germination of wheat seeds. Seed treatment by potassium humate for 6-12 hours and direct planting in greenhouse without seed germination under in vitro caused an increase of seed germination percent and tuber yield of hybrid potato seeds (Hassanpanah & Khodadi,2009). Optimal level of soaking is thought to have enhanced effects on germination and growth, probably, due to hydrolysis of complexes into simple sugars that are readily utilized in the synthesis of auxins and proteins. The auxins produced help to soften cell walls to facilitate growth and the proteins readily utilized in the production of new tissues (Laila Ali & Elbordiny, 2009).

Soaked seeds with potassium humate influenced the number of plants that survived after transplantation due stimulated growth of roots and sprouts and increased survival percentage by 21.3% (Table 4). This suggests that potassium humate enhanced plants immunity and improve plant resistance to the stress factors like negative temperatures registered few nights in spring of 2013. The presence of Humic Substances is important during all stages of plants development but particularly vital in the early stages. That is why the pre-planting treatment of seeds is very important. Even before germination begins, vital forces are awakened, and the immune system is stimulated (Levinsky, 2009).

Foliar application of K-Humate, first time as Vitalic, and second treatment with potassium lignohumate achieved earlier flowering of pepper by eleven days and increased flowers fertility by 19.4% (table 5).

Potassium humate treatment of seeds and foliar treatment stimulated cells development and differentiation tissue, has increased the number of flowers and faster the simultaneous appearance of flowers at various levels along the stems.

Total pepper fruit yield was registered of 5.1 kg/plant for control variant and 6.9 kg/plant to treatment variant. Soaked seeds treatment and foliar application of K-Humate enhanced significant growth of fruit yield/plants and yield/variant so the application of potassium humate improved the production by 35.3% and 64.1% (table 5).

Table 5

Variants	Flowering	Flowers	Thickness of the	Fruits yield	Fruits yield
	time, days	fertility,%	pericarp, mm	Kg/plant	Kg/35 m ²
V1- Control	36	83.1	6.1	5.1	535.5
V2-K-Humate	25	99.2	7.6	6.9	879
V2/V1,%		119.4	124.6	135.3	164.1
			DL5%=1.19	DL5%=0.94	DL5%=79.27
			DL1%=2.02	DL1%=1.17	DL1%=254.70
			DL0.1%=3.21	DL0.1%=3.80	DL0.1%=321.99

Effect of treatment with Potassium Humate on yield of Cil	la hybrid, F1
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Our results are comparable with the results of other experiments (Gadimov et al., 2009; El-Bassiony et al., 2010). Treatment of tomato seeds with 0.01% Potassium humate solution before planting for 24 hours related to variety increased production by 20-25%. Also, Pre-planting seed treatment of cucumber with 0.01% potassium humate solution for 24 hours increased production by 38%; as regard the variety, germination percentage of hazel-nut seeds was increased between 37.08 and 64.14%. Foliar treatment of pepper plants with potassium humate stimulate vegetative growth, fruit yield components and fruit quality expressed as titration acidity, total soluble solids, total carbohydrates, vitamin C, anthocyanin, total polyphenols, tannins and mineral content (Ghoname et al., 2009). High

efficacy of foliar fertilization whit humic fertilizers is due to specific effect those to increase adhesion and penetration into the assimilator parenchyma and by stimulating biosynthesis of chlorophyll, amino acids, dynamics of ergon and other metabolic reactions by cathalyzation and enzymatic stimulation of humic acids (Dorneanu et al., 2011)

Potassium Humate provided significantly thickness of pericarp (6,1 mm to control to 7,6 mm to V2-KH) (Table 5) and increase fruits size (data not shown).

Treatment with potassium humate stimulated chlorophyll synthesis. Data given in the Figure 1 show the chlorophyll medium content of pepper leaf at three level position from the steam. Treatment whit potassium humate enhanced chlorophyll content by 10.2-13.2 SPAD units related to control which means an increase by 20.6%.

Anton et al.,2011 showed that humic fertilizers tested by foliar fertilization on lettuce increased the content in total assimilatory pigments and the content of each pigment in part, especially chlorophyll a and b.



Figure 1 Effect of potassium lignohumate on chlorophyll content of pepper leaf



Figure 2 The effect of potassium humate on dry soluble matter in pepper fruits

Application of potassium humate improved testing quality of the fruits determined as total soluble matter. Data from Figure 2 show that red fruits have more total soluble matter relative to green or yellow fruits. Potassium humate increased the content of total soluble matter with  $0.3^{0}$  Brix degree relative to control variant for green fruits and  $0.7^{0}$  Brix for red fruits. Potassium humate treatement promote more accumulation of total soluble dry matter with 9.36%, whatever the degree of maturation of the fruit.

## CONCLUSIONS

Potassium humate significantly increased germination of pepper seeds soaked three hours before sowing.

Soaked seeds pre-treatment whit potassium humate shorted germination time, increased survival rate of seedling and number of survived plants after transplantation.

Potassium Humate solution – soaking seeds and foliar treatment- shorted the time of flowering and increased the fertility of flowers.

Potassium humate stimulated chlorophyll accumulation in leaf and accumulation of total soluble solid in fruits.

Potassium humate very significantly increased fruits yield per plant and total yield.

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# COMPARATIVE STUDY OF NEW TURKISH TOMATO HYBRIDS ON PRODUCTION CAPACITY AND EARLY MATURITY FOR VEGETABLE PRODUCTION IN ROMANIA

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Keywords: Turkey hybrids, early maturity, productivity, cold greenhouse

#### ABSTRACT

This paper presents preliminary studies in comparative culture orientation of new hybrids of tomato (Lycopersicum esculentum Mill.): Nemesis F1, Giraffe F1, Antalya II F1 Peradur F1, of Turkish origin, developed by Yüksel Seeds Company. Production capacity was tested, the earliness and variability of fruit traits, compared with Antalya F1 hybrid taken as reference. Total production from new hybrids obtained was superior to the hybrid taken as reference Antalya F1, only Antalya II F1 and Nemesis F1 hybrids showed statistically increases. Comparing these hybrids between each other we found that Nemesis F1 hybrid is superior to full production Antalya F1, Giraffe F1 and Peradur F1 hybrids, also Anlalya II F1 is superior to Antalya F1 and Peradur F1, the differences being statistically assured. The earliness production is similar to the control, the first harvest is accomplished 58-64 days after transplanting.

#### **INTRODUCTION**

Decision for choosing the genotypes for cultivation should be taken only after careful studies regarding the market requirements, specific costs applied technology and efficient use of ensemble technological factors, because the same climatic conditions and different production technology require different genotypes of the same species to be grown (Ciofu Ruxandra and et al., 2003).

The increasing demands of the market and high exigency for quality of Romanian consumers to have as early as possible local fruit of tomato request the enlargement of the range of tomato varieties by testing and introducing new hybrids with very early maturity in spring and high yield capacity. (Petrache M. and et al., 2010).

Choosing a new tomato variety suitable of culture needs to be done properly according to local climate and soil. This state of permanent attention of researchers in Romania need to pay more attention to the recommendation of tomato varieties suited for the climatic conditions of the area (Elena Maria Drăghici, Elena Dobrin, 2011).

In Romania, the assortment of cultivated tomatoes expanded during the last years, through the introduction of new hybrids and varieties created in our country or abroad (Maria Călin and et al., 2009)

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#### MATERIAL AND METHODS

In 2013 we performed a comparative cultural orientation study on early tomato hybrids, placed in a cold greenhouse in SC SERELE ADRIAN Rusanesti - Olt, an area with large surfaces of protected vegetable crops in Romania.

The origin and type of the hybrids grown in the study are shown in table 1, and the variants obtained were:V1 - Antalya F1 (control); V2 - Nemesis F1; V3 - Giraffe F1; V4 - Peradur F1; V5 - Antalya II F1.

Specific technology used was the early tomato crop in cold greenhouses or solarium.

Each hybrid (experimental variant) was planted on April 5, in four repetitions, on two rows, after the scheme  $50 + 90 \times 40$  cm, the area of the experimental plots of  $10 \text{ m}^2$ , with a total of 36 plants for each repetition.

As phenological observations were recorded the planting date, the first harvest and last harvest, and biometric measurements on early and total production, the weight and fruit shape index.

## **RESULTS AND DISCUSSIONS**

Since the early Antalya F1 hybrid tomato is very much cultivated in our country, Yüksel Seeds Company in Turkey has provided for testing new hybrids, shown in Table 1, compared with Antalya F1 hybrid taken as reference.

Regarding the type of growth, most of them have indefinite growth, as Antalya F1, except Giraffe F1 hybrid that have semi-determined growth type, the growth ceasing after 5-6 inflorescences.

Tabla	1
raute	т

Cultivars, origin and growth characteristics									
Variants	Cultivars	Type of cultivar	Origin	Type of growth					
V1	Antalya F1	Hibrid F1	Yüksel Seeds (Turkey)	indeterminate					
V2	Nemesis F1	Hibrid F1	Yüksel Seeds (Turkey)	indeterminate					
V3	Giraffe F1	Hibrid F1	Yüksel Seeds (Turkey)	semi-determined					
V4	Peradur F1	Hibrid F1	Yüksel Seeds (Turkey)	indeterminate					
V5	Antalya II F1	Hibrid F1	Yüksel Seeds (Turkey)	indeterminate					

Analyzing the obtained average yields per plant (table 2), we found that all hybrids tested had higher production than the reference Antalya F1 hybrid, with outputs ranging from 4.5% to 30.4%. Statistically, only Nemesis F1 and Antalya II F1, hybrids recorded production increases of 30.5% and 24.7% that are statistically distinct significant differences compared to the control, and, respectively, significant, the other hybrids recording productions close to Antalya F1 hybrid.

Table 2

Production results of the studied hybrids									
Hybrids	Average yield kg/plant	Relative yield %	$\begin{array}{c} \text{Difference} \\ \pm \text{kg} \end{array}$	t	P %	Significance degree			
Nemesis F1	4.81	130.5	+0.89	3.7	0.3	* *			
Antalya II F1	4.64	124.7	+0.72	3.0	1.1	*			
Peradur F1	4.23	110.6	+ 0.31	1.3	2.7				
Giraffe F1	4.05	104.5	+ 0.13	0.5	62.6				
Antalya F1 (control)	3.92	100.0							

Production results of the studied hybrids

DL 5% = 0.523; DL 1% = 0.734; DL 0,1% = 1.037

Comparing tested hybrids between them by Duncan test (table 3) there can be noticed that the average yield per plant obtained by Nemesis F1 hybrid is superior to Antalya F1, Giraffe F1 and Peradur F1 hybrids, the difference being statistically assured. Also Antalya II F1 hybrid production is superior to Antalya F1 and Giraffe F1 hybrids.

Table 3

Classification	Variant (Hybrid)	Average yield (X) kg/plant	X – 3,92	X – 4,05	X – 4,23	X - 4,64
Ι	Nemesis F1	4.81	0.89 *	0.76 *	0.58 *	0.17
II	Antalya II F1	4.64	0.72 *	0.59 *	0.41	
III	Peradur F1	4.23	0.31	0.18		
IV	Giraffe F1	4.05	0.13			
V	Antalya F1	3.92				

Multiple comparisons (Duncan test)

 $q_2 = 0,52; q_3 = 0,55; q_4 = 0,56; q_5 = 0,57$ 

Regarding the earliness and quantity of production per unit area (table 4) it reveals that the first harvest was done after 52 days from transplanting at Giraffe F1 hybrid, which proved to be the earliest, followed by Peradur F1 with 58 days, they are 7 days earlier and, respectively, one day earlier, compared with the reference taken Antalya F1 hybrid. At Nemeis F1 and Antalya II F1 hybrids, the first harvest was made 2 and 5 days after Antalya F1 hybrid.

Harvesting time of the tested hybrids ranged from 43 days with Peradur F1 hybrid and 50 days for Antalya II F1 and production on the surface of 10  $m^2$  ranged from 141.12 kg to 173.16 kg for Antalya F1 and Nemesis F1.

Table 4

Hybrids	1 st harvest DAT	Last harvest DAT	Days of harvesting	Total Tomato Yield kg/10 m ²
Nemesis F1	61	109	48	173.16
Antalya II F1	64	114	50	167.84
Peradur F1	58	97	43	152.28
Giraffe F1	52	102	44	145.80
Antalya F1 (control)	59	105	46	141.12

The earliners and Total Tomato Yield of the studied hybrids

DAT = Days after transplanting

Analyzing the early productions of the tested hybrids obtained in the first decade of June in the experimental plot area (table 5) there can be observed that all hybrids have lower yields than the control variant (Antalya F1), between 15.16 kg/10 m² at Giraffe F1 hybrid and 18.12 kg/10 m² at Antalya II F1 hybrid, the share of total production being from 10.2 to 11.2% for the new hybrids compared with 13.8% as Anlalya F1 has recorded.

Table 5

Assessment of total and early tomato production							
	The yield at the	Total Tomato	Percent of early				
Hybrids	1 st decade of June	Yield	yield from total				
	kg/10 m ²	kg/10 m ²	production				
Nemesis F1	17.66	173.16	10.2				
Antalya II F1	18.12	167.84	10.8				
Peradur F1	17.05	152.28	11.2				
Giraffe F1	15.16	145.80	10.4				
Antalya F1 (control)	19.47	141.12	13.8				

Referring to the main physical properties of tested tomato fruit hybrids (table 6) there can be seen that the shape given by the value of the form is globular-flattened at Antalya F1 and Nemesis F1 hybrids (shape index value of 0.92 and 0.94) round, slightly flattened at Giraffe F1 and Antalya II F1, and oval with Peradur F1 hybrid (IF = 1.12).

The average weight of round-oblate fruit hybrids ranged from 171.1 g Giraffe F1 to 208.6 g Nemesis F1 and with Peradur F1 hybrid that have ovoid fruit, of 105 g.

The variability of the form index has been low to all hybrids and the fruit weight has recorded a middle coefficient of variation the hybrids having a good uniformity.

Table 6

Urshaida	Index form			Weight (g)			
nyblius	х	s	s %	Х	S	s %	
Antalya F1	0.92	0.08	8.4	182.4	33.9	18.58	
Nemesis F1	0.94	0.07	7.8	208.6	40.1	19.22	
Giraffe F1	0.98	0.06	6.3	171.1	28.7	16.77	
Peradur F1	1.12	0.08	6.8	105.8	16.3	15.41	
Antalya II F1	0.96	0.09	9.3	195.2	37.7	19.31	

The variability of tomato fruit features with studied hybrids

#### CONCLUSIONS

Based on the analyzed data there were issued the following conclusions:

- All tested hybrids showed higher production related to control Antalya F1 hibrid ranging from 4.5 % to 30.4 %, only Nemesie F1 and Antalya II F1 hybrids which recorded distinct significant and, respectively, significant differences to control;

- the average yield per plant ranged from 3.92 kg with Antalya F1 hybrid to 4.81 kg with Nemesis F1 hybrid;

- the earliest production was obtained by Giraffe F1 hybrid wich was first time harvested 52 days after transplanting, 7 days earlier than Antalya F1control hybrid;

- the variability of the form index presented, to all hybrids, a low coefficient of variability and medium fruit weight coefficient of variation, the hybrids having a good uniformity.

We recommended for cultivation the Nemesis F1 and Antalya II F1 hybrids for productivity and Giraffe F1 hibrid for earliness.

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# RESEARCH ABOUT METHOD OF TRANSMISSION THE "COLUMNAR" CHARACTER TO DIFFERENT APPLE HYBRID COMBINATION

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**KEYWORDS**: apple, hybrid combinations, columnar character

#### ABSTRACT

At SCDP-Vâlcea, hybrid combinations have been used, where the parent genitors have been columnar breeds: Trident, KV 8, Wijcik, KV 42, Telamon and Red Grieve Newman. In the year 2007, a number of 1365 hybrids were obtained and planted. After 6 years of growth on their own roots, it was found that: 65.6% are columnar type plants, 12.1% plants with semi-erect port and 22.3% plants with displayed port. Most columnar descendants have been given combinations that had the maternal genitor KV 8 (77,6%), KV 42 (64,3%) and Trident (60,0%).

Resistance of columnar hybrid to attack of Endostigme inequalis was 100% for hybrids derived from Telamon and up to 93.3% for those from Trident. At the columnar hybrids, regardless of hybrid combination, the buds have a large density (1 to 3.3 cm) and at those with lax port, the distance between nods is higher (1 to 5.7 cm).

The columnar character is transmitted relatively easy to maternal genitor in descending, proving to be a dominant character type.

## **INTRODUCTION**

Apple is one of the most important fruit crops in the world and also in our country. The tendency is both to increase fruit production, by enriching their quality and by creating new varieties.

One of the objectives of genetic improvement programs worldwide is to create columnar varieties port, which allow super intensive crops, and fruit to meet consumer demands. Such programs are conducted at: University of Geisenheim (Germany), University of Bologna (Italy), Rutgers University (USA), INRA Angers (France), ICDP Pitesti (Romania), etc. (Braniște and Șerboiu 1991; Cociu et al, 1999 Hough, 1994; Sestraș, 2004).

The results obtained in England, Russia, Germany and others were completed by obtaining the columnar-type varieties, but not yet with the fruit quality adapted to the market requirements. (Trident, Telamon, Starcats, Redcats) etc.

In this regard, we proposed at the University of Craiova - SCDP Vâlcea to obtain columnar genotypes, with improved quality and resistance to illness.

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# MATERIAL AND METHODS

The program was developed from 2007 to 2012 and included the following maternal genitors of columnar type with different origin.

- Maternal genitor: Trident, Wijcik, KV 8, Telamon, KV 42;

- Paternal genitors: KV-71.

Pollination was free, with mixed pollen from different varieties (Topaz, Rubinola, Goldstar, etc.) and in addition that was used in combination with the Red Grieve Newman (standard) variety which was pollinated with pollen from KV-71 (columnar).

Obtained hybrids were planted in the hybrid field at  $3,5 \ge 1$  m distance between them. On the plants were made observations and measurements about the mode of transmission the plant character (columnar, semi erect, lax), the growth vigor (SST) and the attack of scab (*Endostigme inequalis*) at the age of 6 years.

#### **RESULTS AND DISCUSSIONS**

In the 6 hybrid combinations, where one type of genital was been columnar, the columnar port occurred in progenies with different percentages. Columnar type progenies hybrids were obtained in 52% percentage at (Telamon x pole. Lib.) to 86% percentage at (Red Grieve Newman x KV-71) (Table 1).

Columnar nature in the progeny is generally dominant (65.6%), beside to semi erect type (12,1%), or lax type (22.3%). From Telamon x pol. combinations lib. and Red Grieve Newman x KV - 71 hybrids were not obtained erect type, descendants were been often, lax or columnar type.

Columnar character is transmitted very easily in lineage and it appears as a dominant character.

At six years of growing on their own roots, it is found that the trunk section area (SST) is lower at columnar hybrids (87.2 cm2) than to those with semi erect port (109.2 cm²) or with lax port (124,0 cm²) (Table 2).

The average percentage of hybrid columnar to SST is only 70.3% from the lax type hybrids and 88.0% to semi erect type from the lax type hybrids. In all hybrid combinations, at the progenies with columnar port, the SST was lower compared to semi erect or lax types.

Comparison between hybrid progenies environments on each combination, showing us that the lowest values were recorded SST Wijcik x pole. lib.  $(90,0 \text{ cm}^2)$  and KV 8 x pole. lib.  $(98,7 \text{ cm}^2)$  and highest in KV 42 x pole. lib.  $(128,0 \text{ cm}^2)$ . To KV42 x pol.lib., combination, the columnar hybrids showed a only 54.2% on SST, compared with those who wear lax port.

Another important element that has been pursued in the hybrids progenies, it was the frequency of scab attack on leaves (*Endostigme inequalis*) (Table 3), to 4 hybrid combinations. To columnar hybrids the attack frequency varied from 2.6% (KV 8 x pole. Lib.) to 6.7% (Trident x pol.lib). Reporting the number of attacked plants, to the total number of progenies provided from every type of plant, results that only 2.3% of the lax port hybrids and to 3.8% in those with columnar port were attacked by apple scab.

Scab resistance gene was transmitted to all hybrid combinations in over 93.0% of cases, the explanation being that genitors are holding it. To the columnar, semi erect and lax type progenies combination of Wijcik x Pol. lib. It is found a good scab resistance, even if the genitor Wijcik has no gene with resistance to disease. The resistance can be attributed to the character transmission from the pattern genitor.

Nr.	Hybrid combinations	Hybrid plants	Type of growth to descending hybrids					
crt.		total	Columnar		Semi erect		Lax	
			No. of hybrids	%	No. of hybrids	%	No. of hybrids	%
1	TRIDENT X POL.LIB	125	75	60,0	25	20,0	25	20,0
2	KV 8 X POL.LIB	490	380	77,6	40	8,1	70	14,3
3	WIJCIK X POL. LIB	370	200	54,1	50	13,5	120	32,4
4	KV 42 X POL. LIB	280	180	64,2	50	17,9	50	17,9
5	TELAMON X POL. LIB	50	26	52,0	-	-	24	48,0
6	KV - 71	50	43	86,0	-	-	7	14,0
TOTAL		1365	904	65,6	165	12,1	296	22,3

# The growth type at apple plants according to the hybrid combination (2007-2012)

Table 1

Nr.	Hybrid combinations	The hybrids progenies trunk section average area(cm ² )			In combination media
crt		Columnar	Semi erect	Lax	
1	TRIDENT X POL.LIB	85,0	131,0	116,0	110,7
2	KV 8 X POL.LIB	88,0	99,0	109,0	98,7
3	WIJCIK X POL. LIB	80,0	96,0	94,0	90,0
4	KV 42 X POL. LIB	96,0	111,0	177,0	128,0
5	TELAMON X POL. LIB	-	-	-	-
6	KV - 71	-	-	-	-
MEDIA (cm ² )		87,2	109,2	124,0	106,8
DIFFERENCE SST (%)		70,3%	88,0%	100%	

# Increasing the thickness of the trunk at apple progeny hybrids depending on the plants growth type (6 years after planting)

Table 2
Nr.	Hybrid combinations	Total	Endostigme inequalis attack frequency (%)								
crt.		no. of	(	Columnar			Semi ere	ect		Lax	
		hybrid plants	Total no. of hybrids	Of witch: attacked	%	Total no. of hybrids	Of witch: attacked	%	Total no. of hybrids	Of witch: attacked	%
1	TRIDENT X POL.LIB	125	75	5	6,7	25	2	8,0	25	-	-
2	KV 8 X POL.LIB	490	380	10	2,6	40	-	-	70	2	2,8
3	WIJCIK X POL. LIB	370	200	11	5,5	50	1	2,0	120	4	3,3
4	KV 42 X POL. LIB	280	180	6	3,4	50	-	-	50	-	-
5	TELAMON X POL. LIB	50	20	-	-	-	-	-	30	-	-
6	KV - 71	50	40	-	-	-	-	-	10	-	-
	TOTAL	1265	835	32	3,8	165	3	1,8	265	6	2,3

# Apple hybrids response to scab attack (Endostigme inequalis)

Table 3

#### CONCLUSIONS

The hybrid combinations of apple, where one of the genitor is columnar type, transmitting this character to progenies with an over 65,6% rate, compared to semi erect port progenies with (12,1%) or lax port with (22,3%).

The columnar character was transmitted to progenies, where the mother genitor was columnar and so, where the pattern genitor was columnar too. Columnar descendants achieved lower increases in trunk area ( $87,2 \text{ cm}^2$ ) than those semi erect ( $109,2 \text{ cm}^2$ ) or lax ( $124,0 \text{ cm}^2$ ), after 6 years from planting.

*Endostigme inequalis* resistance gene was transmitted in progeny with more than 96,2% percentage (columnar) to 98,2% (semi erect). Differences in the frequency of attacks was reduced (3,8 to 1,8%) regardless of the progenies port.

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# STUDY ON THE VEGETATIVE PROPAGATION OF SOME BEGONIA L. SPECIES

Manda Manuela, Nicu Carmen¹

Key words: Begonia sp., vegetative propagation, rooting medium

#### ABSTRACT

The present paper presents the results regarding the vegetative propagation of some Begonia species. The rooting duration and the quality of the cuttings rooted at the studied species were monitored in two kinds of media: peat and sand; peat and perlite. Parameters investigated include the rooting duration and the quality evaluation of the cuttings according to the used medium, by determining the length of the cuttings, the number and the length of the roots. The rooting duration was different from a species to another: between 69 days at Begonia rex 'Fairy' and 111 days at Begonia masoniana. The most indicated rooting medium according to the species was peat and sand for Begonia masoniana and peat and perlite for B. rex 'Fairy' and B. rex 'Inca Fire'.

#### **INTRODUCTION**

Begonia is the largest genus of flowering plants, with 1400 - 1500 species and hybrids with an infinite selection of sizes, growth habits, foliages, and flowers. It consists of herbaceous perennials that are widely cultivated as ornamental houseplants, mainly for their showy flowers and colorful leaves (Hvoslef A. K. and Munster C., 2006; Doorenbos et al., 1998; Tebbitt C.Mark, 2005; Tjia B. and R. J. Black, 2003). In recent years, many field surveys on begonias had been carried out in the world and new species continues to be discovered and described. It is estimated that there are about 10,000 Begonia hybrids and cultivars worldwide (Peng et al., 2010). The genus is widely distributed and mostly pantropical (Forrest et al., 2005).

Most foliage begonias, especially the Rex types, are very useful design elements because of the large selection of colors and patterns available. In many cases, the foliage begonias are excellent alternative to some of the flowering potted plants because they offer different colours, textures, and a product which outlasts most floral products by a considerable margin (Tjia B., 2003; Poole and Henley, 1989).

Given the wide diversity of species and varieties within the Begonia genus, the knowledge of the biological characteristics of plants and their response to various environmental and agro-technical works are essential in the design and application of the advanced technologies (Selaru Elena, 2000).

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Begonias can be propagated using a number of different methods, but most commonly from seeds, stem cuttings and leaf cuttings. (Anton D., 2006; Cruceru S., 2006; Hartmann T.H , 1993; Vidraşcu P., 2002). From "leaf cutting" method of propagation is often considered the method of choice amongst growers and is more common with rex and rhizomatous begonias. (Oyeyemi 0., 2011)

The researches in our country are scarce and worldwide are not systematic; the optimal medium for rooting differ from author to author and from species to species. Common mediums recommended for rooting cuttings of begonia are perlite and vermiculite or a combination of both. Another medium for rooting is peat moss or various combos of peat moss, perlite, and vermiculite. (Thompson, B., 2002). Claude Ferry, 2004 recommends a nutritive ground of 60% peat, 30% leaves soil and 10% sand plus a slow releasing fertilizer of 16-11-11 type, the appropriate pH being 6-6.5. (Cruceru S., 2007)

This paper presents the results of the vegetative propagation (by leaf cuttings) of two species of the *Begonia* genus: *B. masoniana* and *B. rex*, for the last species being also studied two varieties: 'Inca Fire' and 'Fairy'. The vegetative propagation of the studied species of Begonia was monitored using two media for rooting: peat and sand (1:1); peat and perlite (1:1).

#### MATERIAL AND METHODS

The initial biological material consisted of *Begonia masoniana*, *Begonia rex* 'Inca Fire' și *Begonia rex* 'Fairy' cuttings, obtained from the Floriculture discipline collection of the Faculty of Agriculture and Horticulture in Craiova.

*Begonia masoniana Irmsch.*, originally from China, is a robust plant with large (16-20 cm length and 10-12 cm width) cordiforme, embossed leaves with red hairs on the top folds and a brown drawing in the form of a cross on the line of the main veins, hence the name "iron cross".

*Begonia x rex cultorum Bailey.* includes many hybrids with rhizomatic, pulpy, creeping way of growing stems, and it is the most common begonia cultivated for its ornamental leaves. The leaves are very beautiful, cordiforme-asymmetric, embossed, sharply pointed, with a wavy and slightly serrated edge. The colour varies from green to reddish purple or silver and has concentric zones, spots or mosaics in shades and different colours, which are also very attractive. The pink or red flowers are insignificant compared to the leaves. (Anton Doina, 2006)

*Begonia rex* 'Inca Fire' is 25 cm high and 40 cm diameter. The leaves are 13 cm long and 9 cm width, are skew-asymmetric, ovate, of red colour with metallic luster. The petiole is red and covered with pink hairs.

*Begonia rex* 'Fairy' is 20 cm high and 40 cm diameter. The leaves are 17 cm long and 12 cm width, are skew-asymmetric, ovate, of olive green colour with large silvery spots on the upper side and red on the lower side. The petiole is red covered with red hairs.

The research was conducted between 2011 and 2012 in the greenhouse of the Floriculture discipline of the Faculty of Agriculture and Horticulture, a greenhouse with average temperatures ranging between 18 and 20°C, during experimentation. The vegetative propagation of the studied species of Begonia was monitored using two media for rooting: peat and sand (1:1); peat and perlite (1:1), resulting in the following experimental variants: V 11- B. masoniana/P+P; V12-B.masoniana/P+N; V21-B. rex 'Inca Fire'/P+P; V22-B. rex 'Inca Fire'/P+N; V31-B. rex 'Fairy'/P+P; V32-B. rex 'Fairy'/P+N. There were used leaf mature cuttings, harvested in November 2011. The cuttings were reaped, shaped and treated with Radistim1. Parameters investigated include the rooting

duration and the quality evaluation of the cuttings according to the used medium, by determining the length of the cuttings, the number and the length of the roots.

#### **RESULTS AND DISCUSSIONS**

The data presented in Table 1 show that the percentage of rooting of the Begonia cuttings was not influenced by the rooting substrate, the recorded values being maximal (100%) for all the experimental variants.

Analysing the effect of the substrate on the rooting duration of the cuttings, there was observed that for *B. masoniana* the best results were obtained in the peat+ sand substrate, when the rooting duration of the cuttings was of 107 days (V11), compared to 115 days for the peat + perlite substrate (V12), Table 1.

The best substrate for the rooting of the *B*. *rex* 'Inca Fire' cuttings was peat + perlite, the rooting duration of the cuttings being of 77 days in comparison to 87 days for the peat + sand substrate.

For the *B. rex* 'Fairy' the best results of the rooting of the cuttings were obtained in the peat + perlite substrate (66 days), compared to 72 days when the cuttings were rooted in peat + sand.

Making a comparison between the two species, the results show very large differences in terms of the rooting duration of the cuttings. The best results in this respect were obtained for *B. rex* with values ranging between 69 days and 82 days, as compared to *B. masoniana* which rooted after 111 days. (Table 1)

Between the two varieties of *B. rex* the difference in terms of the rooting duration of the cuttings was of 15 days for the 'Fairy' variety, the average values ranging between 69 and 82 days.

Table 1

Variants	Data harvesting leaf cuttings	Data rooting cuttings	Rooting duration	Media	
V11- B. masoniana/P+P		27.02.2012	115	111	
V12-B. masoniana/P+S		19.02.2012	107	111	
V21-B. rex 'Inca Fire'/P+P	5 11 2011	20.01.2012	77	82	
V22-B. rex 'Inca Fire'/P+S	5.11.2011	30.01.2012	87		
V31-B. rex 'Fairy' /P+P		9.01.2012	66 (0		
V32-B. rex 'Fairy'/P+S		15.01.2012	72	09	

The effect of the substrate on the rooting duration of *Begonia sp.* 

In figure 1, the average number of roots for the two species of Begonia recorded the highest values of the cuttings rooting in the peat + perlite substrate, the values ranging between 8.5 and 12.25 roots/plant, in comparison to 8.5 -10 roots/plant, the latter values corresponding to the peat + sand substrate.

Regarding the average length of roots for the *B. masoniana* cuttings, the highest values were recorded for the peat + sand substrate (V12-10.06 cm), and for the two varieties of *B. rex* the maximum values of the root length correspond to the peat + perlite substrate (V21-9.08 cm for *B. rex* 'Inca fire', V31-9.2 cm for *B. rex* 'Fairy').

The figure 2 shows that the *Begonia masoniana* seedlings developed better in the peat + sand substrate (4.74 cm high and 11.42 cm diameter), in comparison to the rooted cuttings in the peat + perlite substrate (4.42 cm high and 9.87 cm diameter).



Figure 1. The effect of the substrate type on the rooting of the Begonia masoniana, B. rex 'Inca Fire' and B. rex 'Fairy' cuttings



Figure 2. The effect of the rooting substrate on the height and diameter of the seedlings

For the two varieties of *Begonia rex* the best results in terms of the vegetative growth were obtained using the peat + perlite substrate (4.58 cm high, 11.25 cm diameter for 'Inca Fire' and 4.92 cm high, 13.2 cm diameter for 'Fairy').

#### CONCLUSIONS

The differences between the two species of *Begonia* in terms of the rooting duration of the cuttings were significant, ranging between 69 and 82 days for *Begonia rex*, in comparison to *Begonia masoniana* which rooted after 111 days.

The best results in terms of the rooting duration were obtained for *Begonia* masoniana in the peat + sand substrate, and for the two varieties of *Begonia rex* in the peat + perlite substrate.

For *Begonia rex* species there is a difference of 15 days between the average values of the rooting duration in the favour of the 'Fairy' variety.

Regarding the average length of roots, the highest values were recorded by B. *masoniana* for the peat + sand substrate, and for the two varieties of B. *rex* the maximum root length values correspond to the rooted plants on the peat + perlite substrate.

The average number of roots, depending on the used rooting substrate, recorded the highest values for rooting the cuttings in the peat + perlite substrate, for the both species of Begonia.

In terms of the effect of the rooting substrate on the average height and diameter of the seedlings, the best substrate for *Begonia masoniana* and *B.rex* 'Fairy' was the peat and sand, and the best results for *B rex* 'Inca Fire' were recorded in the peat + perlite substrate.

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# THE SWEET CHESTNUT (CASTANEA SATIVA Mill.) AND ITS PRESPECTIVES OF GROWING IN GREECE AND ROMANIA

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Keywords: Castanea sativa, sweet chestnut, fruit growing

#### ABSTRACT

The sweet chestnut (Castanea sativa Mill.) is a fruit tree species that can be found in forests and orchards from Greece and Romania. The species originated in Europe since the Tertiary and Quaternary, but after repeated glaciations during 70,000-20,000 BC its area was confined to a few areas called "survival refuges" located also on the actual territory of Greece (in the Peloponnese and in the north east) and the eastern and northwestern Bulgaria. In Romania sweet chestnut were introduced during the Roman Empire but most authors consider more reliable its introduction to several monasteries between the years 1400-1500 AD. Chestnut spread in both countries by natural migration into surrounding areas and through planting into forest areas. Subsequently, modern plantations were established in Greece (over 8600 ha) and Romania (several tens of hectares). Suitable climatic conditions from both countries and the increase of demand for chestnuts, which are edible fruit with high nutritional value, favor the expansion of areas planted with this fruit tree crop.

## **INTRODUCTION**

The sweet chestnut (*Castanea sativa* Mill.) is the most important species of the *Castanea* genus for countries in Europe and Middle East. It is found in all the forests of Central and South Europe and in the chestnut orchards and it is used for its valuable timber and fruits, which are very important for food and trade.

Bounous et al. (2002) mentions that this species is valuable for other items considered valuable: nectar, pollen, bark (for tannin extraction), etc.

Sweet chestnut growing occupies relatively large areas worldwide (540.732 ha), China being on the first place (310,000 ha), followed by Bolivia (43.856 ha), Turkey (38.440 ha), Portugal (36.648 ha), etc (FAO Stat Database, 2013). The FAO statistical data does not refer to areas of chestnut forests, each country separately communicating the chestnut forests or groves and the chestnut orchards (Conedera et al., 2004).

World production of chestnuts in 2011 was 2,023,019 tons. The most important producers in the world are the following countries: China (1,700,000 t), South Korea (73,660 t), Turkey (53,313 t), Italy (50,496 t), etc (FAO State Database, 2013).

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Chestnut trees grown in China, South Korea and Japan belong to the C. mollissima, C. crenata and less to C. sativa species.

Major trend is global expansion and modernization of new plantations for productivity and high quality fruits.

The North American chestnut (C. *dentata*) and in European sweet chestnut (C. *sativa*) are subjected to massive and destructive attack of Cryphonectria parasitica (chestnut blight). In recent years, solutions for biological control of the fungus causing chestnut blight based on hypovirulent strains were implemented.

Greece and Romania are two countries with tradition and perspectives for chestnut culture and benefiting of quite favorable ecological conditions.

## MATERIAL AND METHODS

The work was conducted during 2008-2012 period and included scientific data referring to the situation of sweet chestnut in different areas of Greece and Romania, the history of this fruit crop, evolution of production and areas harvested, etc. The main statistical sources used came from FAO Web Page (FAO State Database, 2013) and National Statistical Service of Greece (2005).

# **RESULTS AND DISCUSSIONS**

# A. History of sweet chestnut in Greece and Romania

With about 40 million years ago, the genus *Castanea* colonized the entire northern hemisphere (Groot and Groot, 1962), and the top of the spread and the maximum number of individuals was in the Miocene (25.0 to 5.0 million years) and Pliocene (2.5 to 5.0 million years) (Mai, 1995).

At the end of the Tertiary and early Quaternary, with the advent of ice ages, the extinction of chestnut began from the northern regions of Europe to the Alps (Tomskaya, 1980; Mannion, 1999).

With 70 thousand years ago, when the major glaciations started, until 20 thousand years ago (Last Glacial Maximus) the chestnut survived in few refuge areas and the recolonization of the European continent occurred after the last glacial age (Bennett et al., 1991).

In case of chestnut survival during ice age on the actual territory of Greece there are different views; Bottema (1974) and Willis (1992) believe that this species has not survived, but others (Jahns, 1993) state that sweet chestnut perpetuated in the western Peloponnese and Thessaly. Moreover, Conedera et. al. (2004) and Krebs et al. (2004) affirm that chestnut survived both in southern Greece and in the SE of Balkan Peninsula and Macedonia.

During antiquity, in ancient Greece and in the Roman Empire chestnut has acquired a special importance and that time was called "chestnut civilization" (Pitte, 1986).

On the other hand, Theophrastus (fourth century BC) and other ancient authors believe that chestnut was introduced in Greece from the Middle East (Iran, Syria, etc.) and distinguish between cultivated chestnut and the chestnut forests. From Greece, the sweet chestnut expanded later in the Roman Empire (in Sicily, Italy, North Africa, etc.).

In Romania fossil chestnut pollen was reportedly dating from the Miocene (Oltenia) and Pliocene (Maramures), but it is difficult to believe that chestnut survived the glaciations. Introduction of chestnut in Europe occurred during the Roman Empire (Conedera et al., 2004), coming from Greece (Botu, 2009). During that time chestnut was probably introduced by Romans also in Dacia (actual Romania), which was a province of the Roman Empire from 106 until 275 AD.

Historic sources dating from the 13th and 14th centuries mention the introduction of chestnut in SW of Romania from Mount Athos (Greece) and its cultivation around the monasteries from northern Oltenia. In the Northern part of Romania (Maramures county), the sweet chestnut probably was introduced from west (Botu, 2009, Botu et al., 2013).

# B) Chestnut culture in Greece and Romania

Greece and Romania are European countries where chestnut is cultivated for a long time in coppices and orchards. Their share in the world regarding the harvesting area and production of chestnuts is different (Table 1).

Table 1

Specification	Voor	Area harvested		Production		
specification	Tear	No. of hectares	%	Tons	%	
Romania	2008	2	-	44	-	
	2009	3	-	35	-	
	2010	2	-	29	-	
	2011	-	-	32	-	
Greece	2008	10,600	2.13	9,800	0.54	
	2009	10,618	2.05	14,000	0.74	
	2010	7,400	1.40	20,900	1.07	
	2011	8,938	1.58	21,500	1.07	
World	2008	498,513	100	1,788,995	100	
	2009	517,968	100	1,886,511	100	
	2010	526,615	100	1,949,885	100	
	2011	543,359	100	2,023,736	100	

# Sweet chestnut area harvested and production* (source: FAO Stat Database, 2013)

*No data on forestry use of sweet chestnut is included

The area covered by sweet chestnut in Greece in 2011 is 8,938 ha, with over 1660 ha less than in 2008. This situation is due to the attack of chestnut blight (caused by *Cryphonectria parasitica* fungus) that devastated the orchards in several areas of the country (Diamandis and Perlerou, 2003).

Greece has a share of 1.58% of the world harvested area of sweet chestnut. The chestnut production in Greece was 21,500 t in 2011 (1.07% of world production).

Out of compact plantation areas in Greece there are 579,987 (36.8%) chestnut trees scattered in pastures and other surfaces covered with different crops. Chestnut is grown predominantly in the mountainous areas, up to altitudes of 700-800 m. In the mountain areas the chestnut occupies over 7948.8 ha and achieve a production of 18 039 t (83.9% of total) (Table 2). Very few plantations and scattered trees are in the plains (60.2 ha) and hills (926.5 ha).

If all areas from Greece where chestnut is present (including forests) are taken into account the total area is 33 281 ha (Table 3). The largest areas occupied by chestnut can be found in the following regions: Magnisia (5065 ha), Agio Oros (7785 ha), Eros (2155 ha), Larissa (1793 ha), Kozani (1730 ha), etc (Diamandis, 2010).

In Romania the sweet chestnut is considered as minor fruit crop. The FAO Stat Database (2013) mentions only 2-3 ha of orchards and a production of 32 t chestnuts (Table 1).

# Table 2

No.	Specification	Total	Trees in orchards		No. of	Total chestn	ut fruit
		number of			individual	producti	on
		sweet	Area	No. of	trees	Production	%
		chestnut	(ha)	trees		(t)	
		trees					
1	Total	1,574,235	8,938.5	994,248	579,987	21,500	100
2	Plains	13,304	60.2	9,688	3,616	451	2.1
3	Hills	136,694	926.5	106,469	30,225	3,010	14.0
4	Mountains	1,424,237	7,948.8	878,091	546,146	18,039	83.9
Dif	fferences (%)	100	-	63.2	36.8	-	-

# Area harvested and production of sweet chestnuts in Greece in 2011 (source: Nat. Stat. Serv. of Greece, 2012)

# Table 3

# The chestnut growing areas in Greece, depending on regions, including those in forests (source: Diamandis, 2010)

Region	Area	Region	Area (ha)
	(ha)		
Etolocacarnania	286	Imathia	1160
Eubia	759	Thessaloniki	1197
Euritania	112	Kastoria	1120
Fthiotida	1124	Kilkis	530
Arcadia	239	Kozani	1730
Laconia	269	Pieria	1059
Messinia	607	Serres	838
Ioannina	448	Chalkidiki	1317
Karditsa	223	Agio Oros	7785
Larisa	1793	Evros	2155
Magnesia	5065	Ksanthi	239
Trikala	448	Rodoni	239
Drama	718	Levros	416
Kavala	1197	Chania	208
	Total area =	= 33,281 ha	

The area occupied by chestnut was about 3260 ha of which 3090 ha in Maramureş, Gorj, Vâlcea, Mehedinți, Caraş - Severin, Bistrița - Nasaud, counties. Pure chestnut orchards did not exceed a few hundred hectares (in Baia Mare, Horezu, Tismana, Rm.Vâlcea localities etc.). In the last years most of these orchards were neglected because of change of land property and were pulled out after the massive chestnut blight attack. The spread the individual chestnut trees or presence of massive clusters in many localities show the potential for expansion in Romania of this valuable species due to favorable environmental conditions.

# C) Tendencies in chestnut culture

Chestnut propagation during time was done through seeds in both countries and this created a very diverse genetic base due to the existence of many natural hybrids on their own roots.

Clonal multiplication of varieties and selections is a valuable way to set up modern fruit orchards with important productive potential. In Romania propagation by grafting is carried out by the SCDP Vâlcea nursery belonging to the University of Craiova. In Greece chestnut propagation is done in various small nurseries.

The expansion of the chestnut forests and especially of organized plantations is necessary in both countries because the chestnut fruits demand is important and they have high marketing value.

Nowadays, the major problems in all countries are to set up orchards using planting material, which is resistant or tolerant to Cryphonectria parasitica fungus (causing chestnut blight), and to find a more efficient technology to reduce the effect of this disease.

On the other hand, suitable land is required for the establishment of new plantations into the micro zones with the most favorable ecological conditions for chestnut growing.

#### CONCLUSIONS

Greece and Romania, together with other 20 countries in Europe, grow sweet chestnut (*Castanea sativa* Mill.) and have interest in the development and expansion of this species in forests and orchards with higher yield fruit.

To extend the chestnut culture the use of clonally propagated material of valuable native varieties or selections and high tolerance to chestnut blight caused by *Cryphonectria parasitica* fungus is taken into account.

New chestnut orchards have be planted under optimal climatic and soil conditions.

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#### IN VITRO MICROPROPAGATION METHODS OF SOME IRIS SPECIES

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Key words: meristem culture, tissue culture, cell suspension cultures.

#### ABSTRACT

In vitro tissue culture techniques have been largely used in the last decades for commercial propagation of plants but also for conservation of the many rare and endemic species from different regions. Knowing the fact that the Iris species are reproduced through vegetative propagation using rootstocks, with a long term reproduction rate and the cross-pollination and extremely long seed germination of many species makes the seed reproduction inefficient a more valuable reproduction methods of irises use the in vitro tissue culturing along with the traditional vegetative propagation. This paper describe a number of in vitro effective protocols that have been developed for regeneration of irises plants by using various explants sources of the plant, via somatic embryogenesis, organogenesis, or both processes at the same time depending on media composition and plant species.

#### **INTRODUCTION**

Iridaceae (Juss.) family is one of the best studied of monocotiledons and probably one of the largest families of the superorder Lilianae (Reeves *et al.*, 2001). Iridicaeae family comprise aproximatelly 1800 species classified in 60 genera (Goldblatt, 1990, 1991, 2000) or 2025 species in 65 genera (P. Goldblatt, personal communication, cited by Ascough *et al.*, 2009). Members of Iridaceae are perennial geophytic herbs, with a great heterogenous morphology (Tillich, 2003), typically characterized by the presence of rhyzoms or bulbs, by the possession of isobilateral, equitant leaves, styloid crystals, inferior ovaries, and flowers with three stamens (Reeves *et al.*, 2001). Although worldwide in distribution, with high adaptability for diverse natural habitats, the family is particularly diverse in Africa where there are some 1000 species, most of which are restricted to southern Africa (Reeves *et al.*, 2001).

The largest and most complicated, but, in the same time well-known, genus *Iris*, belonging to the Iridaceae family, is widely spread out cross the northen hemisphere (Kohlein, 1987; Mathew, 1989; Waddick and Zhao, 1992). Taxonomically, genus *Iris* is very complicated containing between 200 and 300 species, depending on the particular authors (e.g. Webb and Chater, 1980; Köhlein, 1981; Mathew, 1981, 1989; Rodionenko,

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1987; Al-Gabbiesh, 2006), widespread in temperate regions of the northern hemisphere. In the territory of Europe, most frequent is subgenus *Iris* L., followed by the subgenus *Limniris*, spread especially in the Southern part of Europe (Köhlein, 1981; Mathew, 1989, cited by Mitić *et al.*, 2013.)

Species of Iridaceae family is characterized by an exceptional variety of sizes, shapes and colors of flowers and leaves, representing good sources for obtaining new horticultural varieties with commercial importance. Irises are widely used as ornamental garden plants or cut flowers.

Besides the ornamental value, some *Iris* species accumulate secondary metabolites, making them highly valuable to the pharmaceutical and perfume industries. *I. padilla* Lam. and *I. germanica* L. contain essential oils composed partly of irons (Jéhan *et al.*, 1994; Kohlein, 1987), which are very expensive and are commonly used in medicin (William and Harbourne, 1985) and, cosmetics and perfumes indistries (Gozu *et al.*, 1993). A potential free radical scavenging and antibacterial activity of flavonoids, terpenoids, saponins, alkaloids, tannins and ethanolic extract of other different *Iris* species was allready demonstrated (Chikhi *et al.*, 2012, Al-Qudah *et al.*, 2012).

*Iris* species are propagated vegetatively, through rhizomes or bulbs (Jéhan *et al.*, 1994; Radojević and Subotić, 1992), the number of plants obtained per year, per rhizome or bulb, ranging from 2 to10. Thus, the plant material which can be use, with different goals, is limited. *Iris* species are allogamous, and in addition, the germination rate of seeds is reduced so that propagation by seeds is inefficient and leads to a segregation of undesirable characters (Jéhan *et al.*, 1994).

Furthermore, because of their economic importance plants are frequently collected from their natural habitats, which leads to reducing the number of individuals in natural populations and their transition to extinction. In this context, *in vitro* micropropagation is a viable alternative to increase rapidly the number of plants. Furthermore, large-scale plant multiplication, germplasm conservation, pathogen elimination, genetic manipulations and supply of selected plants are other advantages provided by different *in vitro* micropropagation techniques (de Fossard, 1983; Hussey, 1983; George and Sherrington, 1984, Niederweiser *et al.*, 2000).

#### MIGROPROPAGATION OF IRIS SPECIES

Embryo culture was the first method used for micropropagation of some species of *Iris* and has been reported by Werckmeister (1936) in Germany, Randolph and Cocs, 1943 and Randolph, 1945 in the United States. Embryo rescue has become a widely used aseptic culture producers for overcoming delayed germination in *Iris* (Randolph and Randolph, 1955; Werckmeister, 1956, 1962; Arditti and Pray, 1969; Farrington, 1969; Stoltz, 1977; Warburton and Hamblin, 1978).

This method offer the advantage of reducing the time between generations from a minimum of two to three years for soil-sown seed to as little as ten months by overcoming dormancy. Moreover, growing immature iris embryos is an useful for certain hybrids, which otherwise would have perished in the developing ovule due to a failure of the endosperm (Lenz, 1954).

Use of apical meristem culture to eliminate virus was first achieved for Iris 'Wedgwood' by Baruch and Quak, 1966 and 10 years later, Hussey ,1976 break apical dominance and induced the formation of multiple shoots in *I. hollandica* Hort. using 6-benzylaminopurine (BAP, 0.5  $\mu$ M). Stem and scale explants have been the choice in many *in vitro* micropropagation protocols (Hussey, 1976; Van der Linde et al., 1986, 1988). Best

response in shoots producing from scale explants culture (Hussey, 1977) was obtained on Murashige Skoog (MS) medium supplemented with 0.5  $\mu$ M of BAP and 5.4  $\mu$ M of 1naphtalene acetic acid (NAA). Similar results have been reported by Nasircilar *et al.* 2011, for *Iris stenophylla* Hausskn & Siehe ex Baker subsp. *allisonii* B. Mathew. Maturation of somatic embryos of *I. hollandica* Hort. obtained starting from twin-scale explants, cultured in 9 or 18.1  $\mu$ M 2,4 – dichlorophenoxyacetic acid (2,4-D) was improved when 0.95  $\mu$ M BAP was added to the regeneration medium (Fidalgo *et al.*, 2005). Bulb formation was promoted by increased sucrose levels (6–38%), increased Phytagar concentrations (0.6– 0.8%), continuous darkness, and repressed by reduced temperature (16 °C), auxin (IAA, NAA and IBA) and/or cytokinin (kinetin, 2iP and BA) addition (Mielke and Anderson 1989, cited by Ascough *et al.*, 2009).

Plants of several *Iris* species have been regenerated from callus culture (Fujino *et al.*, 1972; Gozu *et al.*, 1993; Hussey, 1976; Jéhan *et al.*, 1994; Laublin *et al.*, 1991; Meyer *et al.*, 1975; Radojević and Landré, 1990; Radojević *et al.*, 1987; Radojević and Subotić, 1992; Reuther, 1977; van der Linde *et al.*, 1988; Yabuya *et al.*, 1991).

2,4-D and kinetin (Kin) proved to be the most convenient auxin for the induction (from isolated embryos) and subculturing of morphogenic callus in *I. ensanta* Thunb. (Boltenkov *et al.*, 2007). Callus from explanted shoot apex of *I. germanica* L., induced on a medium according L/S supplemented with 1 ppm 2,4-D + 1 ppm Kin, developed a great number of non-zygotic embryoids. These somatic embryos revealed later the typical features of monocotyledonous zygotic embryos (Reuther, 1977).

Using various explants from the inflorescences Laublin and Cappodocia 1992, obtained *in vitro* callus induction in a number of genotypes of *I. pseudacorus* L., *I. setosa* Pall., *I. versicolor* L. Best callusing across all species was observed with 45  $\mu$ M 2,4-D or 4.5  $\mu$ M 2,4-D and 4.5  $\mu$ M Kin when ovary explants were grown under low irradiance. Callus and meristematic nodules regenerated shoots when subcultured to 22  $\mu$ M BAP. Callus formation from explants of the ovary has been reported at *I. sibirica* L. by Para and Baratti (1992).

Similar results were obtained by Kereša *et al.* 2009, for the Croatian endemic *Iris adriatica* Trinajstić ex Mitić. Callus induction from leaf base explants occurred in the dark on three media with MS mineral solution containing 4.52 mM 2,4-D, 4.83 mM NAA, 0.46 mM Kin and calli from ovary culture were achieved on MS mediam containing 45.25 mM 2,4-D, respectively. All embryogenic calli were formed on MS media containing 0.45 mM 2,4-D, 4.44 mM BAP and 0.49 mM indole-3-butyric acid (IBA) under low light intensity (25 mE m⁻²s⁻¹).

Laublin *et al.* 1991, reported that root explants were superior to leaf explants for producing embryogenic callus in *I. pseudacorus* L., *I. setosa* Pall. and *I. versicolor* L. The best results in callus induction have been induced on media containing 4.5  $\mu$ M 2,4-D, 5.4  $\mu$ M NAA and 0.5  $\mu$ M Kin and regeneration of embryos occurred with 2,3,5-triiodobenzoic acid (2 or 4  $\mu$ M, TIBA) and BAP (9 or 22  $\mu$ M) or Kin (5  $\mu$ M).

Culturing embryo explants of *I. setosa* L. on medium with high sucrose concentrations (5%) and 2,4-D (22.6  $\mu$ M) has allowed the formation of somatic embryos and shoot development occured on media with lower sucrose levels (2%) and 2.9  $\mu$ M gibberellic acid (GA₃) (Radojević and Subotić, 1992).

The high level of sucrose has been mentioned as a critical factor, along with 0.5  $\mu$ M kinetin and 4.5  $\mu$ M 2,4-D, in producing embriogenic callus from young flowers, leaves and apices in *I. germanica* by Jéhan *et al.* 1994. Furthermore, Jevremović and Radojević (2006) reported that supplementing the culture medium with proline (2.9 gl⁻¹) improved embriogenic callus formation in *I. germanica* L. and *I. pumila* L.

Jéhan *et al.* 1994, regenerated plants via somatic embryogenesis from leaves, rhizome apices, and immature flowers. For *I. germanica* L. (Shimizu *et al.* 1996) and *I. hollandica* Tub. (Hida *et al.* 1999) protoplast generation and growth from embryogenic callus has been successfully accomplished. For both species, glucose in the 0.2–0.5 M range gave the highest protoplast division rates. Colonies were readily formed on solid media in response to 4.5  $\mu$ M 2,4-D and 4.7  $\mu$ M Kin. Plantlet regeneration occurred through embryogenesis in *I. germanica* L. (Shimizu *et al.* 1996) and by shoot morphogenesis in *I. hollandica* Tub. (Hida *et al.* 1999). In addition, *I. germanica* L. has been regenerated from both protoplast (Shimizu *et al.*, 1996) and suspension culture (Shimizu *et al.*, 1997).

During somatic embryo formation from suspension cultures, and also during plantlet regeneration, addition of 2.9  $\mu$ M GA₃ significantly enhanced formation and regeneration of somatic embryos (Shimizu *et al.* 1997).

According to the results of studies made by Uzun *et al.*, with immature embryo explants of *I. sari* and *I. schachtii* Markgr. showed best response when cultured on the MS medium supplemented with TDZ and NAA. The same combination of plant growth regulators induced the largest number of regenerated shoots per explant. The lowest number of shoots was suitable for culture medium containing Kin, what is in agreement with the finding of Shimizu *et al.* (1997), who reported that Kin had an inhibitory effect on somatic embryogenesis, but no effect on shoot development in *I. germanica* L.. On the other hand, Kin was generally used in the induction of embryogenic callus in irises (Gozu *et al.*, 1993; Shimizu *et al.*, 1996; Wang *et al.*, 1999).

Another kind of protocol for plant regeneration from cell suspension cultures of *I. germanica* 'Skating Party' was developed by Wang et al. 1999.

Accordind to this regeneration protocol, for efficient *in vitro* plant regeneration of *Iris* suspension-cultured cells should be grown in MS-L medium, and shoots/clump can be regenerated from 1 g of *Iris* suspension-cultured cells in approximately 4 months.

Agrobacterium tumefaciens-mediated and microparticle bombardment transformation was achieved using suspension cultured cells or callus culture of *I. germanica* L.

This method includes growing suspension culture and the clusters in MS-L medium supplemented with an auxin and a cytokinin, in the dark for a period of time (for instance, six weeks). Differentiated clumps can then be isolated, and placed on shoot elongation and development MS-D medium with 1.25  $\mu$ M BAP, under light (for until regenerate shoots and/or plantlets. Prior to being regenerated by this method,*transformation of Iris* cells, with a recombinant nucleic acid molecule, include co-cultivation with *A. tumefaciens* or microparticle bombardment. Hygromycin and geneticin were found to be the best markers for selecting transformed cells (Jeknic *et al.*, 1999).

#### CONCLUSIONS

*Iris* species are highly valuable to the pharmaceutical and perfume industries. Micropropagation of irises has been successfully accomplished by culturing zygotic embryos, different flower parts, and leaf base tissues as starting explants or by cell suspension culture. Plantlets are regenerated via somatic embryogenesis, organogenesis, or both processes at the same time depending on media composition and genotype. The possibility of combining *in vitro* tissue culture technology with recombinant DNA techniques, represents a special opportunity for irises improvement.

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# UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# THE DETERMINATION OF THE DISCRIMINANT CAPACITY OF SOME MORPHOLOGICAL DESCRIPTORS SPECIFIC TO VINE FLOWERS

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Key words: ampelometry, descriptors, Vitis.

#### ABSTRACT

This study proposes an extrapolation of the ampelometric method on the biometric traits of grapevine flowers, so as to be further used for describing and identifying the sorts and varieties of the genus Vitis.

The biologic material studied was represented by 13 autochthonous grapevine sorts which are part of the ampelographic collection of S.C.P.V.V Drăgășani.

As a result of the determinations regarding the morphological traits of the flower, the conclusion was that the mean values of the studied grapevine sorts differ regarding the length of the stamen and the length and diameter of the pistil.

# **INTRODUCTION**

Nowadays, when there are between 10 thousand and 14 thousand cultivated grapevine sorts, (Olteanu I. et al., 2002; Ghidra V. et al., 2004), not to mention interspecific hybrids and their synonymies, grapevine cultivation conditions require approaching complex methodologies of description and identification.

That was achieved by. O. I. V, U.P.O.V. and I.B.P.G.R. (Bioversity) who elaborated "*The List of Descriptors for the Varieties and Sorts of the Genus Vitis*", the second edition, which included the changes suggested in "The final proposal regarding the modification of the OIV Chart (147 descriptors), presented at the 29-th session of OIV experts meeting in 1997".

The suplimentary descriptors added concerned the ampelometric traits of the adult leaf (18 descriptors), ampelographic traits (3 descriptors), fitopathological descriptors (4 descriptors), izoenzimes (2 descriptors) and SSR markers (6 descriptors).

By including the 18 ampelometric descriptors on the O.I.V. Descriptors List, the methodology of grapevine varieties description becomes more comprehensive, the ampelometric method itself being thus reconsidered.

Ampelometric studies have been made since 1986, concerning mostly the grapevine leaf (phyllometry) and, to a lesser degree, the seeds - carpometry (Cid-Alvarez N. et al. 1994, Araujo J.A. 1996, De Micheli et al. 1997, Liliana Rotaru 2002, Irma Tomazic et al. 2003).

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The ampelometric method proves its reliability and is used extensively nowadays thanks to IT development and the multiple ways of processing data (J. Cunha et al. 2007, Marti C.et al., 2007, Gheorghita Mîndrila et al. 2009).

#### MATERIAL AND METHODS

This study consists of the measurements and observations made of flower components in an attempt to bring attention to the necessity of including these traits on the IVO descriptors list.

The biological material analysed was represented by 13 Romanian grapevine sorts belonging to S.C.P.V.V Drăgășani ampelographic collection.

We considered the following ampelometric descriptors of the flower: stamen length, pistil length and ovary diameter.

In addition to these traits, two more were referred to in our study and they can be really helpful in grapevine varieties description and identification: the values of the ratio between the stamen length and the pistil length, and the values of the ratio between the stamen length and the ovary diameter.

# **RESULTS AND DISCUSSIONS**

The Romanian literature in the domain (The R.S.R. Ampelography, vol.I - VIII) provided a database containing the values of the studied traits for 27 Romanian grapevine sorts (Table 1). Depending on the variation amplitude of the studied traits, class boundaries have been set, applying a grading system in accordance to IVO, UPOV and Bioversity methodology.

The variation amplitude of the considered traits was relatively reduced, and, consequently, there have been set three classes for each trait:

*Stamens length* value ranged from 1.5 to 3.5 mm, the proposed marks being 1, 2 and 3, for the three classes:

- 1 less than or equal to 2.5 mm;
- 2 between 2.5 and 3.5 mm;
- 3 more than or equal to 3.5 mm.
  - *Pistil length* values ranged from 2 to 2.5 and were marked accordingly:
- 1 less than or equal to 2.5 mm;
- 2 from 2.5 to 3 mm;
- 3 more than or equal to 3 mm.
  - Ovary diameter varied between 1 and 2 mm, the marks being:
- 1 less then or equal to 1.5mm;
- 2 between 1.5 and 2 mm;
- 3 more than or equal to 2 mm.

*The ratio between the stamens length and the length of the pistil* ranged from 0.6 to 2 mm, the three classes having the following boundaries:

- 1 -less than or equal to 1.3 mm ;
- 2 between 1.3 and 1.8 mm;

3 - more than or equal to 1.8 mm.

*The ratio between the stamens length and the ovary diameter* ranges from 1 to 3 mm and the markes were:

- 1 less than or equal to 1.5 mm;
- 2 between 1.5 and 2.5 mm;
- 3 more than or equal to 2.5 mm.

Grapevine varieties	Stamen length (mm)	Pistil length (mm)	Ovary Diameter (mm)	Ratio between stamen length and pistil length	Ratio between stamen length and ovary diameter
Majarcă albă	3.0	2.0	1.0	1.5	3.0
Mustoasă de Măderat	3.5	2.5	1.5	1.4	2.3
Negru moale	3.5	2.5	1.5	1.4	2.3
Negru vârtos	2.0	2.0	2.0	1.0	1.0
Plăvaie	3.4	2.5	1.5	1.4	2.3
Razachie albă	3.0	2.0	1.5	1.5	2.0
Razachie roșie	3.0	2.0	1.5	1.5	2.0
Rosioară	3.0	2.0	1.5	1.5	2.0
Tamăioasă românească	3.0	2.0	2.0	1.5	1.5
Tamaiosă albă de Bohotin	3.0	2.0	1.5	1.5	2.0
Tamaioasă roză	3.0	2.5	2.0	1.1	1.5
Zghihară	4.0	2.0	2.0	2.0	2.0
Băbească neagră	4.0	2.5	1.5	1.6	2.7
Bătută neagră	3.0	2,5	2.0	1.2	1.5
Berbecel	3.0	2.0	1.5	1.5	2.0
Braghină	2.0	2.0	1.5	1.0	1.3
Coarnă albă	1.5	2.5	1.5	0.6	1.0
Coarnă neagră	2.0	2.5	1.5	1.0	1.0
Creață	3.0	2.0	1.5	1.5	2.0
Crâmpoșie	2.5	2.5	1.5	1.0	1.7
Fetească albă	3.5	2.0	1.5	1.7	2.3
Fetească neagră	4.0	3.0	2.0	1.3	2.0
Ardeleancă	3.5	2.0	1.5	1.7	2.3
Frâncuse	3.0	2.0	1.5	1.5	2.0
Frunza de tei	3.5	2.5	1.5	1.4	2.3
Fetească regală	3.5	2.0	1.5	1.7	2.3
Galbenă de Odobesti	3.0	2.5	1.5	1.2	2.0

Mean values of the traits studied, registered considering Romanian grapevine sorts (according to RSR Ampelography, vol. I-VIII)

Table 1

The measurements of the grapevine sorts considered were made during the blooming season, when more than half of the flowers were open. There were gathered 10 flowers from each of the 10 vines selected. The vines were normally developed, typical and healthy.

For each trait, there were measured the components of the flowers located on the middle third of the inflorescence. The stamen length was measured considering as extreme points the insertion point and the extremity of the anther, respectively. The pistil length was

measured considering as extreme points the base of the ovary (above the nectariferous glands) and the extremity of the stigma. The diameter of the ovary was measured in its median area. The data were statistically processed to determine the variation index, necessary to establish the homogeneity of the samples. The results obtained show that for the three flower descriptors considered, the variation index does not exceed 10%, ranging from 2.82% in the case of Romanian Tămâioasă considering the trait stamen length, and 8.31% for the grapevine sort Roșioară, considering the trait ovary diameter (Table 2).

Table 2

Grapevine varieties	Statistic	Stamen	Pistil	Ovary
_	indexes	length	length	Diameter
Negru moale	Mean	3.63.	2.49	1.50
	Var. Index	3.68%	2.96%	7.70%
Negru vartos	Mean	2.10	2.30	2.10
	Var. Index	7.10%	5.02%	7.78%
Rosioară	Mean	3.30	2.10	1.50
	Var. Index	6.06%	5.50%	8.31%
Tămâioasă românească	Mean	3.37	2.20	2.10
	Var. Index	2.82%	5.25%	5.50%
Babească neagră	Mean	4.20	2.50	1.50
	Var. Index	3.55%	3.27%	7.70%
Bătută neagră	Mean	3.10	2.50	1.90
	Var. Index	6.08%	4.62%	4.30%
Braghină	Mean	2.5	2.61	1.5
	Var. Index	4.99%	5.25%	7.7%
Coarnă albă	Mean	1.50	2.50	1.50
	Var. Index	7.03%	5.96%	7.03%
Coarnă neagră	Mean	1.90	2.5	1.6
	Var. Index	6.08%	4.62%	7.22%
Crâmpoșie	Mean	2.5	2.5	1.5
	Var. Index	4.62%	4.62%	7.70%
Fetească albă	Mean	3.5	2.42	1.7
	Var. Index	3.30%	4.69%	6.79%
Fetească neagră	Mean	4.3	3.7	2.2
	Var. Index	2.45%	3.12%	3.71%
Fetească regală	Mean	3.5	2.5	1.5
	Var. Index	4.62%	4.42%	7.70%

Variation index values corresponding to the proposed biometric traits

The variability of the traits being low within the same sort proves the representativity of the analysed samples.

The proposed traits have been marked according to OIV, UPOV and Bioversity methodology (Table 3).

# Table 3

# Grapevine varieties Image: Stamen length (mm) Pistil length (mm) Ovary diameter (mm) Ratio between stamen length (mm) Batio between (mm) Image: Stamen length (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm) Image: Stamen (mm)

The mean values of the determined ampelometric traits of the flower and the corresponding

Grapevine varieties	(mm)		(mm)		(mm)		length		length and ovary diameter	
	Mean value	Mark	Mean value	Mark	Mean value	Mark	Mean value	Mark	Mean value	Mark
Negru moale	3.6	3	2.5	2	1.5	1	1.44	2	2.40	2
Negru vârtos	2.1	1	2.3	2	2.1	3	0.91	1	1.00	1
Roșioară	3.3	2	2.1	1	1.5	1	1.57	2	2.20	2
Tamăioasă românească	3.4	2	2.2	1	2.1	3	1.54	2	1.62	2
Băbească neagră	4.2	3	2.5	2	1.5	1	1.68	2	2.80	3
Bătută neagră	3.1	2	2.5	2	1.9	2	1.24	1	1.63	2
Braghină	2.5	1	2.6	2	1.5	1	0.96	1	1.66	2
Coarnă albă	1.5	1	2.5	2	1.5	1	0.60	1	1.00	1
Coarnă neagră	1.9	1	2.5	2	1.6	2	0.76	1	1.18	1
Crâmpoșie	2.5	1	2.5	2	1.5	1	1.55	2	1.66	2
Fetească albă	3.5	2	2.4	1	1.7	2	1.45	2	2.05	2
Fetească neagră	4.3	3	3.7	3	2.2	3	1.16	1	1.95	2
Fetească regală	3.5	2	2.5	2	1.5	1	1.40	2	2.33	2

#### CONCLUSIONS

The results obtained show the fact that for the three flower descriptors considered, the variation index did not exceed 10%, the variability of these traits being low within the same vine sort.

The analysed data show that the sorts studied had different values regarding the flower biometric traits, the marks given being relevant in this respect.

The proposed traits can be introduced on the IVO Descriptors List, being useful in describing and identifying vine sorts.

The Romanian sorts used for the determinations can constitute reference sorts.

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# Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# STUDY OF THE TECHNOLOGICAL POTENTIAL OF THE ASSORTMENT OF CHASSELAS TABLE GRAPES IN THE DRĂGĂȘANI VINEYARD

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Keywords: the process of maturation, assortment, table grapes, technological indices

#### ABSTRACT

This paper is conceived on the comparative study of varieties belonging assortment Chasselas grapes. Were studied following varieties: Chasselas Dore, Chasselas Rose, Chasselas de Cegled, Chasselas de Băneasa, Chasselas Crocant, Chasselas Rose Royal, Chasselas Rose de Palestine. Determinations aimed physical-mechanical technological of the grapes followed by calculating indices and parameters of quality, sugar content and acidity.

#### INTRODUCTION

Rich in phenols, vitamins, enzymes, mineral salts, organic acids, carbohydrates easily assimilated, flavors, etc, table grapes are an excellent food and tonic, while being a stimulant for general sensitivity and sensory. Ageing and ripening is a notion which in a broad sense it means the process which the berry of grapes under the action of heat and sunlight becomes good for consumption (Muntean &Ionică-2006).

During maturation, the components of the grapes entered continue changes, which, although, it follows the same laws of growth and maturation process, have some features that distinguish them and individualized. Thus, at some times, on the same plant and grape, berries have the different size and ripening degree. What is more, even within the same tissue accumulation is different. (Cichi et al., 2010).

Maturation as a evolutionary phase is a physiological process whit coordinated genetic, what is characterized by a complex changes such as morphological, anatomical and chemical. Through the technological maturity of the grapes is pursuing the accumulation of large contents of sugar and reduction of the excessive acidity of grapes in order to achieve a more balanced gluco-acidity report (Târdea., 2000).

Full maturity or maturity of the pulp does not correspond always with physiological maturity. They are used to define the various parts of the one and the analysis of the main constituents of the bean with the establishment of ratio between values found. Of all these, the most important and easiest to follow are the berry weight variation providing indication on the quantitative side, variation in carbohydrate content and acidity as well as to establishment of the report that, in fact, give indications on the qualitative side (Olteanu et al. 2002, Ionica & Olteanu, 2004).

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# MATERIAL AND METHODS

Research has been conducted on the varieties Chasselas from collection S.C.D.V.V. Drăgășani Chasselas Dore, Chasselas Rose, Chasselas de Cegled, Chasselas de Băneasa, Chasselas Crocant (Chasselas croquant), Chasselas Rose Royal, Chasselas Rose de Palestine (Raisin de Palestine).

The grapes have been sampled from collection and were concerned the following issue: physico-mechanical analysis of grapes and establishment of technological indicators of grapes, determination of main constituents of grapes at harvest time.

# The methodology of determining the physical and mechanical characteristics of the grapes.

For it was established: grape length -cm, weight of grape-grams weight of the bunch-grams, grain weight- grams, peel weight -gram, seed weight-grams, pulp-gram weight.

On the basis of the technological indices were calculated as follows:

- a) index structure = weight of grains / weight of bunches;
- b) Index of berry= number of berries per 100 grams of grape seeds;
- c) Index of berry composition = weight of grain pulp / marc weight;
- d) Index must yield = weight / weight marc.

# The methodology of determining the level of main constituents on harvesting.

For this we determined the relative content in the wort sugars g / 1 by refractometry (using Zeiss refractometer) relative acid content must titrimetric method, expressed in g / 1 H2SO4.

# **RESULTS AND DISCUSSIONS**

Result concerning the phisico-mechanical composition of the grape varieties soiurilor Chasselas Dore, Chasselas Rose, Chasselas de Ceged, Chasselas de Băneasa, Chasselas Crocant, Chasselas Rose Royal, and Chasselas Rose de Palestina, grown in Dragasani vineyard are presented in tables 1 and 2.

		1 11	ysico-meet	lancal sur	icture of the	grapes at	un maturi	LY
Investigate	items	Chasselas Dore	Chasselas Rose	Chasselas de Cegled	Chasselas de Băneasa	Chasselas Crocant	Chasselas Rose Royal	Chasselas Rose de Palestina
Dunchos	g	4	5	6	7	3	4	2
Builches	%	1,76	2	1,85	1,74	1,75	1,93	1,21
	g	222	245	317	395	168	203	162
Berries	%	98,23	98	98,14	98,25	98,24	98,06	98,78
	nr.	114	96	98	106	52	76	64
Deals	g	215	239	306	389	160	197	156
Peels	%	95,13	95,60	94,73	96,76	93,56	95,16	95,12
Saada	g	7	6	11	6	8	6	6
Seeus	%	3,09	2,40	3,40	1,49	4,67	2,89	3,65
Marc	g	52	36	57	84	34	36	24
Marc	%	23	14,40	17,64	20,89	19,88	17,39	14,63

Physico-mechanical structure of the grapes at full maturity

100 berries have		Chasselas	Chasselas	Chasselas de	Chasselas de	Chasselas	Chasselas Rose	Chasselas Rose de
		Dore	Rose	Cegled	Băneasa	Crocant	Royal	Palestina
Total weight		194,73	255,20	323,46	372,64	323,07	267,10	253,12
Deal	g	35,96	26,04	40,81	66,98	44,23	34,21	25
Peel	%	18,47	10,20	12,62	17,97	13,69	12,81	9,88
Dula	g	152,63	222,91	271,42	300	263,46	225	218,75
Puip	%	78,38	87,36	83,92	80,51	81,55	84,24	86,42
Saada	g	6,14	6,25	11,22	5,66	15,38	7,89	9,37
Seeds	%	3,15	2,44	3,46	1,52	4,76	2,95	3,70

Physico-mechanical structure 100 berries

Table 2

Regarding the berry weight, variety Chasselas de Baneasa holds the record of 395 g and the lowest recorded the variety Chasselas Rose de Palestine 162 g.

The reference varieties of assortment, Chassela dore and Chasselas Rose have intermediate berrz weight of 222 g and 245 g  $\,$ 

Is not the same situation regarding the number of grains of a bunch of grapes. Thus, most beans (114) recorded the variety Chasselas dore and the representative from Chasselas crocant number (52).

Although in terms of technology is not relevant for table grapes, the grape pomace content complies with both weight and bunches of grapes. Consequently, the highest recorded in the Chasselas de Baneasa and the lowest in Chasselas Rose de Palestine.

Quantitative and numerical ratios obtained by physico-mechanical analysis of the grapes are found in a synthetic technological indices

Structure indices presents the highest value to the variety Chasselas Rose of Palestine (81) and lowest in the variety Chasselas Rose (49).

The berry index values between 51.28 (Chasselas Dore) and 26.80 (Chasselas de Baneasa).

Index composition of the grains show values between 7.36 (Chasselas Rose de Palestine) and 3.62 (Chasselas Dore).

Yield Index ranks first in Chasselas Rose (3.35) followed by Chasselas Rose de Palestine (3,31), last fall the variety Chasselas Dore (2,25).

In Table 3 are presented four indices technological calculated for each variety of sassortment Chasselas.

Table 3.

Technolo- gical index	Chasselas Dore	Chasselas Rose	Chasselas de Cegled	Chasselas de Băneasa	Chasselas Crocant	Chasselas Rose Royal	Chasselas Rose de Palestina
index structure	55,5	49	52,83	56,42	56	50,75	81
Index of berry	51,28	39,21	30,95	26,80	30,95	37,45	39,52
Index of berry composition	3,62	6,90	5,21	4,13	4,41	5,34	6,36
Index must yield	2,25	3,35	2,28	2,47	2,58	2,28	3,31

Technological index of grape Chasselas

Because quite high yield index between 2.25 and 3.31, Chasselas varieties can be used to produce wine.

In Table 4 shows encoding technology indices at the assortment of table grape Chasselas in relation to ICVV.

Observations to calculate the index of berry and berry composition index (descriptors introduced by ICVV) are made at technological maturity at 100 berry, by 10 berries taken from the middle of the 10 grapes.

Observations for the calculation of the structure and yield index (descriptors introduced by ICVV) are made at technological maturity, to 1 kg grapes - sample average.

Table 4

Descriptor	Coding I.C.V.V.							
	Chasselas	Chasselas	Chasselas	Chasselas	Chasselas	Chasselas	Chasselas	
	Dore	Rose	de Cegled	de	Crocant	Rose	Rose de	
				Băneasa		Royal	Palestina	
Index	9	9	9	9	9	9	9	
structure								
Index of	7	6	6	5	6	6	6	
berry								
Index of	1	1	1	1	1	1	1	
berry								
composition								
Index must	1	3	1	1	1	1	3	
yield								

Coding technological indices in relation to ICVV at Chasselas assortment

I.C.V.V.: Institutul de Cercetări Viti-Vinicole

From these varieties can obtained table wines, given the quality parameters reflect the contents of sugars and acidity (Table 5).

Table 5

	1	Coding		Coding
		descriptor	A 11. /1	descriptor
Variety	Glucides g/l	(OIV	Acidity g/l	(OIV 506;
		505;IBPGR	$H_{2}SO_{4}$	IBPGR
		6.2.28.)		6.2.29)
Chasselas Dore	156,4	4	3,19	3
Chasselas Rose	149,6	4	3,03	3
Chasselas de Cegled	153	4	3,28	3
Chasselas de Băneasa	129,2	2	4,41	3
Chasselas Crocant	197,2	7	4,16	3
Chasselas Rose Royal	193,8	6	2,35	3
Chasselas Rose de	136	3	2.08	3
Palestina	130		2,98	

The main quality descriptors and coding at Chasselas varieties at harvest

After analyzing descriptor - sugar content in grape - varieties were ranked in the following order: containing very low (2) the variety Chasselas de Baneasa, low content (3) and (4) respectively Chasselas Rose de Palestin, Chasselas Dore, Chasselas Rose, Chasselas de Cegled, containing medium (6) Chasselas Rose Royal and with high content (7) variety Chasselas crocant.

From the point view of the acid content, it presents limitations oscillation between 2.35 g / L  $H_2SO_4$  (Chasselas Rose Royal) and 4, 41 g/L  $H_2SO_4$  (Chasselas de Băneasa), between varieties analyzed there are a number of differences, although the current coding system proposed by the OIV (code 506) and IBPGR (02/06/29) does not permit framing differentiated varieties, which shows limited discriminant power of this descriptor.

# CONCLUSIONS

Following the completion of this study the following conclusions can be drawn: Due to the attractive appearance, the taste very pleasant and high energy value, table grapes are required among the most valuable fruit is consumed fresh.

For this reason, at present more and grapes entering a growing period of time during the year, the daily diet.

Varieties of assortment Chasselas, Chasselas Dore in particular occupies over 33% of the cultivated varieties of meals, the variety of mixed use for fresh consumption and for obtaining wine consumption.

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# COMPARATIVE AGROBIOLOGIAL CHARACTERISTIC OF INTRODUCED FRENCH CLONES OF CABERNET SAUVIGNON VARIETY UNDER THE CONDITIONS OF PLEVEN TOWN

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Key words: grapevine, variety, clone, fertility and productivity

#### ABSTRACT

During the period 2007 - 2009 in the Institute of Viticulture and Enology - Pleven (Bulgaria), a comparative assessment of fertility and yield of 4 introduced French clones of Cabernet Sauvignon - 15, 337, 338 and 339 was carried out. The newly-selected Bulgarian clone – Cabernet Sauvignon ILV 1/11 was used as reference.

It was found that the introduced clones adapted well to the soil and climatic conditions of the region of Pleven. Better indices of actual fertility showed Cabernet Sauvignon 15 and Cabernet Sauvignon 337 revealing fertility rate, average mass of a cluster and average yield per vine similar and higher than those of the reference. However these clones had insufficient sugar accumulating capacity and low sugars content in correlation dependence on their yield.

# **INTRODUCTION**

Clonal selection is the most widely used method to improve the structure of wine vineyards of *Vitis vinifera L* varieties. Most frequently its aim is searching individual phenotypic differences and / or resistance to various diseases (Meneghetti et al., 2010).

Most of vine varieties have limited distribution, however there are such varieties with a wide area of distribution, grown in many countries and regions, because of their valuable commercial qualities and high adaptability (Вълчев 1978). These are the so called "Cosmopolitan Varieties". Of these, the most widely grown in the world for the production of red wines is Cabernet Sauvignon variety (Clarke, Rande 2001; Robinson 2006). The plantations of this variety differ significantly by their intra-varietal diversity, resulting from its long cultivation and mutation variability of the vine.

According to Caldwell (1998) it is wrong to consider that the individual clone of a variety should have unique differences with the other clones, as differences are often small and relate to a single feature. Differences in fertility between individual clones of the same variety are considerably smaller than those between the varieties, but sometimes they may be very important and significant. The actual fertility represents the average number of clusters per shoot as it is the most accurate index of the productive capacity of each vine variety (clone). Its phenotypic expression strongly depends on the variety genotype, the soil and climatic conditions and the applied agronomic techniques. Fertility has a direct impact

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on the yield (Mokreva, Roychev 2004; Mokpeва 2011). Special attention is paid to that indicator in clone selection of wine and table grapes varieties because of its great importance for the quality of grapes and wine. As a result of ongoing for decades natural selection and focused clonal-sanitary selection in Cabernet Sauvignon variety it was achieved a positive effect on fertility, yield and grapes quality. For enrichment the available vine gene fund, many new clones of the licensed and recommended for the country wine grape varieties, selected in other vine-growing countries were introduced in recent years in Bulgaria. However in their introduction, it is often relied on their recognition, without taking into consideration that to produce quality grapes as raw material, it is of crucial importance the appropriate combination of variety (clone), climate, soil, rootstocks, etc. (Дончев 1991; Катеров и кол., 2005). Nowadays, the need of creating and introducing high-quality and productive vine varieties and clones to respond adequately to the rising agro-technical level and market demands is much more justified.

The objective of this study is to make a comparative statistical analysis of the actual fertility indices and yield of introduced French clones of Cabernet Sauvignon variety.

# MATERIAL AND METHODS

The investigated clones 15, 337, 338 and 339 of Cabernet Sauvignon variety were introduced from France in 2000. The clone investigation was carried out in accordance with established methodology for clone selection (Катеров и кол.,1990) in the clone section of the Experimental Base at the Institute of Viticulture and Enology (IVE) – Pleven. The time-tested Bulgarian clone – Cabernet Sauvignon ILV 1/11 was used as reference. The comparative study of the clones was made with Moser training. The vines were grafted on Berlandier x Ripairia CO4 rootstock, at planting distance 3.00/1.30 m. After the vines were in their fruiting stage mixed pruning was performed, leaving equal number of winter eyes loading per vine - 32 winter eyes (3 x 2 eyes and 2 x 12 eyes).

The following indices were recorded for defining the actual fertility and productivity of the clones and the reference (Cabernet Sauvignon ILV 1/11): developed eyes percentage, percentage of fruit canes with 1, 2 and 3 clusters; fertility coefficient of developed shoot and fruit cane; number of clusters; average cluster mass (g) and yield per vine (kg). The sugars (%) and titratable acids (g/dm³) content in grape must were defined in accordance with the conventional methods in enology for characterizing the grapes quality (Иванов и кол., 1979).

The indices characterizing the actual fertility were recorded annually at the end of May, while the yield indices – at the time of technological ripeness of the grapes. The obtained results for the actual fertility and yield of the French clones and the reference were mathematically processed by analysis of variance (Mokreva, Murgova 1996). The comparative analysis between the investigated clones and the reference (Cabernet Sauvignon ILV 1/11) was performed based on the average values for each index and any significant differences.

#### **RESULTS AND DISCUSSIONS**

From the agrobiological investigation it was determined that the main difference between the clones and the reference (clone ILV 1/11) was in the size and shape of the cluster, and to a lesser extent in the berries size. The cluster of clone ILV 1/11 was medium to large (18.5 / 9.6 cm), cylinder-conical or conical, with a big wing, semi-compact to compact. The cluster of the French clones was medium, varying from 16.2/9.0 cm (clone 338) to 14.8/7.9 cm (clone 339). Of clone 337 and clone 339 it was conical, often with a small wing, of clone 338 - cylinder-conical or conical, without a wing and of clone 15 -
cylinder-conical or conical, with a medium large wing (Table 1). The berry of the introduced clones and the reference was small, spherical, and the size difference was negligible. For clone ILV 1/11 it was 12.23/12.23 cm, while in the French clones it was within the range from 12.86/12.86 (clone 15) to 12.16/12.16 (clone 339). The skin of all investigated variants was dark blue, with abundant wax coating, thick and tough. The consistency was juicy, the taste - harmonious (Table 1). The comparative analysis of the size of the cluster and berries of the investigated clones and the reference revealed some mathematically proven differences for the individual clones and indexes (Table 1).

The values of the cluster length and width were shown to be higher for the reference compared to clones 337 and 339 and only for length – compared to clones 15 and 338. In terms of berry size there were no mathematically proven differences between the investigated clones and ILV 1/11. The exception was clone 15 as its berries were statistically proven to be larger than all other variants.

Table 1

Comparative analysis of the cluster (cm) and and berry (mm) sizes of i	ntroduced french
clone of variety Cabernet Sauvignon average 2007-2009 pe	eriod

Indexes	Clı	ıster	Be	rry
Clone	Length, cm	Width, cm	Length, mm	Width, mm
ILV 1/11 - control	18,5	9,6	12,23	12,23
15	15,3*	8,7 ^{n.s}	12,86*	12,86*
337	15,5*	8,3*	12,43 ^{n.s}	12,43 ^{n.s}
338	16,2*	9,0 ^{n.s}	12,44 ^{n.s}	12,44 ^{n.s}
339	14,8*	$7,9^{*}$	12,16 ^{n.s}	12,16 ^{n.s}

Reliability at deviation  $\alpha$ =0,05

The summarized data of actual fertility indices showed that the reference had a high ratio of developed winter eyes - 83.89% and very high ratio of fruit canes - 90.61%. Most of the fruit canes had 1 and 2 clusters, in approximate correlation  $1 \div 2$  (28.14% with 1 cluster and 68.27% with 2 clusters). Fruit canes with 3 clusters were very few - 3.56% and did not affect significantly the clone fertility and yield (Table 2).

Table 2

Comparative analysis of the actual fertility indexes of introduced french clone of variety Cabernet Sauvignon average 2007-2009 period

	Developed	]	Developed fr	uiting shoots	5	Fertility of	coefficient
Indexes Clone	buds %	total, %	with 1 cluster %	with 2 clusters, %	with 3 clusters, %	developed shoot	fruiting shoot
ILV 1/11 control	83,89	90,61	28,14	68,27	3,56	1,60	1,75
15	76,37*	85,29 ^{n.s}	$18,07^{*}$	68,59 ^{n.s}	13,34*	$1,68^{*}$	1,95*
337	79,57 ^{n.s}	84,59 ^{n.s}	20,97 ^{n.s}	71,26 ^{n.s}	$7,77^{*}$	1,59 ^{n.s}	1,86 ^{n.s}
338	86,40 ^{n.s}	81,93*	27,62 ^{n.s}	60,20 ^{n.s}	12,19*	1,52 ^{n.s}	1,84 ^{n.s}
339	87,17 ^{n.s}	82,21*	29,64 ^{n.s}	65,62 ^{n.s}	4,77 ^{n.s}	1,44*	1,74 ^{n.s}

Reliability at deviation  $\alpha = 0.05$ 

In the French clones it was found a significant difference in the mean values of actual fertility individual indexes. Clones 15 and 337 were characterized with a smaller

number of developed winter eyes -76.37 % and 79.57 % respectively however most of their shoots became fruit canes -85.29 % and 84.59 %. The correlation of fruit canes with 1 and 2 clusters for these clones was higher - almost 1÷3.5. For clone 15 the fruit canes with 1 cluster were 18.07 % and with 2 clusters -68.59 %, and for clone 337 -20.97 % and 71.26 % respectively.

For the other two clones, the ratio of their developed winter eyes exceeded that of the reference - 87.17% (clone 339) and 86.40% (clone 338), but it was much lower for the fruit canes - 81.93% and 82.21% respectively. The correlation of their fruit canes with 1 and 2 clusters was close to that of clone ILV 1/11 – approximately 1÷2. Their fruit canes with 1 cluster were 27.62 % (clone 338) and 29.64 % (clone 339), and with 2 clusters – 60.20 % and 65.62 % respectively.

It was found from the investigation that clone 15 and clone 338 had a tendency to form more fruit canes with 3 clusters - 13.34% and 12.19%, while for the other two clones the ratio of this type of canes was similar or slightly higher compared to the reference - 4.77% (clone 339) and 7.77% (clone 337).

The differences in the number of developed shoots (fruit and fruitless) and clusters between the clones determined the significant variation in the fertility coefficient. The highest fertility rate per developed shoot had clone 15 - 1.68. For the other clones it was close to or lower compared to the reference - from 1.59 for clone 337 to 1.44 for clone 339. The fertility rate per fruit cane of most French clones was very high - from 1.84 to 1.95 and considerably exceeded that of clone ILV 1/11 - 1.75 and clone 339 - 1.74.

The comparative statistical analysis of actual fertility averagely per vine between the reference and the introduced clones of Cabernet Sauvignon revealed that there were no significant differences between most of the studied variants (Table 2).

It was proved that clone ILV 1/11 exceeded the French clones referring the rates of the indices – developed eyes percentage (clone 15); percent of fruit canes (clones 338 and 339), percentage of fruit canes with 1 cluster (clone 15) and fertility rate per developed shoot (clone 339).

The introduced clones of Cabernet Sauvignon variety mathematically exceeded the reference variant concerning the indices - percent fruit canes with 3 clusters (clones 15, 337 and 338); fertility rate per developed shoot and fruit cane (clone 15).

Clone 15 had the greatest number of proven differences related to the indices determining the actual fertility.

From the investigation of the clones' productivity it was found that there was no mathematical evidence of a difference between the clones and the reference in the average mass of a cluster. For clone ILV 1/11 it was 145.8 g, and for the French clones it was in the range of 133.8 g for clone 339 to 152.8 g for clone 338 (see Table 3).

The highest average yield per vine was obtained from the reference 5.074 kg, due mainly to the higher number of fruit canes compared to the French clones. The yield per vine varied for the three introduced clones from 4.529 kg (clone 338) to 4.842 kg (clone 15). The only exception was clone 339, with much lower yield – 3.972 kg. The difference in the yield was a result of the much higher average mass per cluster and the greater number of canes with 3 clusters.

Significant difference between the clones was found in their sugar accumulation capacity and the grapes quality.

The sugar accumulation process was with the lowest intensity in clones 338 and 339. Their average quantity for the period was 18.3 % and 19.7 % respectively as it did not exceed the rates of the reference and the other two clones. The best intensity of sugar accumulation in grapes had clone 337 - 20.6%; comparatively good - clone 15 - 19.8%

and the reference -19.7%. In these variants the grapes sugar content was in direct correlation of yield.

Table 3

Indexes	Average weight per cluster, g	Average yield per vine, kg	Sugars, %	Titratable acids, g/dm ³
ILV 1/11 - control	145,8	5,074	19,7	6,99
15	141,1 ^{n.s}	4,842 ^{n.s}	19,8 ^{n.s}	6,83 ^{n.s}
337	144,6 ^{n.s}	4,676 ^{n.s}	20,6*	7,15 ^{n.s}
338	152,8 ^{n.s}	4,529 ^{n.s}	18,3*	7,23 ^{n.s}
339	133,8 ^{n.s}	3,972*	19,7 ^{n.s}	7,46 ^{n.s}

Comparative analysis of the productivity indexes of introduced french clone of variety Cabernet Sauvignon average 2007-2009 period

Reliability at deviation  $\alpha$ =0,05

The comparative analysis of the mass per cluster, yield per vine and the grapes quality of the investigated clones and the reference indicated the presence of small differences, mathematically proven, in the individual variants (Table 3). The differences of the reference compared to clone 339 for yield per vine and clone 338 for sugars content in the grapes were proven.

The statistically processed data about the indices defining the quality of the grape must revealed proven higher sugars quantity in the grapes of clone 337 compared to the reference and the other French clones.

#### CONCLUSIONS

From the agrobiological investigations of the introduced French clones of Cabernet Sauvignon the following regularities were found:

There was a wide variation in the individual years of the study in the indices of actual fertility between the introduced clones of Cabernet Sauvignon and the reference, revealing their different response to environmental conditions. The rate of developed eyes and fruit canes with 1 cluster were higher for clone ILV 1/11. Clones 15 and 338 displayed a tendency to form more fruit canes with 3 clusters thus their fertility coefficient surpassed the reference and the other two French clones.

No difference in the productivity indices was found between the investigated clones and clone ILV 1/11. The average mass per cluster had very close rates as it was considerably higher of clone 338. Referring the average yield per vine there was no mathematically proven difference however the rates of the French clones were lower compared to the reference.

The investigated clones had insufficient sugars accumulation capacity that was in direct correlation of the yield. However clones 15 and 337 were distinguished for their comparatively higher intensity of sugars accumulation in grapes (vintages 2007 and 2009).

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## THE INFLUENCE OF GROWTH RETARDANTS ON GROWTH AND FLOWERING OF SOME POTTED PLANTS

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Keywords: Beloperone guttata, Plumbago capensis, Cycocel, growth control

## ABSTRACT

Many flowering plant species with fast and excessive growth, grown in pots, require the use of growth regulators for the control of plant height, for obtaining better branched plants with a compact growth and for improving their ornamental appearance in order to produce commercial plants.

The study aims to determine the reaction of some species grown in pots in greenhouse conditions (Beloperone guttata Brandeg. and Plumbago capensis Thumb.) to the application of Cycocel, in a concentration of 0.1% (1000 ppm) and 0.3% (3000 ppm).

The effects of Cycocel treatments on both studied species were the reduction of the vegetative growth, which was more accentuated for the plants treated with Cycocel 0.3% (3000 ppm) and the intensive colour of leaves and inflorescences. There was noticed a positive influence at Plumbago capensis Thumb. regarding the number of shoots and inflorescences formed per plant.

#### **INTRODUCTION**

The treatments with growth retardants are applied on many species, decorative by flowers or leaves, grown in pots, to control the plant height in order to obtain quality plants with a commercial aspect.

The retardant substances are used in ornamental horticulture for: producing vigorous plants with smaller size, by reducing the stem elongation (Quattrini et al. 1995, Warner & Erwin 2003, Lewis et al. 2004, North et al. 2010, Zakrzewski & Zakrzewska 2011); stimulating the plant branching and obtaining compact plants, with an uniform growth of shoots (McDonald & Arnold 2001, Meijon et al. 2009, Karunananda & Peiris 2010, Currey & Erwin 2012); earlier flowering, forming a larger number of flower buds and flowers per plant (Banko 2003, Banko & Landon 2005, Marosz & Matysiak 2005); the intensification of leaves and bracts colour (Bañon Arias et al. 2001); increasing the chlorophyll and carotenoid pigments content (Lodeta et al. 2010); increasing the plant tolerance to stress during handling and shipping (Mackay & Sankhla 2006).

This paper presents the results regarding the effect of Cycocel treatments in different concentrations on the growth and flowering of the *Beloperone guttata* Brandeg. and *Plumbago capensis* Thumb. plants, highly valued flowering ornamental species with rich flowering, grown in pots and used for decorating the interiors, terraces and balconies or

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placed in the garden during summer, having also a medicinal use (Ariyanathan et al. 2011, Ruijter 2006).

## MATERIAL AND METHODS

The biological material consisted of cuttings from the plants of *Beloperone guttata* Brandeg. and *Plumbago capensis* Thumb., from the collection of the Floriculture discipline of the Faculty of Agriculture and Horticulture of Craiova. The research was conducted in the greenhouse of this discipline, between 2011 and 2013.

*Beloperone guttata* Brandeg. from the Acanthaceae family, is a species native to Mexico, which is a shrub with slender, flexible, 1-1.5 m high stems, with ovate-elliptic leaves, pointed at the top, light green and with small tubular white flowers. The spiciforme inflorescences composed of pink or reddish-brown bracts, overlaid and persistent, scaly in appearance, hence the name of "shrimp plant", are the main decorative elements.

*Plumbago capensis* Thumb., Plumbaginaceae family, is a small shrub native to South Africa, grimpant or with pendent growth, thin shoots and simple, alternate, elliptic or ovate-elliptic, 5 cm long, persistent leaves. The flowers are small, light blue, very decorative, and grouped in terminal clusters of 10-15 cm diameter.

The shoots were harvested from the mature plants of *Beloperone guttata*, in April 2011, and from the plants of *Plumbago capensis*, in May 2012, and cuttings were obtained, which were treated with a biostimulator (Radistim 1) and then planted in a 1:1 mix of peat and perlite, the rooting substrate having a temperature of 20-22°C.

The rooted cuttings were planted in pots with a diameter of 12 cm, in a substrate made of 2:2:1 peat, manure and perlite. Three Cycocel treatments in various concentrations were done, applied to the substrate, at an interval of two weeks.

The experimental variants were: V1-control (water); V2-Cycocel 0.1% (1000 ppm); V3 - Cycocel 0.3% (3000 ppm).

For the both studied species, the vegetative growth rate was monitored under the influence of treatments applied, during 6 months after the experiment placement.

To estimate the influence of Cycocel treatments on the growth and flowering process of the plants, the observations and measurements were: for *Beloperone guttata* - the average height of the plants, the average number of shoots per plant, the average leaves size (length and width), the number and the average length of inflorescences; for *Plumbago capensis* (the average length of shoots, the average number of shoots/plant, the average number of inflorescences/plant, the decorative duration).

## **RESULTS AND DISCUSSIONS**

In the first experiment, the effect of Cycocel treatment was observed on *Beloperone guttata* plants by reducing the height, in both variants, in comparison with the control plants. The results obtained show that after 5 months from the placement of the experimental variants, the lowest average height values of the plants were recorded at V3 - Cycocel 0.3% (11.5 cm) and at V2 - Cycocel 0.1% (14.8 cm) and the highest value (37.3 cm) at the untreated plants (graph. 1).

The Cycocel treated plants had a slow growth throughout the assessment period of the applied treatments influence. The recorded growth from their transplantation in pots until the latest measurements and observations, made in September, was of 3.5 cm at V3 - Cycocel 0.3% and of 6.2 cm at V2 - Cycocel 0.1% in comparison with the control plant - V1, which recorded the highest increase, of 28.2 cm.

Regarding the average number of shoots per plant, similar values were recorded for all the variants, in the first two months after planting in pots. From this point of view,

the application of Cycocel treatments in various concentrations, had no positive effect, the lowest number of shoots was recorded at V3 - Cycocel 0.3% (3 shoots per plant), in comparison with V1 - the control plant (10 shoots per plant) (graph. 2).

The Cycocel influence on the leaves size was observed by their reduction for the treated plants in comparison with the control plant. In graph. 3 and 4 we notice that the lowest values of the average size of leaves were recorded at V3 - Cycocel 0.3% (4.7 cm length and 2.8 cm width), followed by V2 - Cycocel 0.1%, with very small differences, and the highest values at V1 - the control plant (7.5 cm length and 4.1 cm width).

The dynamic of the average number of inflorescences per plant shows lower values at the treated plants, i.e. 6.3 inflorescences/plant at V3 - Cycocel 0.3% and 8.0 inflorescences/plant at V2 - Cycocel 0.1%, in comparison with control - V1, with 15.0 inflorescences/plant (graph. 5).

Regarding the average length of the inflorescences, the obtained results show that the Cycocel treatments led to a decrease at the variants treated with Cycocel 0.1% (V2-8.0 cm) and 0.3% (V3-7.0 cm), the recorded values being lower than the ones of the control plant (V1-10 cm), (graph. 6).

At the variants that have been treated with Cycocel, the plants had a more compact growth and there was observed an intensification of the leaves colour as a result of the increase in the content of chlorophyll pigments and an intensive colour of the inflorescences.



Graph 1. The average height of plants



Graph 3. The average length of leaves

Graph 2. The average number of shoots/plant



Graph 4. The average width of leaves



Graph 7. The average length of shoots Graph 8. The average number of shoots/plant

1.IX.2012

15.X.2012

15.XI.2012

18.XII.2012

U1 (Control) V2 (Cycocel 0,1%) V3 (Cycocel 0,3%)

15.L2013

15 || 2013

15.X.2012 15.XI.2012 18.XII.2012

□ V1 (Control) □ V2 (Cycocel 0,1%) ■ V3 (Cycocel 0,3%)

1.IX.2012

15.1.2013

15 || 2013



The second experiment was intended to observe the influence of Cycocel treatments on the growth and flowering of the *Plumbago capensis* Thumb. plants.

The analysis of the obtained results shows that the applied treatments determined at the *Plumbago capensis* plants, a reduction of the shoot length with increasing the Cycocel concentration. The average length of shoots had the lowest values for the plants treated with Cycocel 0.3% (V3 - 40.6 cm), being reduced to half, in comparison with the untreated plants (V1 - 80.7 cm) (graph. 7).

The average number of shoots per plant increased throughout the experiment for all the variants. The last measurement shows that the highest value was recorded at V2 (11 shoots/plant), where Cycocel 0.1% treatments were applied and the lower value was recorded at V1 - control (8 shoots/plant) (graph. 8).

To determine the influence of Cycocel treatments on the flowering of the *Plumbago capensis* plants, observations were made on the inflorescences appearance, on the average number of inflorescences per plant, on the decorative period. The inflorescences appearance and the flowering were observed with 4-7 days earlier at the treated plants in comparison with the control plants.

The Cycocel treatments had a positive effect in terms of the number of inflorescences per plant, which recorded higher values at V3 - Cycocel 0.3% (26 inflorescences/plant) than those of the control plant. At this variant the average number of inflorescences/plant increased by 50%, in comparison with the control - 12 inflorescences (graph 9).

Regarding the decorative duration there was noticed that it was higher at the treated plants than control. The longest decorative duration was recorded for the plants treated with Cycocel 0.3% (V3 - 131 days), in comparison with the untreated plants (V1 - 56 days) (graph. 10).

#### CONCLUSIONS

At *Beloperone guttata* Brandeg., the Cycocel treatments reduced the plant height, the lowest values being recorded at the plants treated with Cycocel 0.3% (3000 ppm). At *Plumbago capensis* Thumb., the application of Cycocel treatments determined on the one hand the reduction of the shoots length, and on the other hand an increased number of shoots and inflorescences per plant, and an increased decorative duration, in comparison with the untreated plants.

The obtained results show that the Cycocel treatment improved the ornamental appearance of the plants for the both studied species, observing a compact growth and an increase in the leaves and inflorescences colour.

It is necessary to continue the research; the ornamental value of the plants is influenced by the reaction of each species to a particular type of plant growth regulator, to the applied concentrations, the number of treatments, the time period and the method of application.

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## FRUIT THINNING BY USING NAA AGENT ON THE JONAGORED APPLE VARIETY

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Keywords: Apple, thinning, NAA, yield, mean fruit weight, size classes.

### ABSTRACT

The experimental plot is placed in the orchard "Dacfruct" Ltd. founded in 2006 with trees of a "knip boom" canopy type. The study subject of the experience was Jonagored apple variety grafted on M 9 weak vigor rootstock. The trees were trained as slender spindles. The distance of plantation is  $3.5 \times 1.2 \text{ m}$ .

The research was conducted during the period of 2011 year. The aim of this study was to evaluate the effectiveness of thinning agent in reducing fruit set in Jonagored apple trees. The tested agents were NAA, which was sprayed at two different times during the thinning period. During the research, it was studied the yield, mean fruit weight, average fruit diameter, the number of first class fruits.

It was established that, in the 2011 year, the two treatments have a significant effect on yield, mean fruit weight and on the distribution of apples in size classes.

#### **INTRODUCTION**

Early fruit let thinning removes excessive fruitless from apple trees and is one of the most effective measures to increase fruit size, color, and quality in the year of application, and to promote flowering the following year (Childers et al.,1995; Tromp, 2000).

Chemical thinning is an established and essential practice performed by fruit growers each spring. In recent years, the apple market has caused chemical thinning to become the single most important cultural practice that many growers undertake. Among the many agents used for thinning, the most frequently used is auxin naphtylacetic acid (NAA) (Stopar and Lokar, 2003; Radivojević et al., 2011).

The mechanism of action of NAA is based on the stimulation of ethylene synthesis taking place in fruit let tissue. It has also been ascertained that the natural drop of fruit lets is always connected with an increase in ethylene synthesis and evolution (Dennis, 2000). The effectiveness of fruit let thinning with NAA is often unsatisfactory. The efficacy of the treatment depends on the weather, which affects the intensity of the agent penetrating into leaf tissue (Basak, 2004). This can influence the intensity of ethylene synthesis and evolution, and ultimately determine the effectiveness of the treatment. The most effective time to apply NAA as a chemical thinner is when fruit diameter is 7to 9 mm or 11 to 13mm (Greene, 2002).

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## MATERIAL AND METHODS

The researches were made during the period of 2011 year in the apple super intensive orchard founded in autumn 2006 at the company "Dacfruct" Ltd. with "knip boom" type apple trees crown formation.

The study object of experience was Jonagored apple tree variety grafted on weak vigor M9 rootstock. The crown was conducted on ameliorated thin spindle system. Distance of plantation is  $3,5 \times 1,2 \text{ m}$ .

The chemical growth regulator used was Dirager, containing 37 g/l active ingredient NAA, the preparation by the "L. Gobbi Ltd." producer from Italy. To optimize the fruit load of the apple trees were experimented the following variants:

V1 - Control variant- without chemical treatments;

V2 - Dirager - 20 ml/hl + 20 ml/hl;

V3 - Dirager - 30 ml/hl.

On experimental section in accordance with the experiences scheme in the second variant were performed two treatments (05/14/11; 05/20/11) with a dose of 20 ml/hl. The first treatments was made at central fruit dimension in diameter in diameter of 6-7 mm (05/14/11), the second one being made at central fruit dimension in diameter of 10-12 mm (05/20/11). In the third variant was also performed a single treatment (05/18/11) with a dose of 30 ml/hl when the central fruit size diameter is 8 - 9 mm. The treatment was performed by a portable watering tool during the hours without wind in the morning, at a temperature of +20-25°C, with tendency of temperature growth.

The effects on the fruit set, the yield, fruit quality at harvest and subsequent blooming were recorded and evaluated according to the following measurements: the number of inflorescences and the number of fruitless on each tree; the fruit yield produced by each tree, and, for 1 ha orchard of apples; weight of 1 apple; the share of fruits (in %) in size classes based on their diameter from 50 mm to 85 mm with the intervals of 5 mm and relative effectiveness of treatments.

## **RESULTS AND DISCUSSIONS**

Investigations made demonstrate Jonagored apple trees had differentiated a sufficient amount of bud fruit that after grafting during the resting period in the spring of 2011 have formed 152-162 inflorescences (tab. 1). The obtained inflorescences amount in the crown demonstrates us that in the given plantation can be mounted experiences for testing the Dirager growth regulator.

Table 1

Total inflorescences number (TIN), formed (FIN) in Jonagored apple tree variety crown and fruit weight in an inflorescence

Nr	Experience	TIN,	FIN,	Fruit weigh	t per an inflo	rescence, %
INI	variants	pcs/tree	pcs/tree	1 pc.	2 pcs.	3 pcs.
1.	Control variant, without thinning	152	138	78.2	18.8	3.0
2.	Dirager, 20ml/hl+20ml/hl	160	74	86.5	13.5	-
3.	Dirager, 30ml/hl	155	56	75.0	19.6	5.4

In the blossom period the total numbers of inflorescences on variants do not essentially differ. If in the control variant the inflorescences total number constituted 152 pcs/tree, then in the variants where is foreseen to use the treatment with Dirager growth regulator, it varied between 155-160 pcs/tree.

Continuing to study the formed inflorescences number, we register a difference at variants taken into study, i.e. a studying index decrease in case of treating with Dirager growth regulator.

In the control variant from total inflorescences number of 1,52 pcs/tree have formed 138 pcs/tree which is 90,7%. Growth regulator Dirager had essentially influenced on the formed inflorescences number in the favor of their diminishment. In these variants, the greatest formed inflorescences number was obtained in the variant Dirager 20 ml/hl+20 ml/hl constituting 74 pcs/tree, or a diminishment with 2,16 times than the total inflorescences number registered in summer. In Dirager variant in a dose of 30 ml/hl the mentioned index indicated 56 pcs/tree, it means a decrease of 2,78.

The most rational fruit weight in an inflorescence was registered in case of Dirager growth regulator in a dose of 20 ml/hl+20 ml/hl, where 86.5% of fruits were one by one in inflorescence and only 13.5% were in two inflorescences. The control variant and Dirager variant in a dose of 30 ml/hl had been at the same level, registering even three fruit in an inflorescence.

In conclusion it can be mentioned that a more optimum formed inflorescences number and a more rational fruit weight in an inflorescence was registered in the variant with using Dirager growth regulator with a dose of 20 ml/hl+20 ml/hl.

Fruit production and its parameters quality are some final indexes that are demonstrating how there were made the agro technical works and other technological operation in the given plantation.

The investigations made (tab. 2) demonstrate us that in the result of treatment with Dirager growth regulator the fruit number differ on the varieties taken into the study. The greatest quantity of fruit was registered in the control variant – 185 pcs/tree, where it was not made the chemical thinning. Then continues in a descendent order the variant with a dose of 20 ml/hl+20 ml/hl - 98 pcs/tree and variant Dirager in a dose of 30 ml/hl - 77 pcs/tree. The study on fruit number of 100 inflorescences demonstrates us the same identical rule with the previous one, constituting respectively 121, 61 and 50 pcs.

Due to the greater number of fruit per tree and at 100 inflorescences in the control variant registered, respectively, an increase of production per tree and unit on production constituting a significant value of production, it was registered in variant Dirager in a dozen of 20 ml/hl 18.13 kg/tree and 43.15 t/ha, statistically demonstrated. In case of making the treatment of trees with Dirager growth regulator in a dose of 30 ml/hl, we registered a more essential fruit production decrease to 17.46 kg/tree, or a movement with 10.05 t/ha in comparison with the control variant, but at the level of variant two.

Studying the influence of growth regulator on fruit production we register that in case we use the preparation Dirager in a dose of 20 ml/hl+20 ml/hl then the index into the study increased with 0,67kg/tree in comparison with the variant Dirager in a dose of 30 ml/hl, or with 1,60 t/ha.

Statistic processing data demonstrate that the statistic difference regarding the formed fruit number and average weight of a fruit exists between all variants into the study. If to refer to fruit production, then the statistical difference is registered only in report with the variant control. Between the treated variants with Dirager growth regulator there are no significant statistic differences of the factor into the study.

Investigations made regarding the obtained production reported to trunk transversal surface (TTS) demonstrate us that the highest frut production per 1 cm² TTS was registered in the control variant -1,39 kg, where the fruit trees were not exposed to thinning. In case of chemical thinning, the fruit production reported to the trunk transversal surface diminishes, registering in the variant Dirager (20 ml/hl+20 ml/hl) -1,13 kg/cm², but in variant Dirager (30 ml/hl) -1,12 kg/cm². Treatment with different doses of preparation demonstrates a non-significant decrease of fruit production.

Table 2

Na	Experience		Fruit number,pcs		it product	Average	Average diameter	
INI	variants	tree	100 inflor.	kg/cm ² TTS	kg/ tree	t/ha	g g	, mm
1.	Control variant, without thinning	185	121	1.39	21.35	50.81	115.4	64.3
2.	Dirager, 20ml/hl+20ml/hl	98	61	1.13	18.13	43.15	185.0	77.2
3.	Dirager, 30ml/hl	77	50	1.12	17.46	41.55	226.7	81.4
	LSD 5%	10,28	-	-	0.88	2.05	17.24	

Influence of growth regulator on production and quality parameters of fruits in Jonagored apple tree crown formation at harvest

Fruit quality has indispensably tangency with average weight and diameter of a fruit. Investigations made demonstrate that the growth regulator Dirager, used as a fruit chemical thinning preparation had an essential influence on quality of production. If in control variant, where there was made no chemical thinning, the average weight of a Jonagored variety fruit constituted 115,4 g, then in other variants where it is tested the growth regulator Dirager, it increased to 185,0-226,7 g.

Studying the average weight of a fruit in the variants with chemical thinning, it is registered an increase of the investigated index with 22,5% in case when treating with the preparation Dirager in a dose of 30ml/hl, in comparison with the variant Dirager in a dose of 20 ml/hl+20 ml/hl.

Fruit average diameter is an indicator that has a direct tangency with the average weight of the production obtained. The smallest average diameter was registered in the control variant -64,3 mm, where the trees were without thinning, then, in an increasing order, was situated the variant Dirager with a dose of 20 ml/hl+20 ml/hl - 77.2 mm and variant Dirager in a dose of 30 ml/hl - 81.4 mm.

The results obtained demonstrate us that treating with Dirager growth regulator with a dose of 30 ml/hl at the central fruit diameter 8-9 mm diminished the number of fruit per tree increasing essentially the average weight and diameter of a fruit. More stable values in report with the previous variant were registered in case of treating with Dirager growth regulator in a dose of 20 ml/hl+20 ml/hl.

The investigations made (tab. 3) demonstrate us that in the control variant were obtained a more inferior production quality in comparison with the variants where it was used Dirager growth regulator.

In control variant the highest weight of fruit (43,1%) is placed in the class with a diameter of 61-65 mm, 34,6% have the diameter of 66-70 mm, 10,6% have the diameter of

56-60 mm, 10,2% have the diameter of 71-75 mm and only 1,5% has a diameter less than 55 mm. So, the fruit weight of extra and I-st categories, without thinning, constitutes 44,8%, and 55,2% is referred to the second category and non-standard fruit.

Continuing to study the influence of the growth regulator on fruit quality we register a more rational redistribution in the variant where there were made two treatments with Dirager in a dose of 20 ml/hl+20 ml/hl. In that variant the fruit weight with a diameter less than 65 mm does not exist. Fruit weight of first category, with a diameter of 66-70 mm constitutes 3,7%, and the extra category the mentioned index constituted 96,3% of fruit. Thus, all the fruit are attributed to the extra and Ist categories of quality.

### Table 3

40.7

22.0

29.8

			e	11		2			
	Experience		Fruit w	eight %	dependi	ng on the	e diamet	er (mm)	
Nr	variants	<55	56-	61-	66-	71-	76-	81-	>85
	, un funtos		60	65	70	75	80	85	
1.	Control variant, without thinning	1.5	10.6	43.1	34.6	10.2	-	-	-
2.	Dirager, 20ml/hl+20ml/hl	-	-	-	3.7	23.3	45.6	27.4	-
3.	Dirager,			1.4	25	9.6	22.0	20.9	40.7

Influence of Dirager growth regulator on redistribution of fruit depending on their diameter at Jonagored apple tree variety

In case when treating with Dirager in a dose of 30 ml/hl we registered an increase of fruit weight in the class with the diameter of 81-85 mm - 29,8% and higher than 85 mm- 40,7%, but a decrease of fruit quantity that is referred to the diameter 71-80 mm (30,6%). It decreases also the fruit weight with the diameter 66-70 mm at 2,5%, are formed category II fruit, less than 65 mm 1,4%. Fruit with a diameter more than 80 mm have a less limited period of storage and are affected by physiological diseases.

1.4

30ml/hl

2.5

8.6

Diameter index (R) constitutes the report between the total quantity of harvested production and that with a diameter less than 70 mm. Investigations made (tab. 4) demonstrates that in the variant control between the total fruit production per one tree and that with a diameter less than 65 mm is a non-significant difference. As a result, the diameter, production and global indexes are properly 1.11; 1.0 and 1.11.

## Table 4

Relative efficiency of treatments for chemical thinning with the growth regulator Dirager in Jonagored apple tree plantation

Nr	Experience	Fruit pro kg/	Fruit production, kg/tree		Index		
INI	variants	total	with ø < 70 mm	diameter (R)	production (P)	global (G)	
1.	Control variant, without thinning	21.35	19.18	1.11	1.0	1.11	
2.	Dirager, 20ml/hl+20ml/hl	18.13	0.69	26.27	0.85	22.33	
3.	Dirager, 30ml/hl	17.46	0.68	25.67	0.82	21.05	

As the fruit weight with a diameter less than 70 mm diminishes, so the diameter index (R) increases, constituting in case of treating with Dirager growth regulator a dose of 20 ml/hl +200 ml/hl – 26,27, but in the variant Dirager with a dose of 30 ml/hl are registered values of 25,67.

To obtain higher values of production index (P) it is necessary that the fruit production in the variants with treatments to be higher in comparison with the control variant. Investigations made demonstrate that an index of production which is lower is attested in the variant Dirager with a dose of 30 ml/hl– 0,82, then in an ascendant direction is pleased the variant Dirager with a dose of 20 ml/hl +20 ml/hl -0,85 and control variant, without thinning -1,0.

The lowest values of the global index are registered in the control variant (1.11). Of the treated variants the highest global index was registered in the variant Dirager with a dose of 20 ml/hl +20 ml/hl – 22,33, and the lowest – in variant Dirager with a dose of 30 ml/hl – 21,05.

The researches made demonstrate that the highest values of real efficiency (diameter index, production and global diameter) are registered in the variant with the use of growth regulator Dirager with a dose of 20 ml/hl + 20 ml/hl.

#### CONCLUSIONS

It was observed in the experiments performed that inadequate thinning was influent on fruit weight in inflorescence, number of fruits on tree, fruit production, average weight and average diameter, fruit redistribution according to their diameter and on the relative effectiveness of treatments.

The first treatment to be made, at the central fruit dimension in diameter 6-7 mm, but the second treatment at the dimension of central fruit in diameter of 10-12 mm.

The Dirager growth regulator can be used as a chemical thinning agent to optimize the apple fruit load in a dose of 20 ml/hl +20 ml/hl.

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## FORAGE YIELD AND QUALITY OF PEA AND OAT INTERCROP

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Keywords: intercropping; Dry matter yield; Crude protein yield

## ABSTRACT

The purpose of this trial was to evaluate the dynamics of yield for pea and oat intercropping. Field trials were carried out during the 2012 growing season at Agricultural Research and Development Station (ARDS) Simnic-Craiova on luvosoil in randomized variants, using a mixture of pea and oat of 60:40 (weight basis). Samples were taken at the following stages of vegetative development of oat: booting, heading, milk development and daugh development, in order to study the dynamic of dry matter DM, and crude protein (CP) accumulation. Acid detergent fibre (ADF) and Neutral detergent fibre (NDF) contents were determined for evaluation of nutritional quality of the forage intercrop. According to the results of this study, it was found that good forage yield and good quality characteristics for animal nutrition could be obtained from a intercrop using pea and oat at 60:40 seeding ratio.

## **INTRODUCTION**

Pea (*Pisum arvense L*) is an important legume in many areas of the world, which is used for grain and forage production.

Cereals are used in livestock nutrition for their dry matter production but they have low nutrition value due to their poor protein content. High quality of forage is an important aspect of forage crop production. Legume-cereal intercropping is considered as a management strategy in producing high quality and quantity forage.

Physiological and morphological differences between intercrop components affect their ability of using soil and environmental resources. Atmosferic nitrogen fixation by legumes can reduce the competition for nitrogen in legume-cereal intercropping system allowing the cereal to use more soil nitrogen. This can affect forage quality of intercrop components.

Cereal grains and legumes are some of the most commonly species for intercropping (Arlauskiene et al., 2011; Hauggaard-Nielsen et al., 2000; Kadziuliene et al., 2009; Han et al., 2012).

Research designed to optiomize the intercrop advantage uses a replacement series experimental design, where total plant density is held constant and the relative proportion of each species in mixture is varied (Bedaussac and Juster, 2011; Han et al., 2012).

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The objective of this study was to evaluate pea and oat intercropping in a seeding ratio of 60:40 for forage yield and quality.

## MATERIAL AND METHODS

The research was conducted during the 2012 growing season at ARDS Simnic in Oltenia region. The total precipitation and average temperature data for the experimental area are given in table 1.

Table 1

Month Temperature (°C) Rainfall (mm) Relative Mean monthly Mean montly Mean monthly humidity Monthly multiannual multiannual (%) temperature March 9.8 4.8 6.2 35.0 55.9 13.7 11.4 79.3 42.8 59.4 April May 17.6 16.8 136.7 61.7 _ June 22.7 20.9 15.1 63.8 22.1 July 12.0 54.6 _ _

The total precipitation and average temperature data for the experimental area

Pea (*Pisum sativum L*) and oat (*Avena sativa*) were used as the experimental material in a ratio of 60:40 based on seed weights.

The experiment was established on 3 March 2012. Each plot consisted of 6 rows, each 10 m in length. The row spacing was 25 cm and the seeds were mixed and sown together (144 and 160 seeds per  $m^2$  for pea and oat respectivelly. Samples were collected at the following stages of vegetative development: booting, heading, milk development and dough development. All samples were dried at 65 °C for 72 hours and weighed, then graunded to pass through a 1 mm sieve screen and analysed for nitrogen acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents. The crude protein content was calculated by multiplying the Kjeldhal nitrogen concentration by 6.25 factors. The ADF and NDF concentrations were determined according to Fibretherm Technology (Gerhardt, Germany).

Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_L) were estimated according to the following equations:

TDN = (-1.291 x ADF) + 101.35

DMI = 120 / % NDF (dry matter basis)

DDM = 88.9 - (0.779 x % ADF), dry matter basis)

RFV = %DDM x %DMI x 0.775

 $NE_L = [1.044 - (0.0119 \text{ x \% ADF})] \text{ x } 2.205$ 

## **RESULTS AND DISCUSSIONS**

The results of the experiment are showed in table 2.

The greatest DM yield was obtained in dough development stage 7210 kg/ha, compared with 6990 kg/ha at milk development stage, 4690 kg/ha at heading stage and 3548 kg/ha at booting stage.

The crude protein content decreased from 18.3% of DM in booting stage to 15.2% in heading stage, to 13,1% in milk development stage and 11.2% in dough development.

The crude protein yield increased from 649.28 kg/ha at booting stage to 807.52 kg/ha at dough development stage, because of higher DM yields (table 2).

Crude protein content of forage is one of the most important criteria for furage quality evaluation. Expressing protein per area, which combines the CP content and total biomass produced is a valuable measure; it is important to know the total protein that can be harvested in a forage crop.

Neutral detergent fiber (NDF) concentration showed an increasing trend from boot stage to dough development stage. The rate of increase was more rapid from boot stage to heading stage and from heading stage to milk development stage (3.95 and 4.18%) than from milk development stage to dough development stage (2.0%).

Table 2

# Dry matter and crude protein yield (kg/ha) and NDF and ADF content of pea and oat intercropping

Intercropped		Vegetative development stages						
plants	Boot	Heading	Milk	Dough				
			development	development				
		Dry matter (kg/ha	a)					
pea + oat	3548	4690	6990	7210				
	(	Crude protein % of	DM					
pea + oat	18.3	15.2	13.1	11.2				
		Total protein (kg/h	na)					
pea + oat	649.28	712.88	915.69	807.52				
	Neutra	al detergent fibre (%	% of DM)					
pea + oat	50.60	52.60	54.80	55.90				
	Acid detergent fibre (% of DM)							
pea + oat	37.0	38.4	38.8	40.1				

#### Table 3

Estimation of the furage quality of pea and oat intercropped

Intercropped	Vegetative development stages					
plants	Boot	Heading	Milk	Dough		
			development	development		
	Tota	l digestible nutrien	ts (%)			
pea + oat	53.58	51.78	51.26	49.59		
	Dry matt	er intake % from be	ody weight			
pea + oat	2.37	2.28	2.19	2.14		
	Di	gestible dry matter	(%)			
pea + oat	60.08	58.99	58.68	57.66		
		Relative feed value	e			
pea + oat	110.35	104.23	99.59	95.63		
Net energy for lactation						
pea + oat	1.325	1.296	1.285	1.250		

In this experiment the lowest NDF concentration was in boot stage and the highest in dough development stage (50.60% vs 55.90% of DM).

Acid detergent fibre (ADF) concentrations showed an increasing trend from boot stage to dough development stage (table 2). The rate of increase was more rapid from boot stage to heading and to milk development stage to dough development stage (3.78% and 3.35%), than from heading stage to milk development stage (1.0%)

The TDN refers to the nutrients that are available for animals and are related to the ADF concentration of a furage.

As ADF increase there is a decline in TDN (table 3). The lowest value of TDN in dough development stage are attributed to the high amount of ADF in this stage.

The DMI was higher in boot stage and NDF is used to predict DMI and is negatively correlated.

A similar trend was observed for RFV and NE_L. The RFV was higher in boot stage (110.35) and lowest in dough development stage (95.63). The RFV is an index used to predict the intake and energy value of the forage.

#### CONCLUSIONS

For forage crops it is important to produce greater forage yields per hectare, higher nutritional quality (percentage composition of selected nutrients) or combined nutrient yields.

In this study, it was found that good forage yield and good quality characteristics for animal nutrition could be obtained from the mixture of pea with oat at a 60:40 seeding ratio.

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## AGRONOMIC BENEFITS OF PEA-OAT INTERCROPPING

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Keywords: Land equivalent Ratio; Aggressivity; Competitive ratio

#### ABSTRACT

Intercropping pea (Pisum sativum L.) with oat (Avena sativa) in a ratio 60:40 was compared with pea and oat monocrops for the growing season 2012 at Agricultural Research and Development Station (ARDS) Simnic-Craiova, Romania. The yealds of dry matter (DM), crude protein (CP) and Nitrogen (N) utilization efficiency (NutE) for biomass accumulation and several competition were used to evaluate the intercropping system. DM yield was the highest in pea and oat intercrop (8.41 t/ha) followed by oat monocrop (8.28 t/ha). Pea crop and pea and oat intercrop produced the lowest biomass per kg of N that was taken up, compared with oat monocrop. Land Equivalent Ratio (LER) and Relative crowding coefficient (RCC) values show a yield advantage of intercropping over manocrops in terms of the better use of the land and environment resources for plant growth.

#### **INTRODUCTION**

Intercropping is an agricultural practice of cultivating two different crops in the same place at the same time during a growing season. Benefits to intercropping can lead to greater yield compared to the sole crop.

Carefully planning and suitable conditions need to occur for each crop to be complimentary.

There are contradictory data about the productivity of intercropping system. In some studies there was no significant effect on forage yield (Lithourgidis et al., 2011), whear as in other studies intercrops yielded lower Dry Matter (DM), as compared with cereal monocrops (Yolcu et al., 2009).

One of the most important factors that can have a significant impact on yield of intercropping systems compared with the monocrops of the species used, is competition between intercrop components.

Several indices, such as Land equivalent ratio (LER), Relative crowding coefficient (RCC or K), Competitive ratio (CR) and aggressivity (A) have been developed to describe competition advantage in intercropping (Mead and Willy, 1980).

The objective of the present study was to evaluate pea + oat monocrops as well as pea-oat intercrop for DM and protein yield, and to estimate the effect of competition among the component species.

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#### MATERIAL AND METHODS

A field experiment was made at ARDS Simnic-Craiova, located in the south region of Romania, on a luvosoil having pH 5,7-6,9 and on organic matter content 1.8%, P 54ppm and K 128 ppm (0-20 cm deep).

Seedbed preparation included plouwing, disk harrowing and sowing. Pea (*Pisum sativum L.*) and oat (*Avena sativa*) monocrops as well as intercrops of pea with oat in seeding of 60:40, based on seed weights, were sown in 3 March of 2012 growing season. The seeding rates for pea and oat monocrops were 134 and 150 kg/ha (corresponding to 240 and 400 seeds per m²). The seeding rates for intercrop were 80 to 60 kg (corresponding to 144 and 160 seeds per m² for pea and oat respectively. The row spacing was 25 cm and the seeds of both species were sown simultaneously. The experimental design was randomised complet block with 3 treatments (2 monocrops and 1 intercrop) replicated four times. Climatic data during March-July of the growing season 2012 are given in table 1.

Table 1

Month	Tempera	ture (°C)	Rai	Rainfall (mm)			
	Mean monthly Mean montly		Monthly	Mean monthly	humidity		
	temperature	multiannual		multiannual	(%)		
March	9.8	4.8	6.2	35.0	55.9		
April	13.7	11.4	79.3	42.8	59.4		
May	17.6	16.8	136.7	61.7	-		
June	22.7	20.9	15.1	63.8	-		
July	-	22.1	12.0	54.6	-		

Thermal regime and rainfall March-July 2012

#### Dry matter and protein yield

Pea and oat were harvested in a 1 m2 area of each plot at dought development of oat, and samples of 0,5 kg were dried at 60 oC for 72 hours for DM and protein determination. The crude protein was calculated by multiplying the nitrogen content (determined usig the Kjeldhal method) by 6.25. Nitrogen utilisation efficiency (NUtE) for biomass accumulation

was calculated according to the following formula: NUtE =  $\frac{DM}{N}$ , where DM is the DM

yield (kg/ha) at harvest an N is the total N (kg/ha) that was taken up by the crop.

#### **Competition indices**

For pea and oat intercrop, land equivalent ratio (LER) was determined as developed by Mead and Willey (1980).

LER = LER pea + LER oat

LER pea = 
$$\frac{Yp.int\ ercropped}{Yp.pure}$$
; LER oat =  $\frac{Yo.int\ ercropped}{Yo.pure}$ 

Another coefficient that is used is the relative crowding coefficient (RCC or K), wich is a relative measure of the relative dominance of one species over the other in a mixture (De Wit, 1960 citied by Dhima et al., 2007): Kpea x Koat

$$Kpea = \frac{Yp.int \ ercropped \times Zo}{(Yp.pure - Yp.int \ ercropped)Zp}$$

 $Kcereal = \frac{Yo.int\ ercropped \times Zp}{(Yo.pure - Yo.int\ ercropped)Zo}$ 

Zp is the sown proportion of pea in mixture with oat and Zo is the sown proportion of oat in mixture with pea.

Aggressivity is another index used to indicate how much the relative yield increase in "a" crop is greater than that of "b" crop in a intercropping system:

$$A \text{ oat} = \frac{Yo.\text{int } ercropped}{Yo.pure \times Zo.\text{int } ercropped} - \frac{Yp.\text{int } ercropped}{Yp.pure \times Zp.\text{int } ercropped}$$
$$A \text{ pea} = \frac{Yp.\text{int } ercropped}{Yp.\text{int } ercropped} - \frac{Yo.\text{int } ercropped}{Yo.\text{int } ercropped}$$

 $Yp.pure \times Zp.int \ ercropped \qquad Yo.pure \times Zo.int \ ercropped$ 

Competitive ratio (CR) gives a better measure of competitive ability of the crop.

$$CR pea = \left(\frac{LERpea}{LERoat}\right) \left(\frac{Zo.}{Zp.}\right)$$
$$CR oat = \left(\frac{LERoat}{LERpea}\right) \left(\frac{Zp.}{Zo.}\right)$$

#### Statistical analysis

The data (DM asnd CP and competition indice S) were analysed by the ANOVA method.

## **RESULTS AND DISCUSSIONS**

Pea and oat intercrop gave the highers DM yield, 8.45 t/ha, compared with pea or oat monocrops, 7.21 t/ha and 8.28 t/ha respectively (table 2). Pea and oat intercrop produced on average 16,64% more DM yield than pea monocrop, and 1,15% more DM yield than oat monocrop.

Crude protein concentration was the highest in the pea monocrop 138 g/kg of DM, compared with 112 g/kg DM in pea and oat intercrop, whereas oat monocrop had the lowest CP concentration 84 g/kg of DM (table 2). NutE was the lowest in the pea monocrop and pea 60 and oat 40 intercrop, 45,3 and 55,7 respectivelly. This means that these crops produced the lowest biomass per kg of N that was taken up, compared with oat crop (table 2).

Table 2

Dry matter yield, crude protein concentration and yield and nitrogen utilisation efficiency (NutE) of monocrops and intercrop of pea with oat

	Dry matter (DM)			Crude protei	n (CP)	NUtE
	t/ha					kgDM / ha
	Vegetables	Cereal	Total	Concentration	Yield	$\frac{0}{1-N/1-n}$
				g/kg DM	Kg/ha	kgin / na
Pea	7.21	-	7.21	138	995	45.3
Oat	-	8.28	8.28	84	696	74.6
Pea + Oat	3.48	4.93	8.41	112	942	55.7
LSD (0.05)	-	-	0.50	-	49.4	-

The LER ratio is used to determine the efficiency of intercropping for using the resources of the environment compared with monocropping. The LER in the case of Pea 60 oat 40 (1,07) indicates that up to 7% more area would be required by the monocroping system to equal the yield of intercropping system. The K value in pea and oat intercropped was higher than 1.00. LER and K values show a yield advantage of intercropping over monocrops in terms of the better use of the land and environment resources for thwe plant growth.

In the intercrop, the oat value of A (positive) and CR greater than pea, indicated that oat was the dominant specie in pea-oat intercrop, probably because of the positive effect of pea on cereal when grown in association (table 3).

Table 3

## Land equivalent ratio, relative crowding coefficient, aggressivity, and competitive ratio of pea with oat intercropped

Intercrop	LER			RCC (K)			Aggress	ivity (A)	CR	
	LER	LER	Total	K pea	K	K	А	А	CR	CR
	pea	oat			oat		pea	cereal	pea	oat
pea 60 +	0.48	0.59	1.07	0.860	2.207	1.898	-0.680	+0.680	0.541	1.84
oat 40										3

#### CONCLUSIONS

The present study demonstrates that pea and oat intercrop had yield advantage of intercropping for exploiting the environmental resources. This mixture seem promising in the development of sustainable crop production and can be used by the farmers in Oltenia region.

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## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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## NEW ASPECTS CONCERNING ROOTING SUBSTRATES INFLUENCE ON GROWTH AND DEVELOPMENT OF GRAPEVINE CUTTINGS IN PROTECTED SPACES

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Keywords: substrat, components, organic, mineral, variety

## ABSTRACT

The objective of this study was the aspects clarification about the composition of rooting substrates, mostly organic or mineral, in direct correlation with the variety or group of grapevines varieties. The mixture, mainly organic, used in the production of vegetable seedlings but also used frequently for rooting grapevine cuttings may be improper to cuttings.

The study on different substrates showed that white wine varieties Feteasca regala and Feteasca alba, prefer mainly organic substrates, red wine varieties as Feteasca neagra prefer mineral and organic substrates, while varieties for quality wines like Muscat Ottonel and even table varieties as Muscat d'Adda prefer for rooting mainly minerals substrates, in both cases it is important proportion of components.

## INTRODUCTION

Grapevine can be propagated vegetatively by several methods: cutting propagation, marcotage, grafting and "in vitro" micropropagation, due to the easiness with which develop the roots. The simplest method is vegetative propagation by cuttings which is based on the possibility to obtaining a plant from a bud and from a piece of cane. Commonly are used portion of the organ (that have one or more buds), taken from a plant in order to obtain other with similar characteristics. In this multiplication participate a single individual, so genetic information is unilateral, based on recovery and growth processes of organs through cell division (Martin T. 1966, Mihaela Bucur, 2011).

Propagation by cuttings of vines was a current technique used in prefiloxeric. In present the method is used on sandy soils where phylloxera not met favorable conditions for development, at the rootstocks propagation, of a hybrid direct producers and also at the rapid multiplication of varieties and new clones, valuable, under the conditions of a small amounts of biological material (Dejeu L., 2010).

The rooting cutting is the process in which are releases adventitious roots from rhyzogene layer called pericycle, in the right medullary rays. The roots occur near the base of the cuttings, usually in the node, but may appear on the internode. The success of propagating cuttings is conditioned by genetic (species and variety), ecological, biological and techniques factors (Rădulescu et al. 2010, Tiţa I., 2003).

If ecological, biological and techniques factors have been relatively well studied, genetic factors, in the case of varieties of *Vitis vinifera* pertain primarily to of variety and its

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descendants, influence of rooting by preferences for different types of soils or substrates and the adaptability to different climatic conditions . This study brings some clarification on rooting of some clones for white and red wines, and for table grape on different substrates for rooting.

## MATERIAL AND METHODS

16 St.

Rooting substrate as technical factor, along with other technical, biological and ecological factors, has an important role in the formation of adventitious roots. In order to establish mixes recipes for substrates, suitable to cuttings rooting of one bud from the high quality biological material (G0, G1 and G2) in protected spaces was created an experimental device which took into account the following two factors:

Factor A - The nutrient	Factor B - Variety
A1- mineral mixture, 100 % (0-3 mm washed sand)	B1- Muscat d'Adda 22 St.
A2 - mineral and organic mixture, 75 % mineral +	B2- Feteasca regala 72 St.
25% organic	B3- Feteasca alba 97 St.
A3- homogeneous mixture, 50 % organic + 50%	B4- Muscat Ottonel 16 St.
mineral	B5- Feteasca neagra 6 St.
A4 - organo-mineral mixture, 75 % organic + 25 %	
mineral	
A5 - organic mixture, 100 % (manure and peat in	
equal parts)	

Experience is bifactorial 5x5 type. By combining experimental factors resulted 25 variants, each experimental variant being randomized into 4 repetitions arranged together.

During the experiment were conducted stratification stages - acclimatization and cuttings transfer on nutritional substrates in order to strengthen. The rooting substrates content in organic matter and nutrients were determined on media samples of soil for each substrate in part by current methods in internal lab. Biometric measurements, respectively the amount of annual wood removed from cutting and appreciation of cuttings phytoproductivity during the vegetation period, were made from annual increases of cuttings planted and rooted in spring and harvested in the fall after the leaves fall and offshoots maturation and vegetative mass in full vegetation (16-17.05.2013) by cutting them above the first bud of the base and weighing it. To determine the influence of rooting substrates on the maturation degree of cuttings offshoots, was harvested offshoots portions at which was determinated the content of soluble sugars, starch, total water, protein and total nitrogen.

#### **RESULTS AND DISCUSSION**

### The influence of rooting substrates on the quantity of wood removed from

annual pruning. The quantity of annually wood (g / cuttings), removed after cutting in the rest period after leaf fall provides reliable information about the influence of rooting substrate on the vegetative growth and development of rooted cuttings. In table 1 is observed, compared to the variants average, that organic and predominantly organic substrates achieved larger increases of the vegetative growth by 41-49% above average, while mineral and predominantly minerals substrates made smaller increases of 32-47 % below average.

The substrate with homogeneous comosition achieved growth of 12% below average, which shows that the organic and preponderant organic mixtures forces strong development of the vegetative and influences through positivity the media, so interpretation from the homogeneous variant can be more accurate. In this case increases on organic and predominantly organic substrates more important, of 61-70%, but also the growth on mineral and predominantly minerals substrates are higher, of 22-39%, which is closer to reality.

#### Table 1

No.	Substrats	Variaty B1-B5	Average R1-R4 g/cuttting	% than average	% than A3
1	A1, Mineral 100%	Media B1-B5	17,91	53,18	61
2	A2, Mineral 75%, Organic 25%	Media B1-B5	22,99	68,26	78
3	A3, Mineral 50%, Organic50%	Media B1-B5	29,60	87,89	100
4	A4, Organic 75%, Mineral 25%	Media B1-B5	47,55	141,18	161
5	A5, Organic 100%	Media B1-B5	50,36	149,52	170
	Average	B1-B5	33,68	100,00	114

Annual quantity of wood removed from cutting in rest period, wood weight (g)

The influence of rooting substrates on the quantity of vegetative mass (offshoots + leaves), assessed during intensive growth off shoots. For a better appreciation of the grapevine cuttings phytoproductivity on various soil substrates, in the second year, the evaluation of on the total weight of shoots and leaves was done by weighing it on four repetitions (table 2), in which case was proceeded to harvesting of vegetative mass in full vegetation (16-17.05.2013) by cutting them above the first bud at the base.

Table 2

The vegetative productivity of rooted cuttings in the second year by harvesting, in green, the grapevine clones planted on different substrates (g)

No.	Substrats	Variaty B1-B5	Average R1-R4 g/cut.	% than average	% than A3
1	A1, Mineral 100%	Average B1- B5	54	57	53
2	A2, Mineral 75%, Organic 25%	Average B1- B5	84	88	79
3	A3, Mineral 50%, Organic50%	Average B1- B5	107	113	100
4	A4, Organic 75%, Mineral 25%	Average B1- B5	113	119	106
5	A5, Organic 100%	Average B1- B5	116	122	108
	Average	B1-B5	95	100	89

The data obtained highlight the growth differences between rooting substrates and between studied clones. Thus by the relative interpretation to average, the predominantly organic were situated with 19 - 22% above the average, while those based on mineral recorded low values of 12-43% below average. The substrate with homogenous composition, 50% organic and 50% mineral was recorded values with13% higher than average. Interpreting against this (A3), which is the medium variant, the growths on organic substrates are lower by 6-8% over average while the substrates based on mineral have achieved increases under the average with 21-47%.

Both the wood weight eliminated from cutting in dormand period and vegetative mass resulted from harvest in full vegetation look like they are positively influenced by increasing the quantity of organic matter from rooting substrate, but more than 50% it can affect all varieties to a certain degree and at quality varieties and table grapes may cause death.

Regarding the clones used in this experiment the differentiation from medium were below average in Muscat dAdda 22 St. with 62% and at Muscat Ottonel 16 St. with 71%, around the average, at the Feteasca regala 72 St. less then 3%, and above average at vigorous varieties like Fetasca alba 97 St. (30%) and. Feteasca neagra 6 St (117%).

The influence of rooting substrates on glucides and total water content of the cuttings canes matured in 2012 in different table grape and wine clones. Increasing the amount of mineral material in rooting substrate increases the proportion of soluble glucides from 4.0% to 4.8% and starch content increase with increasing of the matter organic percentage from 6.2% to 7.9% (table 3). Total water content increases proportionally with increasing of the amount of organic material at 50% in rooting substrate, from 52.12% to 52.81%, and also decreases when increasing the quantity of organic material at 100%, from 52.81% to 50.18%.

The variants with mixture preponderant mineral ensures a higher total water content, approx. 2%, compared to variants that containing predominantly organic mixture, which means a better accessibility of water in these mixtures and also a weaker maturation of matured canes. After analyzing the media variants calculated as the sum of total carbohydrate and total water percentages, since there is no version of control, it is found that variants with the preponderet mineral mixture exceeded the average with 0.3 to 1.2%, while variants with predominantly organic mixture is below average with 1.3-1.9%.

The homogene mixture, organic and mineral in equal proportions, exceeds the average by 1.9%. The above data is supported with the quantity of annual wood removed by cutting in the rest period and with vegetative mass of cuttings in that it increases proportionally by increasing the quantity of organic matter from rooting substrate.

The differentiated maturation of cuttings in 2012 at different wine and table grape clones under the influence of rooting substrates. Analyzing the data from Table 4 and exemplification from figures 1, 2 and 3 observe how soluble carbohydrate content increases, the starch content decreases and total water content increases with the increasing of the quantity of organic matter in the rooting substrate, with appreciable differences between varieties, depending on their preferences for different types of soils.

#### CONCLUSIONS

Rapid multiplication of valuable varieties from superior biological categories G0, G1 and G2, under the conditions of a small quantity of biological material, is made only with short cuttings of 1-2 internodes in protected spaces strictly controlled.

The method of multiplication and rooting technology must ensure fully valorification of biological material available so that each technological component becomes very important.

The rooting substrate composition must be in close correlation with the varieties requirements and preferences for soils and their adaptability to pedoclimatic conditions, which requires a differential rooting technology from variety to variety or from a group of varieties to another.

The compositional structure of rooting substrates determine proportional the grapevines growth and development both in terms of vegetative growth and the quality of wood maturation from rooted cuttings.

The varieties for white wine and table grapes prefer homogeneous rooting substrates and predominantly organic while the varieties for quality white wines and for red wines prefer mineral preponderant rooting substrates.

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Substrat Average B1-B5	Soluble sugar %	Starch %	Total sugar %	Total water %	Total %	% Than average
A1, Mineral 100%	4,8	6,2	11,0	52,12	63,12	100,3
A2, Mineral 75%, Organic 25%	4,6	6,4	11,0	52,66	63,66	101,2
A3, Mineral 50% Organic 50%	4,3	6,9	11,2	52,81	64,01	101,7
A4, Organic 75% Mineral 25%	4,0	7,5	11,5	50,24	61,74	98,1
A5, Organic 100%,	4,0	7,9	11,9	50,18	62,08	98,7
Average A1-A5	4,3	7,0	11,3	51,60	62,92	100,0

The content of soluble glucides, starch and total water of matured grapevines cane

Table 3

Variaty on differents substrats	Soluble sugars %	Starch %	Starch/ Soluble sugars	Total Sugars %	Total water %	Total %	% from the average
B1-M. Adda 22St/ A1-A5	3,90	7,15	1,83	11,05	51,82	62,87	99,87
B2-Fet.reg.72 St/ A1-A5	4,28	7,18	1,68	11,46	51,35	62,81	99,78
B3-Fet.alba 97 St/ A1-A5	4,30	7,36	1,71	11,66	51,89	63,55	100,95
B4-M.Ott.16 St/ A1-A5	4,23	6,80	1,61	11,03	53,22	64,25	102,07
B5-Fet.neag.6 St/ A1-A5	4,66	6,82	1,46	11,48	49,78	61,26	97,32
Average B1 – B5	4,27	7,06	1,65	11,34	51,61	62,95	100,00

## The content of soluble glucides, starch and total water of matured grapevines cane

Table 4



Figure 1 The evolution of the soluble glucides content in Feteasca alba 97St. clone on different rooting substrates.



Figure 2 The evolution of the starch content in Feteasca neagra 6 St. clone rooting on different rooting substrates



Figure 3 The evolution of the total water content of the Fetească regala 72 St. clone on different rooting substrates.

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## THE BEHAVIOUR OF GRAPE VARIETIES FOR WHITE WINE IN THE FIRST THREE YEARS AFTER PLANTING ON SANDY SOILS IMPROVED

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Key words: range, sandy soil, vine variety.

#### ABSTRACT

Improvement of the assortment of vines, in the sandy soils of southern Oltenia, in order to improve the supply of production requires the introduction of the culture of those varieties and clones, which in addition to quality parameters to adapt at local conditions and especially the weather conditions and permanent changes. For this purpose at CCDCPN Dăbuleni founded a collection ampelographics with 50 varieties of white grape varieties with 17.

The results of the first three years have demonstrated a growing capacity to adapt, to the sandy soils of southern Oltenia, depending on variety.

All varieties showed sensitivity to negative extreme temperatures, of  $-27 \, {}^{0}C$ , registered in 2012, being totally destroyed, the buds are above the snow layer and even inside it.

It was noted, with precocity and production, from noble varieties group, the Alb aromat variety, and modern varieties group Brumăriu and Blasius, which showed a big capacity of bear fruit on fruit short elements.

## INTRODUCTION

Increasing food security and quality of primary agricultural products suppose the improving the supply involves the production of ecosystems. In our country grape varieties for white wines hold share in national range (Oşlobeanu M, et al., 1980). In the former ampelographyque collection from CCDCPN Dăbuleni - tried to make a large number of varieties on the basis of which it was established the current assortment (Vlădoianu Em., 1979). However, the improvement of the current assortment, it is necessary, by establishing and introducing the culture of those varieties and clones, which in addition to quality parameters to adapt of local conditions and especially the weather conditions and changing permanent (Vlădoianu Em., 1984; Anca Onache and al., 2008; Cichi & Costea, 2008; Stroe & Bucur, 2012; Artem & Antoce, 2013).

To this end, in 2008, to CCDCPN Dăbuleni were laid the groundwork for the establishment of a collection ampelography with recent varieties from our country and from abroad. The ampelographyque collection, founded in 2010, comprises several varieties and clones with tablegrapes, grapes for red wines and grapes for white wines, the majority of which are new varieties and clones for sandy soils.

## MATERIAL AND METHODS

The varieties studied, in this experience, founded in 2010 year, were as follows:

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Columna, Donaris, Fetească albă Cl. 1 Od., Fetească regală Cl. 21 Bl., Riesling italian, Riesling de Rhin, Neuburger, Alb aromat (figure 1), Grasă de Cotnari, Brumăriu (figure 2), Blasius, Selena.



Figure 1. Alb aromat variety grapes



Figure 2. Brumariu variety grapes

From every varieties *was* planted 40 vines. Distances of planting were 2,2/1,2 m. They made the following observations and experimental determinations:

- entrance to the vegetation of the vines after planting;
- percentage of viable vines at planting;
- the number of eyes trained on the vine during the year;

- the total length of the annual increases;
- the number of shoots with a thickness exceeding 6 mm at the second

internode;

- the yield production and their quality in three year;
- the main vegetative stage.

Before of planting was fertilized with 60 t/ha manure and 400 kg/ha 15 15 15 Complex. In the initial period of the growing season, in May month, were administered 100 kg/ha 15: 15: 15 Complex.

## **RESULTS AND DISCUSSIONS**

All 40 vines planted from each species were viable, proof that all came together in the growing season (table 1). During the growing season some perished because of their capacity to adapt, so that at the end of the growing season have recorded a percentage of rooted normal developed between 80 and 100.

Table 1

The percentage of normal developed vines at the end of vegetation and vigor to the varieties of grapes for white wines in the first year of planting

Variety	Number of planted	Number of underdevel oped or destructed	Vines no develo	ormal oped	The total length of annual	Number of formated nodes on vine
	villes	uestructeu	number	%	(cm)	
Columna	40	4	36	90	254	71
Donaris	40	4	36	90	219	62
Fetească albă Cl.	40	3	37	92,5	360	93
1 Od.						
Fetească regală Cl. 21 Bl.	40	8	32	80	284	85
Riesling italian	40	3	37	92,5	189	49
Riesling de Rhin	40	3	37	92,5	246	68
Neuburger	40	6	34	85	268	76
Alb aromat	40	2	38	95	306	76
Grasă de Cotnari 40		1	39	97,5	289	82
Brumăriu	40	-	40	100	561	106
Blasius	40	-	40	100	283	83
Selena	40	1	39	97,5	291	75

The percentage of normal developed vines of 80 was recorded at variety *Feteasca Regală* Cl. 21 *Bl.* and a 100 percents was registered in the varieties *Blasius* and *Brumăriu* (these are modern hybrids).

The vigour of vines, differences appear both at the annual increases and the number of nodes formed on vine (Table 1). The largest total annual increases and the largest number of nodes formed on the vine were recorded at *Brumăriu*, 561 cm/vine, and respectively 106 cm.

Vigorous varieties are *Fetească albă CL. 1 Od.* (360 cm annual increases in length and 93 nodes/vine), and *Alb aromat* (306 cm annual increases in length and 76 nodes/vine).

Varieties with reduced vigour are *Muscat Ottonel* (462 cm annual increases in length and 41 nodes/vine), *Columna* (650 cm annual increases in length and 64 nodes/vine), *Donaris* (666

cm and 61 nodes/vine) and *Chasselas d'oré* (672 cm length of annual increases and 63 nodes/vine). In the category of varieties with medium vigour were included *Riesling italian* (854 cm length of annual increases in the vine and 66 nodes/vine), *Fetească albă Cl. 1 Od.* (801.5 cm length of annual increases and 74 nodes/vine). Differences between the varieties are there in terms of the number of shoots with a diameter greater than 6 mm at the base of the shoot. These differences have values ranging from 1 to 2.

In 2012 year, the year third at planting, climatical conditions were unfavourable during the winter and less favourable during the period of vegetation.

In 2011 year, after a autumn and 27 days since January, 2012, quiet in terms of minimum temperatures, there was a period of about 15 days, respectively on 28 January and until 10 February, when the vine was well below the limit of resistance for buds and annual or multiannual wood (Table 2).

Table 2

Climatical elements/ month	X 2011	XI 2011	XII 2011	I 2012	II 2012	III 2012	IV 2012	V 2012	VI 2012	VII 2012	VIII 2012	IX 2012	Average or sum
Average temperature (°C)	10.1	1.7	0.7	0.7	-6.6	7.1	13.9	17.2	23.3	26.4	24.3	19.9	11.6
Maximum temperature (°C)	22.8	-8.7	19.2	1.,9	10.4	24.9	29.4	31.8	37.8	41.4	42.6	32	-
Minimum temperature (°C)	-4.5	15.8	-7.8	-24.3	-27	-7.9	-3.7	7.9	9.8	12.4	6.5	4.4	-
Rainfall (mm)	32.6	0.2	13.6	64.6	0	0.4	66.6	93.8	32.4	8.2	21.8	8	342.2
Relative umidity of air (%)	77.4	86	88.1	84	84.7	62.3	62.1	76.8	65	47.7	52.8	58.5	70.5
Multiannual average temperature (1956-2011) (°C)	11.3	5.4	0.4	-1.4	0.8	5.6	11.7	16.7	21.6	23	22.3	17.7	11.3

The main climatical elements registered agricultural year 2011-2012, at weather Station meteo of Research-Development Centre for Agricultural on Sands Dăbuleni, Dolj

This period began on February 28, when, after a very favourable period for vine culture, with minimum temperatures positive or located in the 0  $^{\circ}$ C point, was registered a minimum temperature of –24.3 $^{\circ}$ C from 31 January, with an daily average temperature of – 19.3  $^{\circ}$ C. The next day, on 1 February, the minimum temperature has reached the minimum negative history for this month, -27  $^{\circ}$ C, with an daily average of -19.3  $^{\circ}$ C. The temperature of -27  $^{\circ}$ C has been repeated on 9 February. In the summer (June-August) the monthly average temperature was greater with 1.7 – 3.4  $^{\circ}$ C comparative with multiannual monthly average. Thus, were appeared the drought and sunstroke phenomenons, which influenced negative the vineyards.

Under the conditions set out, so the buds, but also annual wood, were destroyed, so that the
cutting of vine was effectuated short, 2-3 eyes. However the percentage growth of the buds was not 100% (Table 3).

Although in the third year of culture, the plants have produced grapes, but at a lower level, none of the varieties, failing to come close to the production achieved in a year with normal climatical conditions (Table 4). For a most varieties of grape production was symbolic. Here were framed varieties *Neuburger, Pinot gris* (378 Kg/ha), *Fetească albă Cl. 1 Od, Columna, Donaris, Riesling italian, Riesling de Rhine* (757 Kg/ha). From here it follows that these varieties do not have the ability to produce the grapes on short elements, respectively 2-3 eyes.

Table 3

Variety	Viability of buds to entry in vegetation %	Annual increasing (cm/vine)	The number of eyas formed on vine	The number of offshoots with diameter over 6 mm/vine
Columna	83	650	64	4
Donaris	87	666	61	4
Fetească albă Cl. 1 Od.	92	801	74	4
Fetească regală Cl. 21 Bl.	85	684	63	5
Riesling italian	87	854	76	4
Riesling de Rhin	90	680	63	4
Neuburger	86	764	69	4
Alb aromat	87	1494	122	5
Grasă de Cotnari	84	1294	93	5
Brumăriu	84	1768	135	5
Blasius	91	1644	123	5
Selena	84	784	81	5

Viability of buds at entrance into vegetation and vigour of vines from different varieties of
vines with grapes for white wines in the 3rd year of the planting

Something better in these conditions less favourable for vines this year were the following varieties *Blasius*, which registered a 4544 Kg/ha, *Brumăriu* and *Alb aromat* with a 4166 production Kg/ha, which demonstrates that they have the potential to produce the grapes on short elements, situated on the base of vine. *Brumăriu* and *Blasius* varieties are modern hybrids.

Lower production was due to a small degree and lower weight of the grain of grapes, which registered values between the limits 92 g/100 grain of grapes at *Pinot gris* variety and 313 g/grain of grapes at Alb aromat variety, due to dehydration. Only 2 varieties have recorded a weight of 100 grains of grapes to greater than 200 g, *Blasius* (218 g), *Grasă de Cotnari* (208 g).

Total titrable acidity, expressed in g/l  $H_2SO_4$ , recorded lower values than in normal years with climatical conditions and genetic potential than variety, being influenced by the unfavourable weather conditions specific to the agricultural year 2011/2012. The highest

values were recorded in the two modern hybrids *Brumăriu* and *Blasius*, 4.2 and 4.1 g/l  $H_2SO_4$ .

A higher acidity was recorded at *Grasă de Cotnari* variety, 4 g/l H₂SO₄. Most varieties have been recorded values between 3.1 and 3.9 g/l H₂SO₄. There were, however, and the varieties of total titratable acidity values recorded values below 3 g/l H₂SO₄.

Table 4

Grape production and its quality at different varieties of vines with grapes for white wines in the 3rd year of the planting

Variety	Grapes production Kg/ha	The weight of 100 grains of grapes g	Total sugar g/l	Total titrable acidity g/l H2SO4
Columna	757	180	188,4	3,35
Donaris	757	182	185,4	2,4
Fetească albă Cl. 1 Od.	398	126	184,1	2,5
Fetească regală Cl. 21 Bl.	3029	137	186,9	2,6
Riesling italian	757	133	208,8	3,1
Riesling de Rhin	757	126	191,8	3,65
Neuburger	378	154	200,3	3,9
Grasă de Cotnari	1136	208	185,4	4
Alb aromat	4166	313	189,6	3,1
Brumăriu	4166	159	176,9	4,2
Blasius	4544	218	203	4,1
Selena	1514	149	186,3	3,5

The content of total sugars in grains of grapes has exceeded 184,1 g/l at almost all the varieties in this group. Exception made modern variety *Brumăriu*, 176,9 g/l. Were varieties that have accumulated over 200 g/l total sugar, as are *Riesling italian* (208,8 g/l), *Blasius* (203 g/l), and *Neuburger* (200,3 g/l). They will ensure the wine with an alcoholic strength of more than 11,5% vol. alcohol, getting into the category of superior wines. Most varieties have accumulated in grape berries between 180 and 190 g/l total sugars.

The pursuit of growth and development vegetation stages in 2012, is presented in Table 5.

From here it follows that of entrance in vegetation has been triggered at an early, 05.04.2012 and ended at an early date, the *Selena* variety 11.04.2012. This has triggered the phenophase not later than on the date of 16.04.2012 and completed not later than on the date of 20.04.2012 at *Neuburger* variety. Approximately during the same period, entrance in vegetation, *Riesling italian, Riesling de Rhine, Fetească albă CL. 1 Od. and Blasius.* 

The stage of blooming has triggered some 30 days later after completion of entrance in vegetation and took about 12 days. From the point of view of opening stage blossomed the early was *Alb aromat* variety which blooming started on May 29.05.2012, and ended on June 1st. The later flourished the *Columna* variety that has triggered the phenophase started on 23.05.2012 and ended on 05.06.2012.

The stage of increasing of grains, which means increasing the size of the ovary immediately after fecundation, are initiated at the 5-6 days after the start of blooming,m so that a short period they shall be carried out at the same time due to the blooming sequence.

# Table5

Variety	Entrance to	vegetation	Bloom		Increasing of	Ripe	Maturation
	Beginning	End	Beginning	End	grains of grapes -Beginning-	-Beginning-	of grapes
Columna	13.04.12	18.04.12	23.05.12	05.06.12	28.05.12	22.07.12	02.09.12
Donaris	12.04.12	18.04.12	20.05.12	04.06.12	26.05.12	21.07.12	02.09.12
Fetească albă Cl. 1 Od.	14.04.12	20.04.12	19.05.12	03.06.12	25.05.12	20.07.12	04.09.12
Fetească regală Cl. 21 Bl.	14.04.12	19.04.12	19.05.12	02.06.12	24.05.12	20.07.12	05.09.12
Riesling italian	14.04.12	20.04.12	20.05.12	04.06.12	26.05.12	20.07.12	03.09.12
Riesling de Rhin	14.04.12	20.04.12	20.05.12	04.06.12	26.05.12	20.07.12	03.09.12
Neuburger	16.04.12	20.04.12	19.05.12	03.06.12	25.05.12	19.07.12	03.09.12
Grasă de Cotnari	13.04.12	18.04.12	21.05.12	0306.12	27.05.12	19.07.12	04.09.12
Alb aromat	11.04.12	17.04.12	18.05.12	01.06.12	24.05.12	12.07.12	15.08.12
Brumăriu	09.04.12	15.04.12	21.05.12	03.06.12	26.05.12	22.07.12	04.09.12
Blasius	14.04.12	20.04.12	22.05.12	03.06.12	28.05.12	23.07.12	04.09.12
Selena	05.04.12	11.04.12	21.05.12	03.06.12	27.05.12	20.07.12	04.09.12

Phenological observations at different varieties of vines with grapes for white wines in the 3rd year of the planting in the year 2012

The of ripe has triggered the early days of the ordinary in terms of climate. The earliest came into the *Alb aromat* variety on 12.07.2012. It was followed closely by the *Grasă de Cotnari* variety, on 19.07.2012. The latest was *Blasius* variety, on 23.07.2012.

Harvest maturity was achieved from the *Alb aromat* variety, on 15.08.2012 and ended up on the 05.09.2012 at *Fetească regală Cl. 21 Bl.* variety. The *Alb aromat* variety, which has big grain, firms and flavorful is an early variety.

#### CONCLUSIONS

The capacity to adapt of studied varieties different, on sandy soils in the South of Oltenieia, depending on the variety.

All varieties showed sensitivity to temperature of -27 ⁰C, being completely destroyed the buds are above the snow and even layer inside it.

The level of grape production has been reduced, some varieties being symbolic, Pinot gris and Neuburger (378 Kg/ha), Fetească albă CL. 1 Od, Columna, Donaris, Riesling italian, Riesling of Rhine (757 Kg/ha).

It was noted by the Alb aromat variety, for precocity and production level, this showed a great capacity to produce of grapes on short elements.

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# UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

# Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# VACUUM IMPREGNATION OF STRAWBERRIES IN HONEY SYRUP

Roșca Adrian^{1,*}, Roșca Daniela²

KEY WORDS: experimental equipment, non-thermic preservation, vacuum impregnation

#### ABSTRACT

The paper presents experimental equipment special made for interdisciplinary research studies concerning the influence of rough vacuum impregnation process (up to -0,95bar/50mbar).

The aim of this inter-disciplinary research is to study a non-thermic preservation method to obtain a concentrated strawberry comfiture impregnated in honey syrup, with similar characteristics obtained by using thermic traditional method.

The paper presents experimental results to obtain the speeding of vacuum impregnation process increase, the fruit quality too, depending on the rough vacuum values, and the duration and succession of non-thermic preservation process.

### **INTRODUCTION**

One of the main directions in the alimentary industry is focused on the existing natural compounds preservation either through the minimum processing of the raw materials or through the strengthening of the foods with multiple physiologic active compounds such as prebiotics, probiotics, vitamins, fiber, mineral salts etc. The consumer interest increasing for healthy functional foods consumption has oriented the research and the food industry too, towards the goal of obtaining such products (Larousse, 1993).

Consumers increased demand for better food nutritional and sensorial characteristics, with no "fresh taste loss", made necessary extensively studies for functional food industry research concerning non-thermal preservation methods such as high pressure process (HPP), and vacuum impregnation processing (VIP) (Larousse, 1993; Lozano *et.al.*, 2006; Roşca &. Roşca, 2004; Roşca &. Roşca, 2008).

Isostatic process realized by using high pressure process (HPP), middle pressure process (MPP) and vacuum impregnation processing (VIP) can improve the mass transfer rate in any solid-liquid operations are involved: salting, osmotic dehydratation, addition of preservatives, acidification, etc (Alzamora *et.al.*, 2000; Chiralt *et.al.*, 1999; Fito *et.al.*, 2001; Park *et.al.*, 2005).

Due to the high cost of HPP equipments, the functional food products obtained by using this method are still very expensive. Medium pressure process (MPP) equipments and vacuum impregnation processing (VIP) too, are not very expensive, thus this methods are research subjects to obtain cheaper functional foods products (Larousse, 1993).

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In Romania there are known experimental research data obtained by using MPP, or VIP, or both MPP method VIP, respectively (Roşca & Roşca, 2004, 2008, 2009, 2013; Ursachi *et.al.*, 2009).

A great part of fruits and vegetable internal volume is occupied by gas.

In fruit processing, such minimal processing, vacuum impregnation permits fast compositional changes by introducing appropriate solutions (water activity and pH depressors, concentrated solution with sugar or honey, preservatives, antibrowning agents, etc.) into their porous structure (Sloan, 2002; Xie & Zhao, 2004).

Impregnation process is based on porosity and mechanical properties of the fruit.

The fast mass transfer mechanism during MPP or VIP occur when porous structures are immersed in liquid throughout the pores capillary, controlled by the expansion / compression of the internal gas into the fruit. During the MPP or VIP step, the internal gas into product pores is exhausted and partially flows out.

All this is coupled with the capillary penetration as a function of the internal cells tension that occurs between the liquid and the pores diameter. During the vacuuming steps relaxation or up to atmospheric relaxation, the residual gas is compressed, and the external liquid flows into the pores as a function of the compression / relaxation ratio (Chiralt *et.al.*, 1999; Fito *et.al.*, 2001; Larousse, 1993; Park *et.al.*, 2005; Sloan, 2002; Xie &. Zhao, 2004).

## MATERIAL AND METHODS

The aim of this inter-disciplinary research is to study a non-thermic preservation method based on rough vacuum impregnation process (RVIP) to obtain concentrated strawberry comfiture impregnated in honey syrup.

In order to determine the influence of RVIP to obtain concentrated strawberry comfiture impregnated in honey syrup, experimental research using commercial strawberries were performed.

Fresh strawberries (firm fruits) were bough from a hypermarket where were stored in conditioned atmosphere (2-4°C). The same quantity of strawberries (100g) and honey syrup (100g) were put in transparent food grade plastic bags to be introduced into vacuum processing vessel of the experimental equipment.

To observe the influence of RVIP to obtain concentrated strawberries comfiture impregnated in honey syrup, honey syrup with  $80^{\pm0.5}$ % dried soluble mater content, and strawberries with  $10,6^{\pm0.1}$ % dried soluble mater content were used.

Before and after RVIP, the dried soluble mater (DSM)content in strawberries, and the dried soluble mater content in syrup after the non-thermic impregnation process was determined with ABBE refractometer (model ABBE 90), within specific laboratory of Dolj Departmental Sanitary and Veterinary Agency.

In order to put in evidence the quantitative and qualitative information by using RVIP, experimental equipment was made.

The experimental equipment was designed and made by Unconventional Technologies and Equipment for Agro-Food Industry Laboratory within Faculty of Agriculture and Horticulture, in collaboration with Environmental Protection in Industry within Faculty of Electrical Engineering, within the University of Craiova.

In main, the experimental equipment for RVIP is composed in *vacuum pump*, *vacuum processing vessel*, and *condensed gases dryer module* (figure 1). The main characteristics of the *vacuum pump* (HYVAC type): maximum flow rate up to 40 l/min; absolute pressure up to 50 milibar.

The *condensed gases dryer module*, in main, consists in a stainless steel vessel (designed and made according to Romanian ISCIR norms), containing 13X and 5A type molecular sieve.

The *vacuum processing vessel* (designed and made according Romanian ISCIR norms: stainless steel W1.4571; welding coefficient 1; 100% ultrasonic control for welding assemblies) permits vacuum experiments for absolute pressure up to 0,5militorr.

To observe the inlet vessel during vacuum process, one of the flanges is made in transparent visor (high resistant polycarbonate). In order to observe any vacuum losses, the condensed gases dryer module and the processing vessel are provided with special 1,6 accuracy class manovacuumeter gauges made in stainless steel W1.4571 (figure 1). A rotative mixing device (stainless steel W1.4571) is mounted into the vacuum processing vessel. The rotative mixing device can be put in operation for 1-60 rot/min rotational motion by a special electromechanical transmission with speed variator. Transparent plastic boxes or thin transparent plastic bags can be placed inside the rotative mixing device during the experiments (figure 1).



Figure 1. RVIP experimental equipment

Figure 2. RVIP cycle process

The RVIP cyclic processing steps to obtain concentrated strawberries comfiture impregnated in honey syrup are presented in Table 1, and figure 2, respectively.

		Table 1
Cyclic proce	Vacuum / Time	
1. High vacuuming	Vacuum rate, [bar]	0↓-0,95
	Time rate, [s]	40
2. Maintaining	Vacuum level, [bar]	- $0,95 \rightarrow -0,95$
	Time rate, [min]	4
3. Middle vacuuming	Vacuum rate, bar]	- 0,95 ↑ - 0,5
	Time rate, [s]	20
4. High vacuuming	Vacuum rate, [bar]	-0,5 ↓ - 0,95
	Time rate, [s]	40
5. Maintaining	Vacuum level, bar]	$-0,95 \rightarrow -0,95$
	Time rate, [min]	4
6. Pressurization	Vacuum rate, [bar]	- 0,95 ↑ 0
	Time rate, [s]	20

RVIP cyclic processing steps to obtain concentrated strawberries comfiture impregnated in honey syrup

The paper presents four types of experimental research concerning the influence of RVIP to obtain concentrated strawberries comfiture impregnated in honey syrup:

- short variant consisting in 4 cyclic processing steps (40 min total processing);
- middle variant consisting 6 cyclic processing steps (60 min total processing);
- long variant consisting 8 cyclic processing steps (80 min total processing);
- very long variant consisting 12 cyclic processing steps (120 min total processing).

# **RESULTS AND DISCUSSIONS**

The dried soluble mater content in strawberries and the dried soluble mater content in concentrated honey syrup before and after RVIP by using the described variants are presented in Table 2. For each RVIP variant gas evacuation due to intensive osmosis was observed (figure 3).



Figure 3. Gas evacuation due to intensive osmosis during each RVIP cycle process

Table 2

Dried soluble mater in strawberries and dried soluble mater in honey' syrup, before and after cyclic RVIP

Variant	DS strawber Before RVIP	M in ries, [ ⁰ R] After RVIP	DSM increasing in strawberries, after RVIP, [ ⁰ R]	DSM in honey syrup, after RVIP, [ ⁰ R]
Short	K V II	13.9	3.3	76.7
Middle		16,3	5,7	74,3
Long	10,6	18,4	7,8	72,2
Very long		20,3	9,7	70,3

After cyclic RVIP, dried soluble mater increasing in strawberries and dried soluble mater increasing in honey' syrup is observed (Table 2 and Table 3).

Table 3

Variant	DSM percent increase	DSM percent in decrease
	in strawberries,	honey syrup,
	after RVIP, [%]	after RVIP, [%]
Short	31,1	4,2
Middle	53,77	7,2
Long	73,58	9,8
Very long	91,51	12,1

Percent dried soluble mater increase in strawberries and dried soluble mater decrease in honey' syrup, after cyclic RVIP

The impregnated strawberries obtained by using cyclic RVIP were translucent, with no browning, with good texture and shape, with typical organoleptically standard properties comparable with comfiture obtained by using classical thermic preservation method (figure 4).



Figure 4. Impregnated strawberries obtained after cyclic RVIP

# CONCLUSIONS

Due to experimental research it was observed that rough vacuum impregnation cycle process increase the impregnated fruit quality depending on the process duration and succession.

The non-thermic impregnation process is based on the osmosis process between fruits and concentrated honey syrup. During this process the honey content in fruits is gradually increasing, and in the same time the fruits juice dilutes the honey syrup, until the equilibrium stage is realized.

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# MECHANICAL BEHAVIOURS OF APPLE DURING SHORT PERIOD STORAGE

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Key Words: mechanical behavior, penetration and Warner – Brazler test, firmness

#### ABSTRACT

The paper presents experimental researches concerning flesh and peel firmness of Granny Smith apples determined with penetration / puncture and Warner-Bratzler methods. The data present the apples mechanical characteristics and sensorial behavior after short period storage at conditioned atmosphere and in ambient temperature

#### **INTRODUCTION**

*Mature* means that the apples have reached the stage of development which will insure the proper completion of the ripening process. Before a mature apple becomes overripe it will show varying degrees of firmness, depending upon the stage of the ripening process. The following terms are used for describing different stages of firmness of apples: - *hard* means apples with a tenacious flesh and starchy flavor; - *firm* means apples with a tenacious flesh and starchy flavor; - *firm* ripe means apples with crisp flesh; - *ripe* means apples with mealy flesh and soon to become soft for the variety; - *overripe* means apples which have progressed beyond the stage of ripe, with flesh very mealy or soft, and past commercial utility (ams.usda.gov AMSv1).

Measurements of firmness are used in two ways. First, the absolute firmness value can be used in a regulatory fashion to define the minimum standard of fruit that growers must achieve before their fruit will be accepted by export or retail organizations. Second, change in firmness is used to characterize the rate at which fruit soften (<u>ams.usda.gov AMSv1</u>).

Early hand-held penetrometers were developed (Magness & Taylor, 1925), and from these devices, a range of devices have been developed by different companies (Abbott *et.al.*, 1976, 1992; Bourne, 1974, 1980; Smith, 1985). A number of studies have examined the mechanics of puncture testing (Voisey & Kloek, 1981) and recommendations on using penetrometers (Harker *et.al.*, 1996; Lehman-Salada, 1996; Abbott, <u>http://www.ba.ars.usda.gov</u>).

The wide use of firmness measurements has led to the periodic development of new devices for measuring firmness. Effective experimental design is critical for comparing devices and comparing different ways of using the devices. Previous studies have compared different types of penetrometers and investigated how experimental design (replicate number and sample size) affects detection of firmness differences (Harker *et.al.*, 1996; Schoorl, 1983).

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Previous studies indicate the need for standardization in any regulatory use of firmness measurements. There are reported great differences between machinery (materials testing machine) and operator (hand-held penetrometer, or EPT pressure tester) that may considerably affect the measurement of absolute firmness (Harker *et.al.*, 1996; ASAE, 2000).

### MATERIAL AND METHODS

Commercial apples (Malus domestica L. cv. Granny Smith) were bough from a hypermarket. The apples were harvested in last summer in Turkey, calcium-treated and stored in ventilated controlled atmosphere for export. After imported in Romania, these apples were stored in ventilated controlled atmosphere (2-4°C) in large deposits and in the hypermarket, too. The apples were boughs just immediately after there were got out the ventilated controlled atmosphere deposit of the hypermarket. After buying, for two weeks period, a half of the quantity was stored in controlled atmosphere (cold atmosphere storing  $2-4^{\circ}C - CAS$ ), and the other was stored in  $3-25^{\circ}C$  ambient atmosphere (AAS) during this September. To determine the apples dimension a KLASS digital caliper (accuracy 0,001 mm), and the apples weight losses during storing KERN 220 (50mg-220g; 0,1mg accuracy) balance, were used, respectively. After the measurements the apples were classified in three dimensional and weight categories. To determine the total soluble substance evolution a portable refractometer KRUSS (0-30°R), and the pH evolution a portable pHmeter LUTRON PH-222 (0...14pH / -5^oC...80^oC; resolution 0.01pH / 0.1^oC; accuracy  $\pm$  0.02pH /  $\pm$  0.8^oC), were used, respectively. The apples firmness was measure by using materials testing machine LLOYDS INSTR. LRXPlus (cell accuracy 0.5) that was used in previous experimental researches (Rosca & Rosca, 2011, 2013). In order to determine the flesh / peel firmness, Ø 11 penetrator was used. In order to determine the peel and flesh firmness by using Warner-Bratzler method a 1,5mm cutting flat blade was used. The experimental devices were designed and made by Unconventional Technologies and Equipment for Agro-Food Industry Laboratory within Faculty of Agriculture and Horticulture, in collaboration with Environmental Protection in Industry within Faculty of Electrical Engineering, within the University of Craiova.

To apply penetrometer tests to fruit, the speed of penetration was set at 100 mm/min, and the test was stopped after penetration to 8 mm deep. To apply Warner-Bratzler tests, the cutting speed was set at 100 mm/min, and the test was stopped after fruits total cutting. Slices of skin were removed at two opposite sides' equidistant points around the equator of each fruit, and flesh firmness was determined by using penetration / puncture method. In other two opposite sides equidistant points peel firmness was determined by using penetration / puncture method (figure 1), and peel and flesh firmness by using Warner - Bratzler method (figure 2).



Figure 1. Penetration / puncture test



Figure 2. Warner – Bratzler test

# **RESULTS AND DISCUSSIONS**

The relevant experimental data are presented in Table 1 to Table 10, and representative graphics during penetration tests and Warner-Bratzler test are presented in figure 3 to figure 6.

Table 1

# Apples dimensions for ambient atmosphere storing

Category	Diameter D _{max} /D _{min} ,[mm]	Hight H, [mm]
Ι	82,4/76,6	80,7
II	74,3/69,6	72,1
III	67,5/64,2	65,7

Table 2

# Weight evolution during ambient atmosphere storing

Category	Initial weight	After one	Weight difference	After two	Weight difference
	(buying time), g	week, g	Winit - Ww1, %	weeks, g	W _{init} - W _{w2} , %
Ι	198,256	186,014	5,74	185,945	6,21
II	170,755	161,734	5,28	160,993	5,72
III	128,164	122,586	4,41	122,025	4,79

Table 3

# Apples dimensions for cold atmosphere storing

Category	Diameter D _{max} /D _{min} ,[mm]	Hight H, [mm]
Ι	84,5/78,3	81,2
II	75,8/70,3	73,2
III	68,5/65,8	66,5

Table 4

# Weight evolution during cold atmosphere storing

Category	Initial weight	After one	Weight difference	After two	Weight difference
	(buying time), g	week, g	Winit - Ww1, %	weeks, g	Winit - Ww2, %
Ι	199,868	189,415	5,23	188,755	5,56
II	173,179	164,797	4,84	164,226	5,17
III	131, 345	125,815	4,21	125,501	4,45

Table 5

Total soluble substance evolution during ambient atmosphere storing (Brix), %

Category	Initial TTS	After one	TTS increasing	After two	TTS increasing
Ι	12,7	12,95	1,97	13,15	3,54
II	12,65	12,85	1,58	13,05	3,16
III	12,55	12,65	0,79	12,75	1,59

Table 6

Total soluble substance evolution during cold atmosphere storing (Brix), %

Category	Initial TTS	After one	TTS increasing	After two	TTS increasing
	(buying time)	week, %	TTSw1 - TSSinit, %	weeks, %	TTSw2 - TTSinit, %
Ι	12,75	12,9	1,18	13,0	1,96
II	12,7	12,85	1,18	12,95	1,96
III	12,6	12,7	0,79	12,75	1,19

# Table 7

# pH evolution during ambient atmosphere storing

Category	Initial pH	After one	pH increasing	After two	pH increasing
	(buying time)	week, %	pHw1 - pHinit, %	weeks, %	pHw2 - pHinit, %
Ι	3,39	3,43	1,18	3,49	2,95
II	3,38	3,41	0,89	3,45	2,07
III	3,32	3,34	0,6	3,36	1,21

## Table 8

### pH evolution during cold atmosphere storing

Category	Initial pH	After one	pH increasing	After two	pH increasing
	(buying time)	week, %	pHw1 - pHinit, %	weeks, %	pHw2 - pHinit, %
Ι	3,4	3,42	0,59	3,45	1,47
II	3,39	3,41	0,51	3,43	1,18
III	3,34	3,35	0,29	3,36	0,6

#### Table 9

# Force average range, N

			Pene	Warner-Bratzler			
Category Storing		Flesh		Pe	eel	Peel	
	condition	Initial	After two	Initial	After two	Initial	After two
		(at buying)	weeks	(at buying)	weeks	(at buying)	weeks
Ι	AAS	51,79	38,86	69,31	59,79	46,48	38,01
	CAS	64,28	52,22	77,92	68,35	59,44	45,28
II	AAS	71,36	53,64	84,54	73,42	61,49	53,11
	CAS	76,45	58,87	88,37	82,11	71,67	62,93
III	AAS	84,34	75,22	91,13	86,46	78,65	72,56
	CAS	87,48	82,42	95,46	90,23	85,41	79,73

Table 10

Stress average range, MPa

			Pene		Warner-Bratzler		
Category	Storing	Fle	esh	Pe	eel	Peel	
	condition	Initial	Initial After two		After two	Initial	After two
		(at buying)	weeks	(at buying)	weeks	(at buying)	weeks
Ι	AAS	0,545	0,409	0,808	0,629	0,516	0,422
	CAS	0,676	0,549	0,820	0,719	0,663	0,503
II	AAS	0,751	0,564	0,884	0,768	0,783	0,591
	CAS	0,804	0,634	0,926	0,831	0,795	0,699
III	AAS	0,958	0,789	0,958	0,842	0,873	0,805
	CAS	1,021	0,863	0,994	0,906	0,948	0,884

According to Harker *et.al.* (1996) the apples firmness after cold atmosphere storing (hard fruit) has to be 75-77N, and after ambient atmosphere storing (soft fruit) has to be 61-67N.

For commercial use, in American literature there are recommendation concerning the shelf life evaluation of skin apples firmness that were stored at 31°C (after sample taken from controlled atmosphere chamber): minimum skin firmness 16,5 pounds (73,8N).

In Table 10 is observed that all three categories respect these recommendations when were stored in CAS, and only category III when were stored in AAS.



Figure 3. Penetration force/stress for flesh apple I category, at buying time, before AAS (51,79N / 0,545MPa)



Figure 5. Penetration force/stress for peel apple I category, after two weeks AAS (59,79N / 0,629MPa)



Figure 4. Penetration force/stress for flesh apple I category, after two weeks AAS (38,86N / 0,409MPa)



Figure 6. Warner-Bratzler force/stress for apple I category, at buying time, before AAS (46,48N / 0,516MPa)

### CONCLUSIONS

Texture measurement is widely accepted by horticultural industries as a very important indicator of non-visual aspects of quality. The ability to measure texture has allowed industries to set standards for quality at pack-out and to monitor deterioration in quality that occurs during storage and distribution.

Commercial and research interest in texture has focused primarily on the mechanical properties of the tissues. All the diversity of tissues involved, the variety of attributes required to fully describe textural properties, and the changes in these attributes as the product ripens and senesces contribute to the complexity of texture measurement.

However, instrumental measurements are preferred over sensory evaluations for both commercial and research applications because instruments are more convenient to use, widely available, tend to provide consistent values when used by different (often untrained) people, and are less expensive than sensory panels. These instrumental measurements are widely understood and can provide a common language among researchers, industry, and customers. Destructive mechanical methods generally relate more closely to sensory evaluations than do nondestructive measurements; but, by their destructive nature, they cannot be used for sorting produce.

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# TECHNOLOGICAL POTENTIAL OF CERTAIN VARIETIES OF TABLE AND WINE GRAPES IN AROMATISED WINES TECHNOLOGY

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Keywords: technological potential, grapes for table, grapes for wine, special aromatization

wine

## ABSTRACT

The study consists in obtaining the aromatized wines of different grape varieties. For this purpose it used two varieties of table grapes (Victoria and Coarna alba), a variety of seedless grape (Calina) and two varieties of wine grapes (Cramposie selctionata and Feteasca regala). The use of table grape varieties was an attempt to exploit a remaining production as a result of their use as merchandise, production on various quality steps.

# **INTRODUCTION**

Aromatized wines are part of special wines. They are based on natural wine and fresh grape must, with the addition of extracts or herbs infusions. Beside these flavoring ingredients allowed by current legislation, are also uses the addition of alcohol, sugar and caramel. Are distinguished by specific aromas, bitter taste and a slight astringency. Are consumed as an appetizer (Gheorghiță et al, 2002).

Vermouth is an aromatized wine, tonic and appetizer, very much appreciated by consumers. The name comes from the German "Wermuth" which means wormwood, plant with bitter taste preponderant in macerate used in preparing vermouth (Ţârdea et al, 2000).

The proportion of the wine contained in vermouth, must be at least 75% of the final product. The finished product must have actual alcoholic strength than 15% vol; sugar, at least 40 g / l, characteristic color and flavor vermouth (Pomohaci et al, 1996). Aromatic plant extracts are prepared for vermouth. These contain bitter and flavor substances that give the plesant taste and refreshing effect of vermouth (Mujdaba et al, 1971; Amerine et al, 1972; Kontek et al., 1994). Plants involved in obtaining the macerate, especially the proportions of them, are different depending on the type of vermouth and the secret of manufacturing recipe.

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# MATERIAL AND METHODS

Using in purpose of preparation of vermouth type drinks, the wines that are made from table and seedless grape destined for winemaking can be a superior solution recovery of this material. To obtain the wine- raw material from grape varieties were used difference remaining after the capitalizing on their commodity production.

For this study we used as raw material to obtain the wines, both the table grape -Victoria, Coarnă albă, seedless grape - Călina and two varieties for wine - Crâmpoșie selecționată and Fetească regală.

Macerate for vermouth was conducted out by laboratory tests (laboratory sample), from the combination of the different proportions of species of native plants and essences.

After numerous tests performed under laboratory conditions has been made vermouth macerate in obtaining that involved 11 species of indigenous plants and four essences.

Elements that intervene in different proportions in achieving macerate are:

- 1. Matricaria chamomilla (chamomile) Flower
- 2. Artemisia absinthium (wormwood) stalk
- 3. Millefotium Achillea (milfoil) flowers
- 4. Acorus riding (sweet calamus) root
- 5. Iris germanica (Iris) root
- 6. Tanacettum vulgare (tanaceti) Flower
- 7. Majoranna hortensis (marjoram) stalk
- 8. Coriandrum sativum (Coriander) Fruit
- 9. Melilotus officinalis (melilot) Flower
- 10. Feeniculum vulgare (fenicol) Fruit
- 11. Ocimum basilicum (basil) stalk

Essences used are:

- 1. Cinnamon
- 2. Nutmeg
- 3. Quinine hydrochloride
- 4. Vanillin.

Preparation of plant extract was made by maceration in alcohol solution of 50-60 vol%. The components were coarse grinding, and flavour extraction was performed by repeated pressings (2-3, every 24 hours), using each time a new solution of alcohol. Complete exhaustion of flavors made by distillation. A portion of this material (essences) was added directly in macerate of the plant already done.

Vermouths obtained were compared in terms of organoleptic and physicochemical characteristics with that produced in the country and the main Italian vermouth.

To analyze the components of the quality of grapes and wines composition were used formal methods recommended by OIV - Paris and adopted ICVV.

## **RESULTS AND DISCUSSIONS**

To obtain quality vermouth, in addition to using an appropriately macerated, high importance has also, the choise of wine-raw material.

In Figure 1, 2 and 3 are the main characteristics of table and wine grape varieties used for obating the wines raw-material in technology of aromatized wines type vermouth.

Between the table and seedless varieties, can see that the highest sugar content has variety Călina (180 g / L), followed by Coarnă albă and Victoria in terms of suitable acid of

table varieties. In the two wine varieties, the higher the sugar content and therefore a higher potential alcohol present Fetească regală variety.

The physico-chemical of the wines raw materials for vermouth from table and wine grapes are shown in Table 2.





Figure 2. The alcoholic potential of wines



Figure 3. The acidity content of grapes varieties

From the point of view of potential alcohol and other components (acidity, extract, ash, glycerol, etc.) most appropriate are wines from varieties Călina, Coarnă albă Crâmpoșie selecționată.

Wine made from the variety Victoria has a lower alcohol content and higher total acidity, instead, due to its general characteristics. It can be used with good results for the preparation of white vermouths or may be used in combination with the first two varieties.

Table 2

Chanastanistica	Călina	Vistoria	Coarra alla ă	Cuânan a ai a	Estasas
Characteristics	Calina	victoria	Coarna alba	Crampoșie	Feleasca
				selcționată	regală
Alcohol, vol. %	10.55	9,40	10,40	11,0	12,1
Sugar, g/L	2.20	2,40	1,50	2,30	2,80
Total acidity, g/L	4,18	4,35	3,74	4,10	4,20
Volatile acidity, g/L	0,51	0,49	0.47	0,31	0,36
Extract total, g/L	20,24	20,25	18,82	19,96	22,68
Extract reduced, g/L	19,04	18,85	18,32	18,91	21,42
Ash, g/L	1.60	1.71	1,62	1,73	1,99
Tartaric acid total, g/L	4,95	5,73	5,14	5,90	5,65
Glycerol, g/L	9,47	8,32	8,09	7,0	9,1

The chemical composition of the wines raw materials for vermouth from table and wine grapes

Wine from Coarnă albă variety has lowest residual sugar content, total acidity, glycerol, total extract and reduced extract.

According relative carbohydrate content of the grapes at harvest resulting alcohol levels of between 11% vol (Crâmpoșie selcționată) and 12.1% vol (Fetească regală).

Total acidity presented values above 4 g / L H2SO4 at all other varieties, being in line with rules for implementing the Law on Vine and Wine in force.

Rigorously controlled vinification conditions have attracted in wine of low volatile acid content, between 0.31 g / L (Crâmpoșie selcționată) and 0.36 g / L (Fetească regală). The glycerol content was between 7.0 g / L (Crâmpoșie selcționată) and 9.1 g / L (Fetească regală).

The quality of raw materials caused the highest content in their wine extract (19.96 g / L and 22.68 g / L). Contents in minerals (ash) will concern in generally proportional sizes unreduced extract, less than 2 g / L in wines Crâmpoșie selcționată (1.73 g / L), Fetească regală (1.99 g / L), the lowest value recorded at the seedless variety.

The physico-chemical properties of vermouths made from the three varieties of the table and the two wine varieties are presented in Table 3.

From the point of view of the chemical composition, vermouth made both from the wines made from table grape and the wine grape varieties, comply with the characteristics limits indicated by Garoglio (1965) for Italian vermouth.

#### Table 3

Characteristics	Călina	Victoria	Coarnă albă	Crâmpoșie selcționată	Fetească regală
Alcohol, vol. %	17,10	17,20	17,00	17,10	17,80
Sugar, g/L	157,00	154,00	155,00	160,00	158,00
Total acidity, g/L	3,42	3,32	2,88	3,23	3,55
Volatile acidity, g/L	0,39	0,49	0,39	0,40	0,38
Extract total, g/L	171,10	168,00	169,00	170,00	172,00
Extract reduced, g/L	15,10	15,00	15,00	14,19	16,20
Ash, g/L	1,41	1,49	1,57	1,58	1,54
Tartaric acid total, g/L	3,44	3,81	3,36	3,52	3,45
pН	3,40	3.30	3,50	3,30	3,40

Physico-chemical analysis of vermouths obtained from varieties of table and wine grapes

Alcohol and sugar content is within the limits specific sweet vermouths between 154 g / L and 160.0 g / L from wine vermouth Crâmpoșie.

The total acidity fits to all varieties of values which are generally characteristic of Aromatized beverages, around 3 g / L. The lowest value occurs in vermouth with the wine raw-material from table grape Coarnă albă, and the highest content are recorded at vermouth from Fetească regală wine.

Extract reduced not drop below 11 g / L and ash below 1.1 g / L, minimum thresholds considered for vermouth.

The values of tartaric acid are higher in comparison with the Italian vermouth, due to the fact that we were not prepared frozen.

### CONCLUSIONS

Following the completion of this study the following conclusions can be drawn:

Macerate of vermouth realized and tested in the laboratory submit the superior quality and suits the intended purpose. At its realization involved 11 species of indigenous plants and four essences. In order to achieve a pleasant smell macerate should be used at a dose of 25 to 30 ml / L.

Wines raw-material for vermouth must be healthy, pleasant, with a light color, with alcohol content as raised, an acidity of 3.5 to 4 g / L H2S04, the extract reduced to about 18 to 20 g / L, stable in biological and physico-chemical properties.

Table grapes can be valorized by obtaining wines which can be successfully used as raw material for Vermouth. The three table varieties are suitable for preparation white vermouths.

From the wine grape varieties, wine from Crâmpoșie fits best as raw material to obtain vermouths.

Of the types of vermouth obtained, the sweet, with alcoholic strength of 16.6 to 17 vol%, the sugar content of 145-160 g / L acidity of 2.8 - 3.0 g / L H2S04 are the highest rated.

From the point of view of analytical constants, vermouth made from varieties studied is within international guidelines, the proportion of basic wine not drop below 75%.

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# **RESEARCH REGARDING QUALITY PARAMETERS OF CLONAL** SELECTIONS MUSCAT DE HAMBURG 4 Pt AND MUSCAT D'ADDA 5 Pt IN CORRELATION TO ENVIRONMENTAL FACTORS

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Key words: climatical index, clonal selection, grape varietes

## ABSTRACT

Pietroasa vineyard center, is among the many prestigious and wine tradition research stations in our country, possessing germplasm fund, whose favorability wine attributes are derived from specific ecopedoclimatic very favorable conditions found here and oriented scientific work improved grapes and wine varieties, both global range and local varieties. In the present study was followed the influence of regional climate change on the agrobiologic and technologic potential of clonal selections Muscat Hamburg 4 Pt and Muscat d'Adda 5 Pt, in the period 2010-2012. The results reflect strictly a direct correlation between genotype - complex and show that these specific climate changes of these wine years have led an advance of maturation of 4-5 days, a greater accumulation of sugars (195 g/l - 2012 to Muscat d ' Adda 5 Pt, and 190 g/l - 2010 for Muscat Hamburg 4 Pt) and lower productions, which in these years were much below the limits recorded in the good years.

### **INTRODUCTION**

Table grape quality is often associated with the size, the uniformity and the color of the grapes, but in reality it is a complex term seen from various perspectives: visual perspective, taste, nutrition, health, and so on, which are appreciated and evaluated by the consumer in relation to his demands. But if we refer to the favorability of a wine area regarding the obtainance of quality table grapes, then this must ensure annual repetitivity of the quality and the quantity of the obtained production. In the present study has been followed in which way and how much the area climate changes have influenced the qualitative parameters of the production of the clonal selections Muscat Hamburg 4 Pt and Muscat d'Adda 5 Pt on a period of three wine years (2010, 2011, 2012) considered to be special unde the aspect of the recorded climatic indexes. The observations made show that the climate changes from one year to another have consequences on the growing cycle of the vine and on the growing and productivity balance (Jones et al. 2005, Jean-Luc et al. 2008), that there is a direct correlation between the accumulation and increase of sugar quantities in the grape must and the growing values of the Huglin index (Huglin, 1978, Laget et al. 2008) as well as the correlation between the night coolness index and the flavour accumulations and antocianins in grape skin (Tonietto & Carbonneau 2004). This

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last indicator allows an evaluation of the qualitative potential of the wine areas due to the temperatures importance during the night and the differences from day to night recorded in the last month of grape maturation (September) in forming the flavours and the antocianins.

#### MATERIALS AND METHODS

The experimental variants taken into study are found in an experimental device placed on a slope field, with limestone soil, at a planting distance of 2,0/1,2 meters and have been conducted on a semi-stem with bilateral cord, with a load of 18 buds/square meter, and the rootstock used was Oppenheim 4-4 Selection. Climatic data were gathered from the vineyard own weather station and regarded daily observations of the parameters temperature, precipitations, heatstroke and based on these parameters have been calculated the climatic indexes that establish the favorability degree of a certain wine area, as well as the calculus of Huglin index and night coolness index. To make a more accurate appreciation of these climatic aspects, the data in the study period were compared to the average of the last three years, considered indistinctive for the analyzed wine area. The clonal selections have been followed during the whole phenological spectrum (first determining the percentage of viable buds) and in the harvest moment on a medium grape sample there were made the following carpometric determinations as well as physical and chemical: average weight of a grape, average weight of 100 grapes, production/bud, sugars (g/l), acidity (g/l of tartric acid), the gluco-acidimetric index, full maturityl. The results obtained have been analyzed with the help of the following indexes: arithmetic average, maximum and minimum - indexes that can be applied to most of the carpometric parameters because they have the property to variate in time and space.

### **RESULTS AND DISCUSSIONS**

Climate conditions recorded during the experiment, characterized by a serie of extreme weather events - strong winds, maximum extreme temperatures in summer ( $\geq 35$  ^oC in many consecutive days and 41°C – 15 of August 2012, with consecutive tropical nights and air temperatures higher than 20°C), extreme negative temperatures in winter (-26,6 °C- 2010; -16,1 °C – 2011; -23,1°C - 2012) as well as the freezing rain phenomenon, which had effects on the normal growing cycle of these clonal selections, but also on the viability of winter buds, on the start of all phenophases and also on the production per hectar (table 1).

Analyzing the values of the four synthetic indexes, it is shown that to the high heliothermic resources correspond low hydric resources and that the most unstable index is the bioclimatic index, which large spectrum is situated between the limits of 5,38 - 11,2. Due to this data background it is appreciated that the area has conditions that allow the economic culture of table grape varieties with medium maturation. As for the Huglin index evolution, during the study it is observed that it has a growing tendancy from one year to another, reaching a maximum of 2639,7 in the year 2012, conditions in which the wine area characterized by a warm temperate climate in general (IH4) possesses the aspect of a hot climate (IH5) - (IS1, IH5, IF3). For the wine area of Pietroasa, the values of night coolness index are situated generally between 12-14 and is IF3, meaning cold nights, but in the year 2010 this has lower values - 10,7 (situating the wine area in the very cold nights areas group).

Analyzing the data recorded in table 2, it is observed that Muscat Hamburg 4 Pt selection records a medium value of 48,3% viability of winter buds, with a minimum of only 25% in the year 2012, minimum that was due to the large time interval in which the

temperatures maintained critical, but also to the phenomenon of freezing rain that froze on the vine cords, noticed between 4 and 6 of February.

The medium production of a hectar was diminished much in this year, recording a minimum of 8 tons/hectar, much under the potential of the clonal selection (13 tons/hectar), but also under the level of the years 2010 and 2011. For the carpometric parameters specific to the table grapes varieties that mainly attract the consumer, right before a previous taste analysis and appreciation the selection records lower values in comparation to the other years (270 g/grape, as well as the average weight of 100 grapes - 275 grams).

Regarding the gluco-acidimetric index, this is situated between the normal limits, with the specification that during the 3 years of the experiment, the sugar quantity reached the maximum value of 190 g/l, quantity that overcomes the potential of the clonal selection (167,2 g/l).

Table 1

Specification			Average	Anul	Anul	Anul	Max	Min	
				2007- 2009	2010	2011	2012		
T1	- 11	global		4233,4	3920,4	4019,1	4205,7	4233,4	3920,4
Inermi	c balance	active		3577.2	3473,1	3388,3	3835,5	3835,5	3388,3
		useful		1677,2	1626,2	1616,2	1955,6	1955,6	1616,2
Absolute temperatu	minimum ıre °C		Air	-17,13	-26,6	-16,1	-23,1	-16,1	-26,6
temperati			Soil	-19,5	-27,9	-17,4	-26,7	-17,4	-27,9
The sum (Σir)	of the hours	of real in	solation	2060,7	2036,4	2049,3	2125,3	2125,3	2036,4
The sum of the annual precipitations (mm)		522,6	655,2	601,1	579,6	655,2	522,6		
Numb	er of days of	f active pe	eriod	205,6	214	207	220	220	205,6
s	The coefficient	hydro t CH)	othermic	0,82	1,17	1,19	0,97	1,19	0,82
matics	The real h (IHr)	neliotermi	c index	1,37	1,12	1,05	1,08	1,37	1,05
The viticultural bioclimatic index (Ibcv) Index of the oenoclimatic aptitude (IAOe).		9,05	5,96	5,38	11,2	11,2	5,38		
		climatic	5185,7	4816,4	4373,2	5074,5	5185,7	4373,2	
Ind	Huglin inc	lex		2392	2319,4	2314,6	2639,7	2639,7	2314,6
	Index of c	ooling at	night	10,3	10,7	13,8	12,1	13,8	10,7

Evolution of climatic elements in the wine area of Pietroasa (2007-2012)

Based on the same principle of the data analyzed during the experiment it can be observed that Muscat d'Adda 5 Pt records a minimum of 24% viable buds per stock in the conditions of the year 2012 affecting later the production obtained. It can be observed also that the majority of the maximum values are obtained in the year 2010 mainly for those carpometric parameters that attract mainly the consumer, such as: average weight of a grape (310 g), average weight of 100 grapes (335 g). Also in the year 2010 the selections obtains a large sugar production of 12 t/ha (situated a little under the selections limit - 13,5 t/ha) due to a small sugar quantity accumulated, over the normal limit (170 g/l). The smallest values recorded for these parameters are met in the years 2011 and 2012, special years,

excessively hot, with a high hydric defficit, due to a thermic surplus, that left a mark not also on the physiological and biochemical processes development but also on the quantity and quality of the sugar production.

After the experiment, following the evolution of Huglin indexes in this wine area and the sugar quantities accumulated in the grape maturation process, it is observed a direct correlation of these indexes for both clonal selections, the values recorded and the accumulations overcome the normal limits. It is also observed a precocity of grape maturation (4-5 advance).

Table 2

Specif	ication	% viable buds	Yield (t/ha)	Sugar (g/l)	Acidity g/l H ₂ SO ₄	Index gluco- acidimetric	Average weight of	Weight of one hundred	Full maturity
							a grape (g)	beries (g)	
		Mu	scat de H	lamburg 4	4 Pt - Gra	pe table middle	e maturity		
	2010	71	11	190	5,5	3,4	275	280	18.09
Wina	2011	49	10	188	5,6	3,3	280	275	18.09
year	2012	25	8	188	5,7	3,3	270	280	15.09
	average	48,3	9,66	188,6	5,5	3,33	275	278,3	17.09
	min	25	8	188	5,6	3,3	270	275	15.09
	max	71	11	190	5,7	3,4	280	280	18.09
		Ν	Muscat d	Adda 5 F	Pt - Grape	table middle n	naturity		
	2010	69	12	187	5,8	3,2	310	335	17.09
Wina	2011	47	10	190	5,8	3,3	310	300	17.09
year	2012	24	9	195	5,7	3,4	300	325	15.09
	average	46,66	10,33	190,6	5,76	3,3	306,6	320	16.09
	min	24	9	187	5,7	3,2	300	300	15.09
	max	69	12	195	5,8	3,4	310	335	17.05

Evolution of quality parameters on the varieties analyzed between 2010-2012

As for the general mark obtained after the organoleptic tests of the studied clonal selections as an average for the three study years (table 3), average cummulated as a consequence of the indexes: grape berries detachment, taste and flavour, pulp consistancy, skin thickness, were obtained the following marks: Muscat d'Adda 5 Pt, 9,33 mark, and for Muscat Hamburg only 6,75, marks that show the predisposition of the Muscat Hamburg variety to the "small grains" and "very small grains", as well as the fact that the grape skin is thiner in comparation to the Muscat d'Adda variety.

Analyzing the influence of the night coolness index on the flavour and antocianins accumulation it can be observed that this is reflected in the marks close to maximum (2 and 3) obtained at commercial aspect and taste and flavour index for the years 2011 and 2012

when the values of this index were situated in the normal limits of the wine area of Pietroasa (13,8 and 12,1) and a smaller value for the year 2010 when the index value was situated under the multiannual averages limits (10,7).

Table 3

Experimental	Commerci	Grape	Taste	Pulp	Skin	Taste
variants	al aspect	berries	flavou	consistency	thickness	appreciation
	(0-3)	detachment	r	(0-2)	(0-1)	mark
		(0-1)	(0-3)			
Muscat d'Adda	3	1	2	2	1	9
5 Pt (2010)						
Muscat d'Adda	3	1	2	2	1	9
5 Pt (2011)						
Muscat d'Adda	3	1	3	2	1	10
5 Pt (2012)						
						9.33
Muscat	2	0	3	1	0	6
Hamburg						
4 Pt (2010)						
Muscat	3	0	3	1	0	7
Hamburg						
4 Pt (2011)						
Muscat	3	0	3	1	0	7
Hamburg						
4 Pt 2012)						
						6.75

Organoleptic appreciation of analyzed clonal selections grapes



Figure 1. Morphological aspects of clonal selection Muscat Hamburg, 4 Pt

#### CONCLUSIONS

The results reflect a direct and strict correlation between the genotype and the area and shows that the area climate changes that are specific to these wine years determined a faster ongoing of the whole phenological spectrum and had an effect on the precocity of grape maturation (4-5 days in advance) a higher sugar accumulation (195 g/l - 2012, for Muscat d'Adda 5 Pt and 190 g/l - 2010 for Muscat Hamburg 4 Pt) and not least a good flavour due to the sugars accumulated that offers special organoleptical features.



Figure 2. Morphological aspects of clonal selection Muscat d'Adda 5 Pt

Practically, the productions obtained are much under the normal limits of the clonal selections taken into analysis but, surprisingly they weren't affected majorly those qualitative parameters that attract mainly the consumer before the organoleptic appreciation on one hand (shape and size of the grapes, color and grape size and their uniformity) and the organoleptical parameters (consistancy, taste and flavour, skin thickness) on the other hand.

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## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

# Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# FT/IR STUDY ON CHANGES INDUCED BY CHEMOTHERAPY AND ELECTROTHERAPY APPLICATION IN GRAPEVINE

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Keywords: grapevine viruses, corona effect, antiviral detection, Attenuated Total Reflexion

# ABSTRACT

FT / IR spectral analysis was applied to investigate the changes induced in the cell structure of the plant material treated with electrotherapy and chemotherapy as alternative techniques for viral eradication of grapevine. Electrotherapy may cause the inactivation of the viral protein at the cellular level, as a result of the induced thermal effect, while the use of chemical drugs raises serious questions about possible phytotoxic effects of explants. Samples were taken from axillaries buds of herbaceous cuttings from two varieties infected with some of the most dangerous viruses of grapevine – Feteasca neagra infected with GFLV and Cabernet Sauvignon infected with GLRaV - previously irradiated with a flow of electrical charges of 4800  $\mu$ A·s, 9600  $\mu$ A·s and 14400  $\mu$ A·s produced by corona effect. Detection of antivirals in infected leaves and then treated with chemotherapy, was followed spectrally by FT-IR/ATR technique.

#### **INTRODUCTION**

The presence of viral infections can induce severe damages to grapevine plants and therefore, the use of a virus free material for multiplication towards the establishment of new vineyards is required. Classical methods used for this purpose the meristem culture very efficiently only for certain types of viruses and certain genotypes, and thermotherapy energy consuming method that requires special equipment. Chemotherapy and electrotherapy can be considered as alternative techniques for viral eradication of grapevines.

Removal of viruses by in vitro chemotherapy was tested to some horticultural species but cause a number of problems due to phytotoxic effects observed on cultivated explants by using different concentrations of drugs (Panattoni et al. 2011). Electrotherapy followed by in vitro culture has been applied successfully to eradicate potato virus X from some infected potato clones (Klein & Livingston 1983) or fanleaf virus(GFLV), arabis mosaic virus (ArMV), leafroll associated virus 1 (GLRaV-1), leafroll associated virus 3 (GLRaV-3) and fleck virus (GFkV), in simple or mixed infections at a time from some *Vitis vinifera* varieties. ELISA analysis of regenerated and acclimatized plants presented encouraging results on filamentous virus removal (Guta et al. 2011)

In the specialized literature of the last three decades, there are numerous references to methods for obtain biological material free of virus derived from infected plants by using

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in vitro plant cultures subjected to electrotherapy and then multiplied on media with different levels of viricides (Griffiths et al. 1990, Mahmoud et al. 2009).

Although the results of using electric current and chemotherapy in cleaning of viral diseases of horticultural plants are known and studied, theoretical bases of phenomena are less investigated. The aim of this work was to analyze by FT / IR spectral technique possible changes induced at the molecular level by using electrotherapy and chemotherapy to grapevine plants.

# MATERIAL AND METHODS

*FTIR spectroscopy* has grown in recent years, being used to characterize the supply of agricultural products. The FTIR spectroscopy is a powerful technique for assessing food production, in processed food tasting so studied materials provides fundamental information on the behavior of the spectral metabolites and bio product. Consumers are becoming more aware of the importance of using healthy foods, so it is necessary to obtain high-quality food and processed correctly. Food safety management envisages cultivation and processing of the highest quality so as to produce consistently high quality product to be marketed. Therefore, evaluation of the quality control of food and agricultural products based on audible data are required to obtain high quality products.

For food safety investigation methods should be simple, non-destructive, rapid, quantitative and qualitative simultaneously. Optical and spectroscopic methods satisfy these requirements. UV-VIS and IR spectroscopy (NIR – near IR and MIR –mid IR) are the most widely used research techniques for this purpose. However, the strong absorption of MIR radiation by the water inside the products (foods) is a problem. A Fourier transform infrared spectrometer (FT-IR), equipped with an attenuated total reflectance accessory (ATR) presents a substantial potential and is a very effective tool for such measurements (Kameoka et al 1998). Infrared spectroscopy is a fast and easy method. Kurt et al. have developed a FT-IR/ATR method for detecting three kinds of antibacterial (Kurtz &Bellon 1991). Ishizawa et al. have shown that the FT-IR/ATR method has the potential of rapid and accurate system for measuring the pesticide residues (Ishizawa et al 1966, Ishizawa et al 2000).

Detection of pesticides in lettuce leaves can be achieved by FT-IT/ATR. Figure 1 shows the ATR spectra of lettuce leaves with and without pesticides (Ishizawa et al. 1996).Three pesticides were used: daconil, copper-oxine and Topsin-M. Figure 1 shows significant differences in spectral characteristics.



Figure 1. ATR spectra of the leaves of lettuce. (a) leaves with pesticide, (b) leaves without pesticide

Diffuse reflectance infrared spectroscopy (FT-IR-DRS) by pattern recognition is an efficient method in determining the pesticide in the plant (Ishizawa et al. 2001).

The quality of foods and agricultural products is influenced by not only the molecular structure of the components, but olso the interactions between the molecule and its environment. This thing focuses on the applications of IR spectroscopy for quality evaluations and control through the food stuff production based on the fundamental subjects which are mainly our studies, since the IR spectrum could be reflected by such factors and most of the component have very active in the IR region.

*Electrotherapy*. Although the corona electric discharge is known and intensively studied for its technological applications (electrophotography, electroprecipitation etc) works on the effect of corona discharge on living matter are relatively few and recent (Korge et al 1993, Chang et al. 1996, Aubrecht et al.1999, Gonzales et al. 2006, Giosanu et al. 2005).

Like any electric discharge in gas, corona discharge is based on the ionization of gas molecules and on the extraction of electrons from the cathode metal. Due to the pointed shape of one of the electrodes the applied electric field is highly localized around it. As a result of the concentration of electric field lines around the electrode of low curvature radius, all processes of excitation and ionization of the gas molecules occur in a narrow region around it. The bearers of opposite sign than the potential applied to the filiform electrode are attracted by it, while those of the same sign are rejected. The charge carriers, rejected by the electrode with small radius of curvature, quickly leave the area where the ionization processes take place (crown layer, as is known) by entering a weak electric field area (exterior space). In the exterior space, whose geometric dimensions are much larger than the crown layer, there is only one type of electrical charge carriers. For this reason, the electrical current that exists in this region is said to be unipolar (as opposed to the crown layer which is bipolar). Moving under the influence of a weak electric field, the bearers from outer space migrate under the influence of concentration gradients (possibly air currents) to long distances from interelectrode region. This causes an electrically charged atmosphere, around the corona discharge area, whose volume can incorporate a variety of other objects (such as plants).

## **RESULTS AND DISCUSSIONS**

To study the influence of corona effect on plant material, it was performed the experimental device in Figure 2.



Figure 2. Experimental device to apply corona discharge

The positive electrode was made of a nickeline wire with a diameter of  $9x10^{-2}$  mm, placed in a quartz capillary. Not covered capillary portion of the wire is the active electrode

and has a length of 5 mm. The negative electrode was made of a stainless steel cylindrical plate, 10 cm in diameter. To change the distance between the electrodes, the anode was fixed upright on a stand. The power supply for electric discharge may produce continuous voltage up to a value of 15kV. Voltage applied to the electrodes was read using an electrostatic voltmeter and the electric current by means of a digital microamperometer.

The plant material used was vine cuttings from two varieties infected with some of the most dangerous viruses of grapevine: Feteasca neagra infected with GFLV and Cabernet Sauvignon infected with GLRaV. During influence, they were placed on a moistened filter paper over the negative electrode. There have been four experimental variants as are presented in Table 1. The system geometry was constant, the distance between the electrodes being maintained at a value of 5 cm. It should be noted that in such experiments is significant radiation dose (I x t) and not each term (I or t). Values for exposure time, intensity of discharge currents and irradiation doses are given in Table 1.

Table 1

Experimental variant	V ₁ (control)	$V_2$	$V_3$	$V_4$
Exposure time (minutes)	0	10	20	30
Intensity of current (µA)	0	8	8	8
irradiation doses ( $\mu A \times s$ )	0	4800	9600	14 400

Experimental variants for electrotherapy

FT/IR spectra presented in Figure 3 have been achieved with FT-IR 6300 Jasco equipment in the 4000 - 400 cm⁻¹, apodization Cosine, resolution 4 cm⁻¹. Device control and data acquisition was performed using Spectra Manager II software. The spectra were performed by the technique of transmission by ATR (attenuated total reflection) using FTIR-ATR Gladi with a single reflection on diamond crystal.

Detection of antiviral in infected leaves can be spectral monitored by FTIR / ATR. Figure 3 presents the ATR spectrum of grapevine leaves infected and then treated with an antiviral Tamiflu (Oseltamivir, a Hoffmann-La Roche product).



Figure 3.ATR spectra of leaves infected (solid line) and infected and then treated with Tamiflu (dotted line).

In both cases, the leaves present similar spectral characteristics as we can see in Figure 3. In leaves treated with antiviral drug peaks of medium intensity are observed at

2917,77 cm⁻¹ și 2849,31 cm⁻¹ due to asymmetric and symmetric stretching vibrations CH₂ ( $\nu_{CH2}$ ) and more intense peaks at1257,36 cm⁻¹ and 1019,19 cm⁻¹, characteristic to carbonyl group( $\nu_{C=0}$ ,  $\nu_{C-0}$ ) present in the antiviral whose structure is shown in Figure 4.



Figure 4. Structure of Oseltamivirphosphate (OP)

From axillaries buds of vine cuttings, electrotherapeutic treated by corona discharge, samples were taken which were analyzed by the same technique of FT-IR /ATR spectral analysis. Recorded spectra showed no conclusive changes compared with the control sample, not subject to corona discharge. The electrotherapy could be explained as a thermotherapy at cellular level, starting from which denaturation by mediated inactivation of the viral nucleoprotein happens. Probably, the viral particles inactivation preferentially occurs during their traffic thru the apoplastic space instead at their stay inside the cell. Furthermore, the electric pulses were used to stimulate organogenesis and growth.

# CONCLUSIONS

Detection of viricides in infected leaves can be spectral monitored by FTIR / ATR. Recorded spectra showed characteristic changes due to carbonyl group ( $\upsilon_{C=0}$ ,  $\upsilon_{C-0}$ ) present in the Oseltamivirphosphate antiviral drug.

Electrotherapy by corona discharge can be considered as alternative techniques for viral eradication of grapevines. FTIR/ATR did not show significant changes in samples taken from axillaries buds subjected to corona discharge. The electrotherapy could be explained as a thermotherapy at cellular level and stimulate organogenesis and growth.

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# WALNUT (*JUGLANS REGIA* L.), A FRUIT TREE CROP WITH TRADITION AND OF INTEREST FOR ROMANIA AND GREECE

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Keywords: Juglans regia, walnut, fruit growing

## ABSTRACT

Common walnut (Juglans regia L.) is one of the oldest tree species. There are indications that this species would be present from Tertiary and Quaternary in Central and Eastern Europe. On the other hand, historical sources mention the introduction walnut since antiquity (750-500 BC) in Greece, the Roman Empire and the Balkans. Walnuts are a staple food because of nutritional value (containing 52 to 77.5% fat, 11-25% protein, 5-24% carbohydrates, vitamins, minerals, etc). Worldwide common walnut is grown either in organized orchards (968,596 ha), or as individual trees within agricultural crops or groups of trees in the forests. World production of walnuts recorded in 2011 was 3,423,447 tons. Among countries with tradition and culture interest for walnut are Romania and Greece. In Romania, organized walnut plantations occupy 1435 ha; however, the annual production of nuts is 35037 t, which it ranks ninth in the world. Greece has 11,000 ha walnut orchards (12th worldwide) and a production of 29,800 t, which ranks 11th. Together, both countries represent 1.89% of world production and 43.25% of the European Union walnut production. In recent years, there has been an evolutionary trend of increasing area under this fruit tree crop and increased trade and consumption (2.0 kg / capita / year in Greece and 1.2 kg capita / year in Romania). Walnut populations scattered in different areas of the two countries have been particularly important, being composed of natural hybrids on their own roots and beneficial for biodiversity of this species showing genetic variability.

### **INTRODUCTION**

Common walnut (*Juglans regia* L.), known as English, Carpathian or Persian walnut, is one of the oldest tree species found in Europe since the Tertiary and Quaternary periods. Successive glaciations between 70,000 and 20,000 years BC contributed directly to changes in the natural habitat of this species.

The earliest references in the history regarding the culture of walnut are found in manuscripts of Han Dynasty in China. From China, the walnut was introduced later to Europe (Avanzato, 2010).

Walnuts kernels are an important source of food, their value is extremely high. The consumption of walnut kernel in E.U. is 0.5 kg / capita / year, in Greece is 2.0 kg / capita / year and in Romania of 1.70 kg / capita / year. The EU walnut production reaches 150,000 t

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/ year but the current consumption is of 200,000 t / year, the difference is covered from imports.

Research undertaken on the nutrient content of kernel established that 100 g of walnut kernel has 647 to 660 calories. The kernel has also lipid levels of 52.0 to 77.5%, 11.0 to 25.0 % protein, 5.0 to 24.0% carbohydrates and from 1.3 to 2.5 % minerals (Radu, 1985; Cociu et al., 2003; Şarova, 1971; USDA Nutrient Database, 2001).

Walnuts also contain potassium (441-544 mg/100 g), phosphorus (320-409 mg/100 g), and other minerals. In addition, the walnut kernel is an important source of vitamins: A (retinol),  $B_1$  (thiamine),  $B_2$  (riboflavin),  $B_6$  (pyridoxine), E (tochopherol) and several polyphenols (Ţurcanu and Comănici., 2004).

Besides fruits, the walnut wood is of great value too. It is used for furniture, veneer but also as biomass with quite high value of energy (18.49 to 19.63 mg / kg) (Duke, 1983).

Walnut is grown in over 51 countries worldwide, reaching in 2011 an area of over 965,552 hectares cultivated and a fruit production of 3,423,447 t according to FAO Stat Database, 2013. Large area harvested and important productions are obtained by: China (400,000 ha and 1,655,510 t), USA (99,148 ha and 418,212 t), Iran (64,000 ha and 485,000 t), Turkey (94,959 ha and 183,240 t), etc.

Actual world production of walnuts does not fulfill the market demands and consumption requirements so, increasing the surfaces of walnut orchards, use of productive cultivars and better quality of fruits are needed.

#### MATERIAL AND METHODS

The analysis of walnut situation is based on available statistical data of Romania, Greece and world provided by FAO Stat Database Web Page and on scientific papers referring to the walnut updates from both countries.

Biodiversity of this species is of interest especially for the area of reference and some assumptions about continuity in the Balkan Peninsula and its introduction from other areas were taken into account.

# **RESULTS AND DISCUSSIONS**

# a) Biodiversity of Juglans regia in Romania and Greece

Vavilov (cited by Molnar, 2011) considered that common walnut (*Juglans regia* L.) has the center of origin and diversity in Central Asia. Zeven and Zhukovski 1975, confirm that the Primary center for walnut is Region 5 (Central Asia) and Secondary centers are: Moldova, SE Europe and SW Europe. Later on, FAO modifies the term of primary genetic centres of diversity into "regions of diversity", 17 regions being named. In case of walnut is considered that it belongs to the Central Asia subregion. This subregion was named by Vavilov the Southwestern Asian centre and corresponds to Zeven and Zhukovsky's Central Asian area (FAO, 1997).

Outside regions of diversity, there are important secondary centers. One of the secondary centers includes the Balkan Peninsula (Greece, Bulgaria, Romania, Serbia, Albania, etc), which is closely linked to the west of Turkey and seems to make a connection with walnut left behind by the ice ages, but also with introduced walnut from primary genetic centers (Cociu, 1967; Botu et. al., 1994).

Many scholars insist on walnut introduction from Persia to Europe, this fact being mentioned in the writings of Theophrastus (372-287 BC) and Varo (116-27 BC), who consider that between 750-500 BC walnut was brought from Persia and was later passed into Italy (O'Rourke, 1967).
Because the walnut was multiplied only by seeds in both countries, a highly complex biodiversity resulted based on numerous populations and tens of thousands of individuals who are natural hybrids. This variability is constantly subjected to pressure caused by the climatic and soil conditions from different geographical areas from Romania and Greece. From these natural hybrid populations many walnut cultivars were selected during the last decades ('Sibişel 39', 'Valcor', 'Valrex', 'Jupânești', etc).

Walnut genetic resources were identified across Romania and Greece and were evaluated in order to be introduced into collections or to be used into culture (Deaconu, 1990; Botu et al., 2001; Drăgănescu et al., 2001; Flondor, 2010; Rouskas and Katrinis, 1997; Rouskas and Zakynthinos, 2001, etc). Over 650 genotypes were evaluated in Romania and more than 130 in Greece, this activity confirming once more the rich diversity of *Juglans regia* species.

#### b) Walnut culture in Romania and Greece

Common walnut is found in all the provinces of Romania (Oltenia, Transylvania, Muntenia, Banat and Moldova) for over 2,000 years (Bordeianu et al., 1967). Walnut was cultivated and spread into pastures or along riverbanks.

Documents show that walnut fruits were the subject of intense trade with Austria-Hungary, Turkey, etc. Before the Second World War, Romania was the first walnut producer in Europe and the second walnut exporter in the world after U.S.A. (Botu and Achim, 2013).

Due to extremely low temperatures during the winter of 1940 - 1941 and the massive walnut pulling up until 1950, the number of walnut trees decreased to 1.0 - 1.5 million trees. Until 1989, the number of walnut trees increased to 4,680,000 trees then a second drastic decrease occurred due to massive walnut wood exploitation during 1990 - 1995 period. Currently, in Romania there are 2.06 million walnut trees (Table 1), more than 350,000 trees are in organized orchards, the rest of the walnut trees are scattered (1,720,607 trees).

## Table 1

No.	Country	Total no.	Or	chards	No. of	Total fruit	Average
		of trees	Area	No. of trees in	individual	production	yield
			harvested	the orchards	trees	(t)	(kg/tree)
			(ha)				
1	Romania	2,065,007	1,435	344,400	1,720,607	35,073	17.00
2	Greece	2,720,982	11,000	1,980,000	740,982	29,800	10.95
3	World	-	968,596	193,719,200	-	3,423,447	-

Walnut situation in Romania and Greece

The area covered with walnut orchards in Romania is only 1,435 ha according to the FAO Stat Database (2013); unfortunately, the statistical data does not reflect the real situation. Most walnut fruit production in Romania (35.073 t) is harvested from individual scattered trees (83.3%) only 16.7% (5854 t) comes from organized orchards. In the last two decades, over 400 hectares of walnut orchards were planted using domestic and foreign cultivars. The Romanian cultivars propagated by grafting were: 'Jupânești', 'Mihaela' 'Roxana', 'Valcor', 'Valrex', 'Valmit', 'Germisara', Geoagiu 65', Velnița, etc.

The Romanian walnut production during 2004 - 2011 period ranged from 15,608 t - 2004 to 47,810 t - 2005 according to FAO Stat Database - 2013. The average in-shell walnut production of Romania stands at 32-38 thousand tons. These values represent between 0.99 to 2.75% of walnut world production (Table 2).

Ν	Country	Specification				Ye	ear			
о.			2004	2005	2006	2007	2008	2009	2010	2011
1	Romania	Area harvested (ha)	1,856	2,063	1,678	2,119	1,726	1,523	1,490	1,435
		Production (t)	15,608	47,810	38,471	25,516	32,259	38,329	34,359	35,073
		Share from world production (%)	0.99	2.75	2.28	1.24	1.87	1.45	1.15	1.02
2	Greece	Area harvested (ha)	8,652	8,956	9,193	13,700	13,700	13,700	10,500	11,000
		Production (t)	19,830	21,595	23,786	22,115	15,100	22,000	22,200	29,800
		Share from world production (%)	1.26	1.24	1.41	1.08	0.87	0.83	0.74	0.87
3	World	Area harvested (ha)	651,005	671,888	680,585	736,633	718,327	826,794	910,023	968,596
		Production (t)	1,568,767	1,740,470	1,689,054	2,050,636	1,724,172	2,646,663	2,989,107	3,423,447

# Walnut area harvested and productions obtained in Romania and Greece (Source: FAO Stat Database, 2013)

Table 2

The general trend of walnut culture in Romania is directed to expansion of cultivated areas. The Reconversion Program for fruit tree growing in Romania (2014 - 2020) have as target the growth of walnut areas up to 10% of total fruit tree areas (15,000-18,000 hectares).

Introduction of foreign lateral bearing cultivars, which are susceptible to frosts of -26°C to -30°C, into the continental climate areas (Moldova, Eastern part of Transylvania) lead to important tree loses in the young orchards.

Common walnut is reported to be grown in Greece since ancient times. Walnut is present into the plains of Greece (15-16% share), in the hilly areas (16-18%), mountain areas (66-69%) along river valleys and slopes. Walnuts trees grow all over Greece (38% in Peloponnese, 26% in Center - West and the rest in Macedonia, Thessaly, Corfu, Crete, etc).

The total number of walnut trees recorded in Greece is 2,720,982 (Table 1). Cultivated area reached 11,000 ha and walnut production is 29.800 t/year (0.87 % of world production). Besides the walnut trees, which are present into the orchards, there are more than 740,982 individual walnut trees.

The calculated average yield per walnut tree is 10.95 kg in Romania and less than 17.0 kg / tree in Greece.

Walnut production, number, and surface of new orchards are increasing. The planting material used consist in selected seedlings but also cultivars (especially lateral bearers) introduced from other countries ('Vina', 'Serr', 'Pedro', 'Hartley', 'Chandler', 'Lara', etc).

Between the scattered walnut trees of Greece are found in high proportion genotypes with lateral bearing, probably brought from Persia 2,000 years ago.

They were propagated by seeds over time and form populations of such genotypes, which benefit of the most favorable weather conditions. In the favorable walnut growing areas the temperatures in winter rarely fall below  $-10^{\circ}$ C and therefore there is no problem of susceptibility to low temperatures.

The Program of extension of walnut growing in the hilly and mountainous areas of Greece include the modernization of cultivar assortment, clonal propagation of planting material and better control of diseases like walnut blight (*Xanthomonas campestris* pv. *juglandis*) and anthracnosis (*Gnomonia leptostyla*).

## CONCLUSIONS

Romania and Greece are EU countries with a rich diversity of walnut (*Juglans regia* L.), both countries being responsible of 43.2% of the total EU's walnut production.

The production is very high valued because walnuts kernels are rich in nutrients (lipids, proteins, vitamins, minerals, etc) and have beneficial health effects.

Romania and Greece are targeting walnut intensive culture based on an assortment of valuable cultivars clonally propagated and suitable technology to ensure average walnut in shell yields of 3000 - 4000 kg/ha.

Extension of walnut growing areas will be suggested into the areas and micro zones with optimal environmental conditions. In case of Romania, the cold areas where temperatures drop below -26^oC during winter and where the soils are thin, cold and have high percentage of clay should be avoided for walnut plantings.

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## STUDY CONCERNING THE INFLUENCE OF SOME VITICULTURAL PRACTICES ON THE QUALITY OF RED WINES OBTAINED IN SAMBURESTI VINEYARD

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Key words: pruning, quality, chemical composition, red wine

## ABSTRACT

The study carried out in 2012 under micro winemaking, from clones and varieties for red wines grown in vineyards Sâmbureşti showed that the viticultural technology has a significant influence on the quantity and quality of grape production that is reflected in the chemical composition and sensory characteristics of wines. The pruning type and the bud load have significant influence on the compositional and sensorial quality of Sâmbureşti red wines.

#### **INTRODUCTION**

In modern viticulture, great importance is given to the intrinsic characteristics that can be expressed by a variety, not only in terms of viticultural features but also in productive/enological terms which will in turn influence the quality characteristics of the wine produced. As a result, the presence of differentclones of a single variety within a vineyard has become common practice and international varieties already have a myriad of clones (Van Zellerde Macedo Basto Gonçalves M.I. et al. 2011). As a component of terroir, climate arguably exerts the most profound effect on the ability of aregion or site to produce quality grapes and therefore wine. Worldwide, the average climatic conditions of wine regions determine to a large degree the grape varieties that can be grown there, while wine production and quality are chiefly influenced by site-specific factors, husbandrydecisions, and short-term climate variability (Jones G.V. & E. Hellman. 2003). Grape quality is linked directly to the composition of various fruit tissues (pulp, skin and seed) and indirectly to the winemaking process (Le Moigne M. et al. 2008). Biochemical and physical modifications of grape berries begin during ripening and continue throughout berry maturation altering grape composition (Coombe B.G., 2002). It is a know ledged that a range of environmental constraints may restrict vigour and yield and thereby enhance the winemaking potential of the grape (Coipel J. et a. 2006). A limitation in vine water uptake reduces shoot growth, berry weight and yield and increases berry anthocyanin and tannin content (Koundouras S. et al. 2006).

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## MATERIAL AND METHODS

The aim of the study was to determine to what extent the pruning system and fruit load influence the quality of wine in the vineyard Sâmburești the new range for red wines. For this purpose, it has been studied two varieties: Cabernet Sauvignon clone 685 and Merlot clone 181, both grafted on rootstock  $SO_4$ . For both varieties were followed two pruning systems: Guyot and spur-pruned cordon. Cabernet Sauvignon, for both leading systems were developed 3 versions of fruit load: V1 - 9 loop/m², V2 - 12 loop/m², V3 - 15 loop/m². The Merlot for both leading systems where followed two options for the location of the plantation: the plateau and the slope. The study was conducted in 2012, the grapes were harvested simultaneously, the winemaking was done in laboratory in micro winemaking conditions in Oenology Laboratory from the Faculty of Agriculture and Horticulture from University of Craiova, after a winemaking diagram identical for all experimental variants. Wines were analyzed in terms of composition and organoleptic features at 6 months and 1 year after production, using official methods of analysis.

### **RESULTS AND DISCUSSIONS**

Analysis of the main parameters of the chemical composition (Table 1) shows that there are differences between the versions due to leading systems used and fruit load.

Table 1

Variants	Alcohol, %vol.	Total acidity, g/L H2SO4	Glycerol, g/L	Residual sugar, g/L	Extract
Cabernet Sauvignon, Guyot, 9 loop/m ²	13.7	3.88	10.6	3.6	28.2
Cabernet Sauvignon, Guyot, 12 loop/m ²	13.5	3.55	10.5	3.8	27.7
Cabernet Sauvignon, Guyot, 15 loop/m ²	13.4	4.20	10.5	3.2	27.6
Cabernet Sauvignon, spur-pruned cordon, 9 loop/m ²	14.0	4.25	11.1	2.8	28.3
Cabernet Sauvignon, spur-pruned cordon, 12 loop/m ²	13.5	4.15	10.8	3.1	28.0
Cabernet Sauvignon, spur-pruned cordon, 15 loop/m ²	13.3	3.62	10.7	3.2	27.8
Merlot, Guyot, Plateau	14.4	4.65	11.2	3.0	27.9
Merlot, Guyot, Slope	13.7	4.45	10.9	3.3	27.5
Merlot, spur-pruned cordon, Plateau	14.5	4.36	11.3	2.7	28.0
Merlot, spur-pruned cordon, Slope	13.7	4.24	11.0	3.2	27.3

The chemical composition of wines

The first parameter analyzed of composition was alcoholic strength. In all cases, it is between 13.3 and 14.5% in volume of Cabernet Sauvignon wines, alcoholic strength is between 13.4 and 13.7% in volume for leading system variants Guyot and between 13.3 and 14.0% in volume for leading system variants spur-pruned cordon. Another important

observation is that the variants of Cabernet Sauvignon, with different fruit load, the alcoholic strength is greatest for 9 loop/m² and the lowest is for  $15loop/m^2$ . Therefore, increasing the looploadandhence the production of grapes, was accompanied by lower sugar content that is equivalent to 0.3-0.5% vol. alcohol less.

Total acidity of wines varies between 3.55 and 4.25 g/L  $H_2SO_4$  for Cabernet Sauvignon and between 4.24 and 4.65 g/L  $H_2SO_4$  at Merlot. From this point of view, there is no direct relationship between leading system or fruit load and total acidity content of wines. The factor that made the difference is the malolactic fermentation, after which malic acid is converted to lactic acid.

Sâmburești red wines are recognized for their polyphenolic potential. The maceration time of 7 days in all cases, good health and advanced maturity of the grapes allowed to obtain intensely colored wines with great phenolic structure. Under these conditions, for the wines balance of taste is very important the glycerol content. The data in Table 1 show a parallel evolution between glycerol content and alcohol content of the wine. The variants with the lowest load 9 loop/m² have the highest content in glycerol and are most alcoholic. At Guyot leading system, the difference is minimal, 0.1 g/L glycerol. At Merlot in both leading systems the glycerol content is with 0.3 g/L higher on variants derived from the plateau toward the variants obtained from the slope. Regarding the differences between different versions of leading systems, the differences are minimal, 0.1 g/L glycerol, in favor of the spur-pruned cordon.

All 10 wines, regardless of variety, leading management and fruit load or location are dry wines with residual sugar contents under 4 g/L.

The data on the chemical composition of the wine shows that there are differences between the variants like the content of dry unreduced extract. The wines of Cabernet Sauvignon, the values of this parameter varies between 27.6 and 28.3 g/L, while the Merlot wines between 27.5 and 28.0 g/L. The wines of Cabernet Sauvignon, the highest content of dry unreduced extract occurs in 9 loop/m² variant and the lowest at 15 loop/m² variant for both leading systems. The Merlot wines, dry unreduced extract content is higher for the variants on the plateau to the variants from the slope. The differences are 0.4 g/L for Guyot leading system and 0.7 g/L in spur-pruned cordon leading system.



Figure 1 – Wine tasting results

Tasting wines showed differences between them in terms of sensory profile, determined by the leading system, fruit load and planting location. The scores of the 10 wines tasting obtainat 1 year after the production are presented in figure 1; it shows that the wines of Cabernet Sauvignon, with the Guyot leading system and highest loop load is best appreciated whereas the wine obtained from the lowest loop load obtain the lowest score. The spur-pruned cordon leading system, the situation was different, the most appreciated wine was the one obtain from the smallest fruit load. At Merlot wines, variants obtained with spur-pruned cordon leading system were rated better than those obtain with Guyot leading system. Regarding the location of the plantation at Guyot leading system, the most appreciated wine was the one from plantation located on the plateau, while at the spur-pruned cordon leading system was rated better wine produced from plantation located on the slope.

#### CONCLUSIONS

The study on wines produced in 2012 under the Sâmburești micro winemaking showed that the leading system, the fruit load and vineyard location influence the quality of wine composition and sensory aspect. At the Cabernet Sauvignon it is a direct relationship between fruit load and content in alcohol, glycerol and extract, which are higher as the fruit load is lower. Also, wine obtained from Merlot located on the plateau gives higher levels of alcohol, glycerol and the extract toward the wine obtain from the slope, indifferent of the leading system. Although there were differences between wines tasting in terms of results, being young wine, the sensory characteristics are evolving and no valid conclusions can be drawn than for the moment when tasting was conducted.

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## BEHAVIOR OF VARIETIES OF CHERRY ON SMALL ROOTSTOCKS IN VÂLCEA AREA

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*Keywords*: *Cherry*, *polyphenols*, *antioxidant activity*, *vitamin C* 

#### ABSTRACT

In Romania, the cherry represents a traditional orchards culture with high ecological plasticity, being planted on alignments, interspersed with other species or isolated up to an altitude of 900 m Cherry leverages well sites and soils varied land that creates optimal conditions for expression of agrobiologic its potential. To contribute to zoning within the conditions of Vâlcea, some of the many varieties of cherry culture were performed in the Copăceni, Vâlcea County composed of four varieties on small rootstocks (Kordia, Simone, Regina and Summit).

In order to create the database on the potential of these varieties of cherry and their behavior in terms of growth and fructification and in terms of adaptation to the Vâlcea county were determined biochemical and biometric analysis during the fruiting period (ripping) both on fruit and leaf system.

## INTRODUCTION

Vâlcea county is part with 4 other counties (Dolj, Gorj, Mehedinți and Olt) of the South West development region of Romania situated between the meridians 22°2' and 24°2' and the parallels of 43°3' and 45°3', covering 29 212 km2, or 12.25% of Romania.

In the hills of Gorj and Vâlcea orchards occupy important areas. The most cultivated fruit tree species are plum brandy that produce a beverage specific to the area, also are grown apples, walnuts (Vâlcea being an important research station), apricots, peaches, grapes, etc.Compared with other stone fruit species, cherry is less common in Vâlcea County, although its fruits are sought by locals and tourists.Cherry is grown throughout the country with results more or less satisfactory according to local climatic conditions. It is originally from the region between the Black Sea and the Caspian Sea and in the wild state it grows in Minor Asia, Central Asia, China, Iran, southern and south-eastern Europe.

Lately worldwide in horticulture has placed great emphasis on intensive culture of fruit trees. Part of this category is the cherry that in the past decades were recorded a series of test on reduction of trees waist giving growers many positive aspects (ease of cutting, harvesting, etc.) (Treutter et al.,1993).

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Recommended varieties are characterized by trees with constant production, adapted to regional environmental conditions and resistant to biotic or a biotic stress, which are easy to clean with modern techniques of culture, to be able to produce attractive fruits, consumption market size required, to meet in terms of quality and how to be kept a longer period. In addition to these qualities requires a wide distribution of ages of ripening fruit to cover continuously the market with fresh fruit.

Need to ensure a significant production; a research is carried out on the species of cherry for promoting regional assortment of varieties with a capacity check of them in ecoclimatic conditions of Vâlcea County. In practical terms this can be achieved through the study of comparative cultures of new varieties created in the country or abroad (Cociu, 1990).

### MATERIAL AND METHODS

Study the behavior of the culture in the Valcea of some cherry varieties on small rootstocks has been done in the area of Copăceni located at latitude 45, longitude 23.983 45°0'0" North, 23°58'59" East with an area of 6137 Ha, altitude 329 m and a temperate oceanic climate.

As research material we used four varieties of cherry fruits (Kordia, Simone, Regina, Summit) where were determined main parameters that may help to establish the behavior in culture: determination of fruit diameter was done by measuring with calipers;

determination of edible fruit (fruit yield) was made by collecting 100 randomly fruit of 1 kilo of fruits. After removing the stones, the mass of pulp, seeds and stems were weighted and were expressed as a percentage; determination of moisture content of the fruit was achieved by three methods: (thermo-gravimetric Balance; in the oven at 105°C; with solvent); determination of total soluble substance was made with Abbe refract meter; determination of fruit acidity was done by titration with 0.1 N sodium hydroxide in the presence of phenolphthalein; determination of pH was made with pH-meter; Ascorbic acid was determined (Barros et al. 2010) with 2,6-dichloro-phenol-indophenol, and measuring the absorbance at 515 nm. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.006-0.1 mg/ml), and the results were expressed as mg of ascorbic acid per 100 g of fresh weight (fw) (Barros et al. 2010).

One gram of plant material was extracted with 70% aqueous acetone solution (50 mL) at ambient temperature. The extracts were rapidly vacuum filtered through a sintered glass funnel and kept refrigerated before assay.

The amount of total phenolic in the extract was determined according to the Folin-Ciocâlteu method (Bița-Dumitru et al.,2012).

The antioxidant activity was assessed by the DPPH method. 40 µL of the investigated extract were added to 1460 µL of DPPH solution (1mM) at room temperature. A control sample was prepared by adding 40 µL of methanol to 1460 µL of DPPH solution (1mM). The reaction mixture was allowed to stand at room temperature in the dark for 10 min. Absorbance was measured at 515 nm The antiradical activity (AU₅₁₅) was calculated according to the following equation (Bita-Dumitru et al., 2012):

$$AU = A_0 - A_i$$

Where:

$$= A_0 - A_i$$

AU is the antiradical activity of the extract;

 $A_0$  – the absorbance of the sample at the beginning of the reaction (0 min);

 $A_i$  – the absorbance of the sample after 1 min of the reaction

## **RESULTS AND DISCUSSIONS**

After biometric measurements and biochemical determinations performed on cherry fruit from four varieties following data were obtained (Tables 1 and 2):

Table 1

	Average		Mass, %		Total	Moisture, %			
Variety	fruit diameter, mm	Pulp	Stone	Stem	soluble substance %	Thermo- gravimetric balance	Oven	Solvent	
Kordia	21,5	93,12	4,62	2,26	14,1	80,54	81,0	80,75	
Simone	22,0	91,18 5,96		2,26	15,5	81,7	82,1	81,5	
Regina	23,0	91,13	3 6,36 2,51		16,2	82,3	82,9	82,2	
Summit	25,0	91,54	6,09	2,37	17,0	84,0	83,2	82,9	

#### Experimental results on cherry fruits

Table 2

Experimental results on cherry fruits

Variety	Treatable acidity, g malic acid/100g FW	Total soluble substance/Acidity, °Bx/g malic acid	рН	Ascorbic acid, g/100g FW	Total polyphenols, mg equivalents Gallic acid /100g FW	Antioxidant activity, %
Kordia	0,8	17,5	3,7	10,5	317	32
Simone	0,8	19,37	3,6	12,2	376	38
Regina	0,7	23,14	3,3	9,7	307	29,7
Summit	1,28	13,28	3,1	10,0	298	27,9

*Statistical analysis.* All measurements were performed in triplicate. A one-way analysis of variance was carried out. Significance was accepted at  $p \le 0.05$ .

Cherries are some of the very low calorie fruits, however, are a rich source of nutrients, vitamins and minerals. These nutrients vary depending on the variety and the manner of reaction grown in culture of cherry. This kind of behavior is directly related to soil and climate conditions where it is grown and the treatments applied during the growing season.

Aim of the study was to determine some parameters that can characterize the behavior of the culture of sweet cherry varieties in Vâlcea County. The maximum diameter of the equatorial section of the fruit from the 4 cherry varieties ranged from 21.5 mm for variety Simone and 25.0 mm for variety Summit.

Fruit yield calculated is between 91.13% for the variety Simone and 93.12% for variety Kordia and soluble substance ranging from 14.1% for variety Kordia and 17.0% for variety Summit. Moisture determined by the three methods varied within narrow limits with values between 80.54% for variety Kordia and 84.0% in Summit and *fruit acidity and pH* are characterized by a low value varying depending on the variety.

Polyphenols determined had values between 298 mg Gallic acid/100g acid fresh product for variety Summit and 376 mg Gallic acid/100g acid fresh product for variety Simone, data obtained correlated with those in the literature (Usenic et al., 2008; Jakobek et al., 2009).

The antioxidant activity determined by the DPPH method has values within the range of 27.9% for variety Summit and 38.0% for variety Simone and is in line with the values obtained by other authors (Prvulovic et al., 2001).

Citric acid has values similar to those in the literature (Gundogdu and Bilge, 2012) corroborating with antioxidant activity obtained.

Report the total soluble substance / *acid* present values between 13.28 °Bx / g malic acid in variety Summit and 23.14 °Bx / g malic acid in variety Regina.

Analyzing the data obtained and the importance of each parameter to define product quality we find that Simone and variety Kordia have a value of parameters almost equal followed by Regina and Summit.

### CONCLUSIONS

Biometric and biochemical measurement results on cherry fruits of four varieties Kordia, Simone, Regina, Summit, studied on their behavior in culture in the county of Valcea prove a real possibility of adaptation to environmental conditions in the area. Further research is required for a longer period of studying other key factors in cherry culture.

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## TESTING OF SUBSTANCES TO LIMIT THE SPREAD IN SOIL OF AGROBACTERIUM TUMEFACIENS PATHOGENIC BACTERIA

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Keywords: garlic tincture, sodium hypochlorite, copper

#### ABSTRACT

Crown gall is known since antiquity, meeting in a large number of plants species, in which cause extensive damage. At the establishment of new vineyards, as required by law, must to be used healthy planting material free of crown gall and viruses. Therefore the planting will be made with healthy material in healthy soils. When, after testing, is identify in soil Agrobacterium tumefaciens pathogenic bacteria it must necessarily disinfected. The novelty in this study is the realization of two treatment recipes which significantly reduced the number of Agrobacterium tumefaciens pathogenic bacteria from soil. The recipies had in compozition:  $R_1$ -garlic tincture, sodium hypochlorite, copper sulphate, distilled water and  $R_2$  garlic tincture, alcohol, copper sulphate, distilled water. Both recipes were effective in eliminating the pathogen from the soil.

## **INTRODUCTION**

Agrobacterium spp. and grapevine nematodes, spread in most cultivators countries (Quader et al., 2002, Riley & Walker, 2006), should be identified obligatory even if some of them are not specific to the continental temperate zone of Romania (*Xiphinema* spp., *Meloydogine* spp.). However, should be considered that the movement of planting material is liberalized on the territory of the European Union, and the import of material can not be entirely controlled and there is a risk to spread these pathogens through infected plants.

## MATERIAL AND METHODS

*Collection, transportation and processing of soil samples.* To each sample was taken 1000 g of soil, from which 200 g were used for the isolation and identification of *A. tumefaciens* pathogenic bacteria, and the remaining of 800 g soil were used to test the effectiveness of some treatments for limiting the spread of the pathogen.

Each soil sample of 800 g was divided in half, resulting two smaller samples, of 400 g which have been treated with  $R_1$  and respectively  $R_2$ . Soil samples were taken from three different locations: from pots with grapevine plants diseased with crown gall ( $A_1$ ), places where plants were cleared ( $A_2$ ) uncultivated soil ( $A_3$ ).

*Isolation of pathogenic bacteria and application of the treatment on the ground.* Before to test the substances, with role of limiting the spread of pathogenic bacteria in the

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soil, was performed their isolation on Lieske, PGA (Severin & Cornea, 2009), PGA 3 and PGA 4 culture media and identification on the 1A medium. These steps were carried out in a special box, from windows, inside laboratory. Before starting work, and periodically the instrumentation was disinfected with ethanol 70% and buckling. After application of the treatments, one time with  $R_1$  and  $R_2$ , were made again the steps for isolation and identification of pathogenic bacteria from the soil and from the solution resulting from soil disinfection.

For disinfection the soil samples were placed in gauze, on beaker, over which were applied, on variants, the two treatments (Figure 1).



Figure 1. Treating the soil infected with A. tumefaciens pathogen (original)

*Recipes preparation.* The two recipes recipe used in this study had the following composition:

 $R_1:$  garlic tincture 12,5 ml + sodium hypochlorite (12 %) 5 ml + copper sulphate (5·H_2O) 30 g + distilled water1 l

 $R_2:$  garlic tincture 12,5 ml + alcohol (99,3°) 25 ml + copper sulphate (5·H_2O) 30 g + distilled water 1 l

Garlic tincture was purchased from the Hofigal firm and had the following composition: 20 g of garlic and ethanol 90% v / v for 100 g of solution. The garlic tincture is found in both recipes because allicin, the main ingredient of garlic has an antibiotic, antifungal and antiviral effect and it is used for reduction or prevention of cancerous tumors in humans (Josling P., 2005). Therefore in this paper was pursued the effect of this product in mixture with various substances on *A. tumefaciens* pathogenic bacteria isolated from soil.

#### **RESULTS AND DISCUSSIONS**

In order to isolate and identify pathogenic bacteria that cause crown gall of grape were obtained suspensions from soil samples, collected from vegetation pots (A1, after 1 year from planting infected plants), land resulted after clearing a vineyard (A2) and uncultivated soil (A3), which were seeded onto four culture media: PGA, PGA 3 PGA 4 and Lieske. After 24 hours was observed the formation of bacterial colonies in all culture media variants, but in different ways depending on the origin of the sample. From bacterial colonies isolated from untreated soil was made a bacterial suspension which was passed on 1A culture medium. On this specific media were formed pathogenic bacteria colonies of *A. tumefaciens*.

The soil remaining after the pathogen isolation was disinfected with the treatments  $R_1$  and  $R_2$ . After application of the two different types of treatments on soils in which have been identified the pathogenic bacteria, were made all stages of isolation and identification of them. Thus, were obtained bacterial cultures on all culture media. The number of

bacterial colonies was much lower after treatments application, this were forming in fewer streaks / Petri dish compared to untreated soil, from which the bacteria have been developed in all streaks 9 / Petri dish. From the bacterial colonies formed on the isolation medium was formed a suspension which was inoculated on the identification 1A medium, where again formed bacterial colonies specific *A. tumefaciens* pathogen.

The efficiency of the recipes  $R_1$  and  $R_2$  for each of the three soils  $(A_1, A_2, A_3)$  was determined by the presence of bacteria on the culture medium. The data were statistically interpreted, multiple correlations highlighting the effect of the two recipes on the development of the A. tumefaciens pathogenic bacteria. Thus, the soil from the vegetation pots  $(A_1)$ , where the plants grapevine were affected by crown gall, contained the largest number of pathogenic bacteria (on all of the four culture media) as compared with the soil from uncultivated land (A3) in which pathogenic bacteria have been found in a smaller number. After soil treatment (once) the number of bacteria has been greatly reduced (tables 1, 3). Starting from the needed to find an effective recipe for removal of pathogenic bacteria A. tumefaciens from soil, the results were analyzed by comparison (from untreated soil with those from soil treated with  $R_1$  and soil treated with  $R_2$ ). Since the experiment was carried out on soil from three different locations and the isolation of bacteria was performed on four culture media, the results were statistically interpreted for each location (tables 1, 2 and 3). Statistical analysis performed allowed the identification of significant differences between treated soil and control (untreated soil) at P <0.05. Positive correlations between untreated soil (control) and treated soil with R1 and R2, in terms of efficiency of both recipes, showed that the bacterial number decreased significantly after treatments application on the soil from all study plots  $(A_1, A_2, A_3)$ .

Table 1

Bacteria remainin	ig in the so	l taken fr	om the	vegetation	1 pots (A ₁ )	after treatments
	application	on with R	1 and R	₂ recipes		

No	Soil - A		Culture me	dia isolation		
110.	501 - 11	PGA	PGA3	PGA4	LIESKE	
1	% the bacteria from untreated soil	90,00±5,00ª	92,00±3,00 ^a	100,00±0,00ª	90,00±6,00 ^a	
2	% the bacteria from soil trated with R ₁	22,00±2,00 ^b	25,00±1,73 ^b	14,00±2,64 ^b	25,00±2,00 ^b	
3	% the bacteria from soil trated with R ₂	24,00±3,60°	26,00±3,61°	17,00±1,73°	28,00±2,64°	

The values represent the average of three repetitions  $\pm$  SD, the letters represent the significance of differences compared with the control at P <0.05.

Table 2

The bacteria remaining in the soil taken from the cleared vineyard  $(A_2)$  after treatments application with recipes  $R_1$  and  $R_2$ 

No.	Soil - Aa		Culture media isolation							
110.	50H - H2	PGA	PGA3	PGA4	LIESKE					
1	% the bacteria from untreated soil	69,00±10,58ª	100,00±0,00ª	61,00±2,00 ^a	76,00±3,61ª					
2	% the bacteria from soil trated with R ₁	20,00±1,00 ^b	22,00±1,73 ^b	17,00±1,00 ^b	22,00±1,00 ^b					
3	% the bacteria from soil trated with R ₂	21,00±1,73°	23,00±1,73°	19,00±1,00°	25,00±1,00°					

The values represent the average of three repetitions  $\pm$  SD, the letters represent the significance of differences compared with the control at P <0.05.

### Table 3

			Culture media isolation							
No.	Soil - A ₃	PGA	PGA3	PGA4	LIESKE					
1	% the bacteria from untreated soil	47,00±8,18 ^a	45,00±6,24ª	35,00±2,00 ^a	54,00±3,61ª					
2	% the bacteria from soil trated with R ₁	9,00±1,00 ^b	12,00±2,65 ^b	9,00±2,64 ^b	15,00±3,61 ^b					
3	% the bacteria from soil trated with R ₂	12,00±1,00°	15,00±2,00°	10,00±2,00°	17,00±1,73°					

The bacteria remaining in the soil taken from the uncultivated soil (A₃) after treatments application with recipes  $R_1$  and  $R_2$ 

The values represent the average of three repetitions  $\pm$  SD, the letters represent the significance of differences compared with the control at P <0.05. On all culture media were formed fewer bacteria because the treatments applied had been destroyed the most pathogenic bacteria from the soil due to the R₁ (garlic tincture, sodium hypochlorite, copper sulfate, distilled water) and R₂ (garlic tincture, alcohol, copper sulfate, distilled water) recipes composition. Comparing the evolution of pathogenic bacteria on all culture media the best results had the Lieske medium, followed by the PGA 3 medium. This culture media resulted at the isolation of a significantly higher number of bacteria. Regarding the effectiveness of the treatments on bacteria elimination, the recipe that containing sodium hypochlorite (R₁) leads to the isolation of a significantly smaller number of bacteria on culture media used. This shows that the recipe one reduce, in a greater number the *A. tumefaciens* pathogenic bacteria from soil. Comparing the evolution of pathogenic bacteria in all culture media before and after application of the two treatments it can be seen that both recipes were reduced more than half the number of bacteria from soil.

#### CONCLUSIONS

The treatments with  $R_1$  (garlic tincture, sodium hypochlorite, copper sulfate, distilled water) and  $R_2$  (garlic tincture, alcohol, copper sulfate, distilled water) applied to the soil infected with *A. tumefaciens* pathogen had significant efficiency in the partial elimination of the pathogen and in diminish his potential multiplier.

Lieske and PGA3 culture media led to the isolation of a significantly higher number of bacteria than PGA and PGA4 culture media.

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## TECHNOLOGICAL CHARACTERISTICS OF SOME FRENCH CLONES CABERNET SAUVIGNON GROWN IN PLEVEN REGION, BULGARIA

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Keywords: Cabernet Sauvignon, clones, wine, chemical composition, organoleptic profile.

#### ABSTRACT

The technological characteristics of the French clones 15, 337, 338, 339 of Cabernet Sauvignon variety grown in the region of Pleven (Central Northern Bulgaria) was made. The study covered three consecutive vintages - 2007, 2008 and 2009. In the area of study these clones are late ripening, reaching technological maturity in early October. They showed to have lower sugar accumulation compared to the typical one for the variety but similar high titratable acidity rate. The experimental wines of each vintage had similar chemical composition, colour features and organoleptic profile. Under the influence of climatic conditions of the year, more significant differences were found between the wine indicators of wines from the different vintages.

#### INTRODUCTION

Cabernet Sauvignon is the most widely grown variety in the world for the production of red wines (Clarke and Rande, 2001; Robinson 2006). Under the influence of a complex of abiotic and biotic factors in grapevine varieties of perennial phylogenetic development, as is Cabernet Sauvignon variety, mutational changes occur affecting to varying degrees some morphological, agro-biological and technological characteristics. The best results in improving its commercial qualities and selection of its valuable variations are achieved by the method of clone and sanitary selection. In many vine-growing countries a certain number of Cabernet Sauvignon clones have been selected by that method, covering the constantly increasing demands of the market and the individual consumer in terms of quality of grapes and wine (Galet, 1990; Moretti 1998).

The clone is a variation of the variety which has a distinctive important characteristic that might result in its separate propagation. The proper combination of grapevine propagation material, soil, stocks, etc. is of crucial importance for the grapes quality as raw material for the production of quality wines. Rivas, 1981, Rouquie and Remoue, 1981, Breider, 1967 and Boubals, 1978 believed that the clone was obtained as a result of the impact of specific environmental conditions, and its introducing and cultivation under other, substantially different conditions from the original ones, created prerequisites for the loss of the positive qualities of the clone. In many cases, the characteristics of the different wine grape varieties (clones) are known, but it is unclear how they would develop under new and very different natural-climatic and soil conditions.

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Quite often the wines from certain varieties do not exhibit the expected flavors and tastes. Schoffling (1981) considered that prior to an introduction of varieties or clones from one region to another it should be accurately clarified the interaction between them and the environmental conditions in which they were selected and tested.

The investigation of the adaptability and enological potential of Cabernet Sauvignon clones has been the subject of numerous studies in different countries and regions (Whiting and Hardie, 1981; Troshin and Chuprakov, 1981; McCarthy and Ewart, 1988; Cirami et al., 1993; Crespan et al., 1999; Battistutta et al., 2000; Burin et al., 2011 a, 2011 b).

All this justifies the need of extensive and diverse studies of the newly-introduced vine clones in Bulgaria, so that their agro-biological and technological potential under the soil and climatic conditions of the country could be determined.

The objective of the present study is to make technological and enological evaluation of grapes and wines from introduced French clones of Cabernet Sauvignon under the conditions of Pleven.

#### MATERIAL AND METHODS

The technological characteristics of the French clones 15, 337, 338, 339 of Cabernet Sauvignon variety were studied during the period 2007 - 2009, based on a comparative study of the chemical composition and organoleptic qualities of the grapes and the wines from the respective vintages. Cabernet Sauvignon clone ILV 1/11 was used as reference. The clones were grown in the clone section of the Experimental Base at the Institute of Viticulture and Enology (IVE) – Pleven. The vines of the studied clones and the reference had equal number of winter eyes loading so that regularity could be established in the sugar accumulation capacity and the quality indicators.

The grape harvest of the studied clones and the reference was carried out simultaneously in the first half of October. The grapes were processed in the Experimental Winery of IVE - Pleven, in the amount of 25 kg for each sample under the conditions of microvinification in accordance with the classical technology for red wines production (Янков и кол., 1992; Amerine et al., 1972). The grapes pulp was sulphited with 50 mg/dm³ SO₂. The alcoholic fermentation was carried out with pure culture of dry wine yeast *Saccharomyces cerevisiae* in the amount of 10 g/hl, temperature 28°C. Because of the insufficient sugar accumulation in the grapes from the clones in the studied vintages and for making wines without deviations in the chemical composition, the experimental variants had proportional adjustment of the sugar content before the beginning of fermentation. After the process was finished the young red wines were de-decanted and sulphited with up to 20 mg/dm³ free SO₂.

The chemical composition of the grape pulp and the obtained experimental wines, referring the key indicators, was analyzed by conventional winemaking methods (Иванов и кол., 1979). The young wines were tasted by 9-member commission. The differences in the organoleptic qualities were determined by a 100-point scale (Цветанов 2001) and in accordance with the method of the main characteristics by spider diagrams (Bertrand and Kotseridis, 2004; Проданова 2008).

### **RESULTS AND DISCUSSIONS**

In this study, carried out in the period 2007 - 2009, the differences found in the technological characteristics of the introduced French clones of Cabernet Sauvignon variety

and the reference (Cabernet Sauvignon ILV 1/11) were due to their agro-biological characteristics, as well as the meteorological conditions impact over the years.

The chemical composition of the studied grape clones and the referent one during the period 2007 - 2009, is presented in Table 1.

The data show significant variation of the sugar content in grapes, both between the studied clones and for the different vintages.

In 2007, the highest sugar content, and exceeding that of the reference had only clone 337 (221.00 g/dm³). The lowest sugar content was reported for clones 338 and 339, which had the highest titratable acidity - respectively 6.90 g/dm³ and 7.73 g/dm³. In the other clones their quantity was considerably less – 6.05 g/dm³ (clone 15), 6.25 g/dm³ (clone 337) and 6.50 g/dm³ (reference) (Table 1).

Table 1

Clone	Date of harvest	Sugars, g/dm ³	Total acids, g/dm ³	pН
		2007		
15		202,00	6,05	3,58
337		221,00	6,25	3,52
338	3 October	176,00	6,90	3,55
339		189,00	7,73	3,50
ILV 1/11 control		205,00	6,50	3,21
		2008		
15		170,00	7,84	3,33
337		183,00	7,80	3,20
338	1 October	170,00	7,70	3,19
339		184,00	7,85	3,17
ILV 1/11 control		183,00	7,60	3,23
		2009		
15		220,00	6,60	3,28
337	]	214,00	7,40	3,16
338	15 October	203,00	7,08	3,10
339		219,00	6,80	3,26
ILV 1/11 control	]	203,00	6,88	3,11

Chemical composition of grapes from clones Cabernet Sauvignon, in the period 2007-2009

In 2008, the sugar accumulation in all clones, including the reference, was with the lowest intensity, as a result on the 1st October, the sugars amount was within the range from 170.00 g/dm³ (15, 338) to 184.00 g/dm³ (339). The grapes from that vintage was characterized by the highest acidity rate - from 7.60 g/dm³ (clone 1/11) to 7.85 g/dm³ (clone 339), that was in line with the low sugar content (Table 1).

In 2009, the lowest sugar concentration was recorded for clone 338 and the reference ILV  $1/11 - 203.00 \text{ g/dm}^3$ . For the other French clones the sugar content was considerably higher – from 214.00 g/dm³ (clone 337) to 220.00 g/dm³ (clone 15). The grapes must titratable acidity from that vintage was within the optimal ranges – from 6.60 g/dm³ (clone 15) to 7.08 g/dm³ (clone 338), except only clone 337, which, despite its good sugar content kept higher level of titratable acidity – 7.40 g/dm³ (Table 1).

The differences in the grapes composition of the studied clones determined the differences in the chemical composition and organoleptic characteristics of the experimental wines (Table 2, Figure 1). The wines of the studies vintages had alcoholic content in correlation to the amount of sugars in the grapes. The residual sugars

concentration in the samples was less than  $2.50 \text{ g/dm}^3$ , which confirmed the complete process of the alcoholic fermentation.

For vintage 2007 wines, the stated indicators were within the rates of respectively 12.15 vol. % (clone 338) – 12.85 vol. % (clone 337) and 2.19 g/dm³ (clones 15 and 339) – 2.42 g/dm³ (clone 338). More significant differences were observed in the values of sugarfree extract (SFE), that was directly related to the density and extractability of red wines taste. The lowest SFE rate was recorded in the wine from the reference – 24.01 g/dm³.

In the studied French clones its content was higher, ranging from  $25.01 \text{ g/dm}^3$  (clone 339) to 27.51 g/dm³ (clone 15). The titratable and volatile acids in the experimental varieties were within the characteristic rates for young red wines. The highest acidity had the sample from clone 339, respectively 6.80 g/dm³ and 0.62 g/dm³.

An important component of red wine composition was the content of total phenolic compounds (CFC), and anthocyanins. The studied clones differed substantially from year to year concerning these indicators. Wines from the 2007 vintage were characterized by the best phenolic and colouring content. The highest rates of CFC and anthocyanins were reported in wines from clone 337, and the lowest - in the referent clone (Table 2). There was a correlation between these components of wine and its color characteristics. All samples were distinguished by highly intense color with violet hues, which was confirmed by the color intensity and hue ratios (Table 2). The experimental wines had also different organoleptic features quantified by their tasting assessments. The variants of the studied French clones from that vintage were superior compared to the reference in their organoleptic profile – higher content of SFE, CFC and anthocyanins (Table2).

The highest assessment was awarded to the samples of 15 and 338 clones, distinguished for their typical deep red color and intense flavors of forest red berries (raspberry, blackcurrant, blueberry). The wine taste was extractive, dense, harmonious, with soft tannins (Figure 1).



Figure 1. Organoleptic profile of experimental wines made from clone 15 and clone 1/11 (control), 2007 vintage

	E	l asting assessments		86,63	81,72	83,63	82,36	80,90		74,87	75,66	76,22	74,33	77,88		82,21	79,80	81,79	80,12	78,20
	ur	Tint, T [abs.units]		0,605	0,655	0,645	0,624	0,615		0,558	0,602	0,618	0,613	0,607		0,503	0,578	0,589	0,598	0,533
	Colo	Intensity, I [abs.units]		11,85	12,08	12,00	11,27	10,50		9,52	9,33	9,20	9,16	9,88		10,05	10,73	10,26	10,15	10,10
0		Anthocyans, mg/dm ³		667,80	682,90	676,30	651,20	622,20		456,75	463,99	466,08	480,00	502,24		512,00	560,00	547,00	537,00	535,00
	Car	g/dm ³		2,60	2,78	2,65	2,40	2,10		1,49	1,52	1,53	1,58	1,66		1,82	2,17	2,00	1,95	1,94
		Hd	2007	3,28	3,28	3,36	3,34	3,25	2008	3,27	3,25	3,17	3,18	3,25	2009	3,47	3,27	3,33	3,26	3,30
	Volatile	acids, g/dm ³		0,48	0,59	0,45	0,62	0,59		0.54	0,42	0,48	0,60	0,54		0,42	0,60	0,36	0,42	0,36
I	Total	acids, g/dm ³		5,56	6,00	5,56	6,80	6,10		7,50	7,20	7,30	7,50	7,23		6,30	7,20	6,68	6,28	6,60
	Sugar-	пее extract, g/dm ³		27,51	25,91	26,98	25,01	24,01		25,06	24,06	24,00	24,43	25,27		25,36	25,62	25,16	26,86	25,12
1	C	ougars, g/dm ³		2,19	2,39	2,42	2,19	2,39		1,14	1,64	1,61	1,67	1,33		1,54	1,98	1,44	1,54	1,78
		Alconol, vol %		12,38	12,85	12,15	12,17	12,30		12,06	12,14	12,04	12,10	12,08		12,80	12,50	12,28	12,60	12,22
		Clone		15	337	338	339	ILV 1/11 control		15	337	338	339	ILV 1/11 control		15	337	338	339	ILV 1/11 control

Chemical composition of the experimental wines from clones Cabernet Sauvignon, in the period 2007-2009

Wines from the 2008 vintage were characterized by the lowest alcohol content in the range from 12.04 vol. % to 12.14 vol. %. The quantity of residual sugars in all samples was below 2 g/dm³. SFE rates were also in a narrow range - from 24.00 g/dm³ (clone 338) to 25.06 g/dm³ (clone 15). The most extractive was wine from the reference – 25.27 g/dm³

Due to the grapes higher acidity content the resulting wines were characterized by higher titratable acidity, as no substantial differences were observed between the different variants. The highest acidity ratio was reported in wines from clones 15 and 339 (7.50 g/dm³).

The variants with lower acids content were rated more highly at wine tasting. Young wines had normal volatile acidity, not negatively influencing their organoleptic characteristics (Table 2).

Wines from the French clones did not exceed in the content of phenols and anthocyanins the reference that had the highest ratios  $-1.66 \text{ g/dm}^3$  and 502.24 mg/dm³ respectively. CFC in wines from the French clones varied from 1.49 g/dm³ to 1.58 g/dm³, and anthocyanins were within the range from 456.75 mg/dm³ to 480.00 mg/dm³. All samples were characterized by the lower intensity of the color for that year. That was due to the cool, rainy autumn, leading to not good ripening of the grapes and its low phenol contents.

Wines from the 2008 vintage had also the lowest tasting scores, as the highest was 77.88 points (the reference) (Table 2). In their organoleptic characteristics the wines from the studied French clones did not exceed the reference. They were characterized by a lighter color, suppressed, unexpressed aroma; their taste was unbalanced, with standing out titratable acidity and stringent tannins in the aftertaste.

The next vintage of the studied period (2009) was especially favorable in terms of quality of the grapes and the resulting wines. The alcohol content of the samples ranged from 12.22 vol. % to 12.80 vol. %, with residual sugars from 1.44 g/dm³ to 1.98 g/dm³. The samples from the introduced clones were slightly superior compared to the referent clone on SFE, as the wine from clone 339 had the highest content (26.86 g/dm³). SFE content in the rest of the variants had very close rates. The experimental wines had normal titratable and volatile acidity, with the highest values in the variant of clone 337, respectively 7.20 g/dm³ and 0.60 g/dm³. However that sample had fewer scores in the organoleptic analysis (Table 2).CFC content in the experimental wines were in the range from 1.82 g/dm³ to 2.17 g/dm³, and anthocyanins – from 512.00 mg/dm³ to 560.00 mg/dm³. The lowest values had the wine from clone 15, and the highest - clone 337. These variants had the weakest and the most intense colour respectively. However, it was not found a correlation between these analytical indices and the wines tasting assessment. The best organoleptic profile (distinctive fruity aroma and harmonious taste with soft tannins) had the wine obtained from clone 15 (82.21 points), although its lower values of SFE, anthocyanins and colour characteristics (Table 2).

#### CONCLUSIONS

Under the soil and climatic conditions of Pleven, the studied clones were lateripening. They were characterized by lower sugar accumulation compared to the typical for Cabernet Sauvignon variety while having high titratable acidity.

The identified differences in the chemical composition of the grapes and the resulting wines from the studied introduced French clones of Cabernet Sauvignon and the reference (Cabernet Sauvignon ILV 1/11) were due to their agro-biological characteristics and the influence of meteorological conditions during the study period.

The wines from clones 15 and 339 were reported to have the best organoleptic characteristics (color, aroma and taste).

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# NEW RESULTS FOR OBTAINING NUCLEAR STOCK PLUM MATERIAL

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Key words: plum, virus-free, 'Prebasic' material

## ABSTRACT

During 2008-2011, Fruit Research & Development Station Bistrita had implemented the European standards for obtaining of nuclear stock plum material, succeeding in a first stage certification of 15 plum cultivars as 'Prebasic' category. Starting to 2012, in addition to maintaining the existing nuclear stock material, the work was expanded with the aim to enlarge the range of plum varieties of 'Prebasic' category. Trees selected from seven plum cultivars (Agent, Gras ameliorat, Centenar, Gras românesc, Minerva, Andreea, Delia) have represented the initial planting material for propagation. Subsequently, preliminary steps were followed for the obtaining of 'Prebasic' material according to EPPO scheme. Thus, the 'Candidate' material was tested to Plum pox virus, Prune dwarf virus, Prunus necrotic ring spot virus, Apple chlorotic leaf spot virus, Apple mosaic virus, Mirobolan latent ringspot virus, and European Stone Fruit Yellows phytoplasma. The results revealed infection with Apple chlorotic leaf spot virus on Gras romanesc cv., and consequently the infected plants were removed. The 'Candidate' plants from six cultivars which have been found virus-free are ready to be certified as 'Prebasic' category, enriching thus the existing nuclear stock collection.

#### INTRODUCTION

*Prunus domestica* is the dominant fruit specie in Romania (FAOSTAT, 2011) with a high economic importance. Mainly, the viral infections represent causes for the important economic losses on almost plants. That because once the viruses and/or phytoplasmas have infected plants, there is no curative method for these pathogens in the field conditions. *Plum pox virus* is the most destructive viral pathogen on plum, which is widespread in all plum growing areas from Romania, causing serious yield losses (Zagrai et al., 2010). But this is not the only virus that could affect plum specie. Also, ILAR viruses (*Prune dwarf virus, Prunus necrotic ring spot virus* and *Apple mosaic virus*), Trichoviruses (*Apple chlorotic leaf spot virus*), Nepoviruses (*Mirobolan latent ringspot virus*) and phytoplasmas (*European Stone Fruit Yellows*) could represent a threat for plum growers. Therefore, prophylactic measures are very important in order to reduce the economic losses caused by

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these pathogens. The using of certified virus-free planting material represents one of the main prophylactic measures.

To increase the quality of planting material, the European and Mediterranean Plant Protection Organization (EPPO) established standards for production and maintenance of nuclear stock, production of propagation stock and certified plants [PM 4/30(1)] - (OEPP/EPPO, 2001). Also, EPPO (2004) established standards for diagnostic protocols on regulated pests.

Forty-eight years ago, at Fruit Research & Development Station (FRDS) Bistrita under supervision of the Academy of Agriculture Sciences, has started the first program for the production of virus-free material (Parnia, 1990, 1999). By this program had been established orchards with virus-free scions and rootstocks, in all fruit research stations from the main fruit growing areas. Before 1989 all planting material was propagated by using multiplication material originating from scion and rootstock orchards. The financial support for this program was drastically reduced in the period of 1990-2000, and later on was practically stopped. In this situation, the maintenance of biological virus-free planting material became difficult and therefore, a high probability of infected material propagated in nurseries and subsequently in commercial orchards. After Romania became European Union member, the requirements for biological material necessary for planting have to be in conformity with the European Directives 92/34/CEE and 2008/90/CE (with subsequent amendments), transposed by Romanian legislation (Orders no. 1295/2005 and no. 82/210 with subsequent amendments).

In the period 2008-2011, FRDS Bistrita has established a certification program according to European standards for obtaining of nuclear stock plum material, through a project funded by the Romanian Ministry of Education and Research, succeeding in the first step certification of 15 plum cultivars (Iulia, Matilda, Geta, Zamfira, Ivan, Dani, Doina, Romaner, Elena, Jubileu 50, Flora, Renclod Althan, Stanley, Anna Spath and Carpatin) as 'Prebasic' category, and in the second step 10 plum cultivars (Iulia, Matilda, Geta, Zamfira, Ivan, Romaner, Elena, Renclod d'Althan, Stanley, Anna Spath) as 'Basic' category (Zagrai et al., 2013). In addition to maintaining the existing nuclear stock material, starting with 2012, the work has been expanding with the aim to enlarge the range of plum cultivars of 'Prebasic' category.

#### MATERIAL AND METHODS

**Plum material.** Trees from ten plum cultivars (Agent, Gras ameliorat, Centenar, Gras romanesc, Ialomita, Minerva, Diana, Silvia, Andreea and Delia) grown in experimental orchards were selected for pomological quality, and have represented the initial planting material for testing. Selected trees were visually inspected on spring 2012 for any potential symptoms suggesting possible virus infections and tested for pathogens listed in EPPO standards [PM 4/30(1)] – (OEPP/EPPO, 2001). In this way, one shoot from each selected tree of each cultivar was collected and tested by serological and molecular methods. The biological material found virus-free was grafted onto virus-free Myrobolan 29C. Eight plants of each presumed virus-free plum cultivar has been grafting into the insect-proof house. In parallel, biological indexing was performed. Three plants of each gelected on woody indicator, *Prunus persica* GF 305. All propagated plants, and also indicators, were individually tested one year later (May, 2013) for all pathogens listed in EPPO standards.

*Diagnosis method.* Serological diagnoses were performed by Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) – (Clark and Adams, 1977), using commercial polyclonal antibodies, on the following viral pathogens: *Plum pox* 

virus (PPV), Prune dwarf virus (PDV), Prunus necrotic ring spot virus (PNRSV), Apple chlorotic leaf spot virus (Bark split)-(ACLSV), Apple mosaic virus (ApMV) and Mirobolan latent ringspot virus (MLRV). To increase the sensitivity of PPV detection, DAS-ELISA was combined with Immunocapture-Reverse Transcription-Polymerase Chain Reaction (IC-RT-PCR) (OEPP/EPPO, 2004), using the pair of primers P1/P2 (Wetzel et al., 1991). Qiagen one-step kit (Qiagen, Germany) was used for RT-PCR. Test samples (1:20 in extraction buffer) of leaves from each shoot (before grafting) and from different part of the plants (bottom, mid and top) (after propagation) were used for testing in DAS-ELISA and IC-RT-PCR.

To detect '*Candidatus* Phytoplasma prunorum', the causal agent of *European Stone Fruit Yellows* (ESFY), Nested-PCR method was performed. Nervures of leaves and/or phloem were grind to a fine powder under liquid nitrogen and, subsequently, total DNA was purified by using DNeasy Plant Mini Kit (Qiagen, Germany). Phytoplasma primers P1/P7 (Deng et al., 1991; Schneider et al., 1995) and R16(X)F1/R16(X) R1 (Lee et al., 1995) were used in the first and second Nested-PCR. GoTaq Flexi DNA Polymerase kit (Promega, USA) was used for DNA amplification.

The soil in which the plant material has been produced and maintained was sampled and analysed by Central Laboratory for Phytosanitary Quarantine Bucharest, for potential presence of nematode vector *Xiphinema* sp. and *Longidorus* sp. (OEPP/EPPO, 2001).

#### **RESULTS AND DISCUSSIONS**

Three cultivars (Diana, Silvia and Ialomita) from those ten selected were found infected with PPV, both by DAS-ELISA and IC-RT-PCR (Table 1). Consequently, the three cultivars were not included in the next step for obtaining 'Candidate' nuclear stock. All plants tested by DAS-ELISA for the other viruses (PDV, PNRSV, ACLSV, ApMV and MLRSV) and by Nested-PCR for ESFY have reacted negative.

According to serological and molecular detection, seven cultivars (Agent, Gras romanesc, Gras ameliorat, Minerva, Centenar, Andreea and Delia) were presumed to be virus-free, and consequently grafted onto virus-free Mirobolan 29C rootstock.

The results of serological tests of plum 'Candidate' material and indicator plants are shown in the table 2. Unexpectedly, the serological tests performed were revealed that Gras romanesc cv. was infected with ACLSV, both in 'Candidate' material and indicator plants.

All infected plants were promptly removed from insect-proof house. An explanation for ACLSV escaping in the first round of testing could be the presence of the virus as latent form, which did not allow the detection in the previous year.

All samples tested by Nested-PCR for detection of ESFY phytoplasma were negative (Figure 1).

Soil analysis from pots in which the 'Candidate' material have been grown, confirmed the absence of nematode vectors *Xiphinema* sp. and *Longidorus* sp. as the bulletin of analysis performed by Central Laboratory for Phytosanitary Quarantine Bucharest showed.

As a result of this work, three plants of each virus-free cultivar (Agent, Gras ameliorat, Centenar, Minerva, Andreea and Delia) are ready to be certified as 'Prebasic' category. Taking into account that the tests were performed according with EPPO certification standards and the rules of the national regulatory, we expect to finish soon the certification process under Territorial Inspection for Quality Seeds and Planting Material (ITCSMS) Cluj.

This biological material enrich the existing nuclear stock collection from Fruit Research & Development Station Bistrita (Photo 1), and thereby, the number of 'Prebasic' plum cultivars has risen at twenty-one.

## Table 1

Cultivar			DA	S-ELISA		IC-RT- PCR	Nested- PCR	Prelimi-	
	PPV	PDV	PNRSV	ACLSV	ApMV	MLRSV	PPV	ESFY	status
Agent	-	-	-	-	-	-	-	-	Virus free
Gras ameliorat	-	-	-	-	-	-	-	-	Virus free
Gras romanesc	-	-	-	-	-	-	-	-	Virus free
Centenar	-	-	-	-	-	-	-	-	Virus free
Diana	+	-	-	-	-	-	+	-	Infected
Silvia	+	-	-	-	-	-	+	-	Infected
Ialomita	+	-	-	-	-	-	+	-	Infected
Minerva	-	-	-	-	-	-	-	-	Virus free
Andreea	-	-	-	-	-	-	-	-	Virus free
Delia	-	-	-	-	-	-	-	-	Virus free

## Serologic and molecular tests on selected plum material before grafting - FRDS Bistrita 2012 –

## Table 2

## The results of serological tests of plum 'Candidate' material -FRDS Bistrita, 2013-

Cultivar /	DAS-ELISA					Status	
plant position	PPV	PDV	PNRSV	ACLSV	ApMV	MLRSV	
Agent P1-P7	-	-	-	-	-	-	Virus free
Gras ameliorat P1-P8	-	-	-	-	-	-	Virus free
Centenar P1-P7	-	-	-	-	-	-	Virus free
Gras romanesc P1-P7	-	-	-	+	-	-	Infected
Minerva P1-P8	-	-	-	-	-	-	Virus free
Andreea P1-P8	-	-	-	-	-	-	Virus free
Delia P1-P6	-	-	-	-	-	-	Virus free
Cultivar/Indicator plants (GF 305)							
Agent/GF 305	-	-	-	-	-	-	Virus free
Gras ameliorat/GF 305	-	-	-	-	-	-	Virus free
Centenar/GF 305	-	-	-	-	-	-	Virus free
Gras romanesc/GF 305	-	-	-	+	-	-	Infected
Minerva/GF 305	-	-	-	-	-	-	Virus free
Delia/GF 305	-	-	-	-	-	-	Virus free

#### M 1 2 3 4 5 6 7 8 9 10 C+ C- C+ C- M 11 12

#### M 11 12 13 14 15 16 17 18 C- C+ C+ C- C+ C-



Figure 1. Electrophoresis onto agarose gel of amplified products by Nested-PCR (for phytoplasma ESFY), M-marker, 1-3 – Agent cv. P1-P3; 4-6 - Gras ameliorat cv. P1-P3; 7-9 – Centenar cv. P1-P3; 10-12 - Andreea cv. P1-P3; 13-15 - Delia cv. P1-P3; 16-18 – Minerva cv. P1-P3; C- negative control, C+ positive control.



Photo 1. Nuclear stock collection from Fruit Research & Development Station Bistrita

In the next years, this material will be used to obtain plants of 'Basic' category for establishing mother plantations. A strategy including propagation of virus-free *Prunus* and reinforcing orchards with these materials could help to reduce the impact of PPV in Romania.

## CONCLUSIONS

Three plants of six plum cultivars (Agent, Gras ameliorat, Centenar, Minerva, Andreea and Delia) correspond to criteria for 'Prebasic' category, enriching thus the existing nuclear stock collection from Fruit Research & Development Station Bistrita.

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## Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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## THE EFFECT OF GROWTH REGULATORS ON THE ORNAMENTAL POTENTIAL OF *Hypoestes* sp. PLANTS

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Keywords: growth retardants, ornamental potential, pigments

#### ABSTRACT

The Hypoestes genre included leaves decorative species, perennial herb, native to Madagascar, with ovate leaves marked with bright pink, red or white spots.

Cycocel (chlorocholine chloride) is a retardant typically used in flower decorative plants, in order to control vegetative growth and stimulate flowering. Treated plants are more compact with shorter internodes, stronger stems and greener leaves.

The purpose of the present study were to estimate the changes in growth and pigments amounts in leaves occurring in two varieties of Hypoestes sanguinolenta under treatment with Cycocel.

The researches performed indicated that it foliar spray on various concentrations resulted in decreased plant height, reduced shoots length and growth of plant compactness. It also induced a leaves growth content of pigments (chlorophylls, carotenoids, anthocyanins), and coloring emphasis.

#### INTRODUCTION

The Hypoestes genre includes species of perennials, sub- shrubs and shrubs originating from South Africa, Madagascar and SE Asia.

Species *Hypoestes sanguinolenta* (sin. *Hypoestes phyllostachia*) is the most prevalent culture as indoor ornamental plants. The plant looks like a bush shoots, height between 20 and 50 cm. Leaves opposite, ovate- elliptic with a sharp point are very decorative due to numerous small spots , pink, green amid the upper face. Freckled appearance of leaf gave the popular name of the plant "plant with freckles" (Şelaru Elena, 2006).

Today there are many cultivars of *Hypoestes sanguinolenta* in a different range of colors, from the series "Splash" are: Pink - pink with mottled, Red -red, mottled, Rose - pink - purple, mottled, White - White cream marble.

Interior Fit exploiting decorative valences of species Hypoestes that because look spectacular original highlight all other flower species used.

Interior Fit exploiting decorative valences of the species that because look spectacular original highlight all other flower species used.

By application of the growth retardants for the purpose of obtaining plants with smaller size, the foliage intensely colored with a large number of shoots. Researches on the effect of these chemicals were made to flower plants such as Oleander (*Nerium sp.*),

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Geranium (*Pelargonium sp.*), Laurel (*Laurus sp.*) *Poinsetia*. Cycocel (chlorocholine chloride) is one of the most important retardants, with a wide spectrum of action, with many applications in agriculture, forestry, horticulture. It has a strong activity on the growth of the vegetative organs of plants, the exploit, maturation and increase the production of key metabolic processes, such as, for example, increased plant resistance to the action of unfavorable factors.

The purpose of this research was to observe the effect of the Cycocel on Hypoestes plants and assess induced changes on growth and pigment content (chlorophylls, carotenoids, anthocyanins reflected in foliar coloration emphasis).

## MATERIALS AND METHODS

The experiment was conducted in the Floriculture discipline greenhouse within USAMV Bucharest.

The Cycocel treatment effect was followed on plants obtained from peaks of shoots cuttings. There were studied two varieties of *Hypoestes sanguinolenta*, one pink and one white leaves pigmentation. 10 cm diameter planting pots were used filled with a peat based substrate, for the specific requirements of the plants.

The plants were treated with various concentrations of Cycocel from 0,5 % to 2,5 %, according to the diagram shown in Table 1. Cycocel solution was commercially available and was applied after determining the concentration, according to the schedule adopted (so that no concentration threshold recommended by manufacturers and the literature, up 2.5%). The control variant was not tampered with Cycocel treatments instead of were applied to all the other works of care as those applied experimental variants treated.

Table 1

Experimental variants	V _m	$V_1$	<b>V</b> ₂	<b>V</b> ₃	$V_4$	<b>V</b> ₅
Cycocel concentration	-	0,5%	1%	1,5%	2%	2,5%

The Cycocel application diagram on the Hypoestes sanguinolenta plants

There were carefully pulverized, both in the aerial parts of the plants, as well as on the surface of the substrate. When applying treatment the substrate was moist so it doesn't need watering.

The treatments were carried out at an interval of 10 days between April and June 2013.

Observations and measurements were made on plant growth (height, number of shoots, shoot length, number of leaves).

Biochemical parameters were analyzed using proper methods:

• *Chlorophyll* and *carotenoid pigments* were extracted in 80% acetone and determined spectrophotometrically (wavelenghts 663 nm, 647 nm and 480 nm) using the extinction coefficients and equations described by Schopfer (1989). The results were expressed in mg/100 g fresh weight;

• Anthocyanins content was measured using the pH-differential method (Versari A. *et al.*, 2007). Each plants extracts dissolved in methanol were mixed with 2% HCl (pH 0.6), respectively with citrate buffer (pH 3.5). The absorbance was measured at 520 nm. The results were expressed as cyanidin-3-glucosideequivalents.

### **RESULTS AND DISSCUSIONS**

The average data for each experimental variant, representing the values observed about two weeks after the last treatment with Cycocel application to plants have been summarized in Table 2.

Table	2
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Experimental variants	Plant height (cm)	Shoots number	Shoots length (cm)	Leaves number
Vm	17,00	6,00	15,50	29,00
$V_1$	16,50	6,25	15,00	24 ,00
$V_2$	11,50	7,00	12,00	32,00
V ₃	14,50	6,75	9,50	25,00
$V_4$	13,00	7,50	11,00	35,00
V ₅	10,50	6,50	10,00	30,00

Observations and measurements on the *Hypoestes sanguinolenta* pink leaved plants

Analyzing the data relating to the height of the pink leaved plants, it is noted that they confirm the plant response to treatment with Cycocel namely the variants treated plants have a height less than the height of the control version (17 cm), untreated. Plants with lowest height (10,50 cm) was obtained after treatment with Cycocel at a concentration of 2,5 %.

In terms of the shoots number as the data in Table 2 show, it increased to the value of version control. A larger number of shoots were seen on treated variants in concentrations of 2% (7,5) and 1% (7).

Т	abl	le	3
-			~

Experimental	Plant height	Shoota number	Shoots length	Leaves
variants	(cm)	Shoots number	(cm)	number
V _m	19,50	10,00	16,50	41,00
$V_1$	18,50	8,00	15,50	32,00
V ₂	16,50	11,50	14,00	36,00
V ₃	17,00	12,00	13,50	39,00
$V_4$	16,00	11,00	13,00	43,00
V ₅	13,50	9,00	12,50	35,00

Observations and measurements on the Hypoestes sanguinolenta white leaved plants

The data obtained from measurements made on the length of the shoots show the same trend of decreasing treated variants compared to the control one, as in the case of plant height.

The length of the short shoots of the treated plants at a concentration of 1,5 %, and variations  $V_4$  (2%) and  $V_5$  (2,5%) showed a slightly higher values (11 and 10 cm).

The highest number of leaves per plant (35) was observed in  $V_4$  (2%) and the lowest number of leaves per plant (24) was recorded in  $V_1$  (0,5%).

As for the white leaved plants (Table 3), the indicators followed the same trend as in the case of pink leaved ones.

The effect of Cycocel on the amount of pigments in the both, white and pink leaved *Hypoestes sanguinolenta* plants.

The obtained results indicated that the chlorophyll content in the leaves of *Hypoestes sanguinolenta* was increased by the treatment with all tested concentrations of Cycocel (figures 1 and 2), except the highest one (2,5%). However, the 1,5% and 2% cycocel solutions were more effective than the lower concentrations (0,5 and 1%). So, the experimental variant  $V_3$  (1,5% Cycocel concentration) for both varieties registered the highest value of the amount of total chlorophyll: 385.27 mg/100g f.w. for the white leaved plants and 350.39 mg/100 g f.w. for the pink leaved ones, which was for 1,84, respectively for 1,21 times higher than those measured in control plant (untreated).

Similar results were reported by others authors (Hussanein M.M., 2004; Taha R., 2012), which showed also that treatment with Cycocel determined an accumulation of more chlorophyll in the leaves of some plants.



Figure 1. Effect of Cycocel on content in assimilatory pigments in Hypoestes (white leaved)



Figure 2. Effect of Cycocel on content in assimilatory pigments in Hypoestes (pink leaved)

Regarding the amount of carotenes, an increasing was registered in the leaves of treated plants, the highest value been determined also in the variant treated with 1,5 % Cycocel solution (11,11 mg/100 g f.w. in the leaves of the white leaved variety and 10,03 mg/100 g f.w. in the pink ones.

On the contrary, as a result of treatment with the highest concentration of Cycocel (2,5%) the lowest amount of content in assimilatory pigments was registered in the leaves of the treated plants (figures 1 and 2). So, the values of carotenes concentration determined in the plants which received treatments with 2,5% Cycocel solution were for about 1.3 times lower than the amount of carotenes determined in the control plants for both the analyzed varieties. It appears that growth retardant Cycocel applied in high concentrations blocked some enzymatic systems involved in the pigments biosynthesis.

Concerning the content in anthocyanins of the *Hypoestes* (pink leaves), the researches performed showed that Cycocel treatment stimulated the anthocyanins accumulation, so that the leaves of the pink variety treated with Cycocel were more colored that the ones of untreated plants.



Figure 3. Effect of Cycocel on anthocyanins content in *Hypoestes* (pink leaved)

The concentration of anthocyanins determined in the leaves of planted treated with 1,5 % Cycocel solution registered the highest value (431,63 mg/100 g f.w.). The smallest amount of anthocyanins was obtained under 2,5 % Cycocel application (figure 3), which probable inhibits some cellular metabolic processes involved in anthocyanins production.

### CONCLUSIONS

The treatment with Cycocel inhibited the *Hypoestes* plants height increase, as in all the variants treated plant height was lower than in the case of the control version, untreated, as the cultivation of pink leaved plants as well as the white leaved ones.

The number of shoots was higher compared to control on all treated variants, a larger number (12 shoots) was registered on the variant treated with a concentration of 1,5%. At concentrations of 2 to 2,5% the number of shoots started to decline but not less than the control.

Shoot length had lower values compared to the control variant, the concentration of 2,5% was the lowest recorded in both cultivars.

Treatment with Cycocel as a growth retardant induced a concentrating of foliage color due by an increased content in chlorophyll, carotenes and anthocyanins.

Application of a 1,5 % Cycocel solution determined an increasing of the amount of total chlorophyll for 1,84 times at *Hypoestes* (white leaves), respectively for 1,21 times at the pink leaved plants compare to those measured in control plant (untreated);

Also the content in carotenes reached the highest values in the variant treated with 1,5 % Cycocel solution (11,11 mg/100 g f.w. in the leaves of the white variety and 10,03 mg/100 g f.w. in the leaves of the pink one;

The concentration of anthocyanins determined in the leaves of the pink variety treated with 1.5 % Cycocel solution registered the highest value (431,63 mg/100 g f.w.).

The lowest amounts of chlorophyll, carotenes and anthocyanins were registered in experimental variant with 2,5% Cycocel solution applied, so it appears that growth retardant Cycocel used in high concentrations blocked some enzymatic systems involved in the pigments biosynthesis.

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# **RESEARCH AND PRELIMINARY RESULTS ON THE DECORATIVE POTENTIAL OF THE SPECIES** *Phaseolus coccineus* L.

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Keywords: ornamental potential, runner beans

# ABSTRACT

Runner bean (Phaseolus coccineus L.) is a climbing vegetable species with high waist, cultivated in the open, in cold and humid climates. In the last century this species is listed as part of the group leguminous vegetables grown for the production of green peas and dried beans.

In our country, runner bean is grown with good results by private producers in the hills of northern Moldavia and Wallachia. Bright and attractive colors of flowers, the runner number of showy flowers and leaves of plants have attracted the attention of landscapers who appreciate the decorative characteristics of this species.

This full paper presents preliminary results of research and ornamental characteristics of runner bean plants in the northern Bucharest gardens.

## **INTRODUCTION**

Within the range of vegetables and aromatic species grown in the world are distinguished species or groups of species with only utility (carrot, onion, celery, etc.), only ornamental character (ornamental cabbage) and/or those that combine both directions use, such as beans, zucchini, chrysanthemum, peppers, red cabbage, peas and more.

Genus Phaseolus (from the Latin word Phaseolus) includes about 150 species. In Romania the most important species are *Phaseolus vulgaris* and *Phaseolus coccineus L*. syn. *Phaseolus multiflorus* Willd. – Runner bean.

The species *Phaseolus coccineus* plants root systems, with nodules of 2,5 cm length, high strain 2-7 meters, trilobite leaves, oval and acuminate leaflets. Hermaphrodite flowers, bright red (feuerbohne = fire beans), white, black, purple or light purple, sometimes bicolor, 1, 5-3 cm long are grouped in racemes longer than the leaves (25-30 cm length). The pods are green, slightly flattened, rough (pubescent) employed (Eihfeld I.G, 1948).

Large seeds up to 25 mm long, 13-14 mm wide and 8 mm thick are great for eating and MMB (1000 seeds mass) between 700-1350 g.

Alogam-pollination, carried by insects caused the appearance of growing areas as hybrids that turns local populations (Popa Lorena, 2008). As ornamental plant it is usually to decorate pergolas, fences, walls unsightly (Traian Săvulescu, 1957). Romania flowering period is between June and September.

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Runner bean is a very demanding plant on temperature conditions during flowering and fruiting. Temperatures below 14 °C during flowering causes flower drop. Also, high temperature, accompanied by atmospheric drought causes abortion of flowers and pods fall at the beginning of their training (Munteanu N. 2005).

The present full paper presents preliminary results of an original investigation in which it was tested in the specific area of Romania plain, the biological material from more runner bean local populations in an attempt to obtain useful data for:

- Establish the possibilities of growing plants of *Phaseolus coccineus L.*, producing and planting seedlings;

- Evaluation of the ornamental potential of this species under conditions of high temperatures and low atmospheric humidity specific southern Romania;

- Development of technical recommendations for growing Runner bean as an ornamental plant in the north of Bucharest.

#### MATERIALS AND METHODS

The experiments were conducted in the campus of the University of Agronomic Sciences and Veterinary Medicine (USAMV) Bucharest and the surrounding green spaces Baneasa forest in northern Bucharest.

To conduct this research work were studied four local populations (with colored seeds) received from USAMV-Ion Ionescu de la Brad Iasi, courtesy of Mr. Prof. PhD. Neculai Munteanu, whom we thank for his kindness and promptness.

Table 1

### Experimental variants- studied local populations, Runner bean (*Phaseolus coccineus*), Bucharest, 2013

Variant	Local populations
$V_1$	black beans and violet points (-)
<b>V</b> ₂	black beans (1)
<b>V</b> ₃	violet with black beans (14)
$V_4$	white beans (C ₃ )

The experience is a monofactorial type, based on 4 versions. As a witness of experience, for the characters and traits studied expressed numerically, we used the medium data of the experience.

In the experiment observations and measurements were made on:

- Beans size – there were determinations of the length, width and thickness of the bean, taking the average of these local populations separately.

- The number of days required emergence - according to the date and time of occurrence seed mass seedlings.

- The percentage of emergence - for each local population.

- Data showing the main phenophases-based culture which was established schedule and was established during the growing season. It was determined the vegetative growth of plants assessed by recording the number of leaves, number of inflorescences and plant height.

Before actual sowing, the seeds were subjected for 24 hours, a wetting process that accelerates the emergence of plants with up to 5 days.

After wetting, the seeds were sown in pots with a capacity of 200 ml in a peat substrate (pH 6.2, N: 250 mg/liter 150 mg  $P_2O_5/l$ , K  $_2O$  150 mg/l) to a depth of 3-4 cm. We sowed one seed in pots.

Sowing took place on 24.04.2013 in greenhouses inside USAMV Bucharest. High humidity and temperatures of 20-25°C in the greenhouse provided optimum conditions for seed germination.

#### **RESULTS AND DISCUSSIONS**

### Results and discussion on morphological characteristics of the seeds.

Average values of the seed dimensions for all the experimental variants  $(V_1 - V_4)$ 

• The average length measured in the axis of the seed largest range from 18,86 mm to V₂, reaching a maximum of 20,4 mm V₁, V₃ and V₄ has the above specified values of an intermediate size, namely 19,26 mm (V₃) and 19,2 mm (V₄).

• The average bean width ranging from 9,73 mm to 12,83 mm  $V_2$  to  $V_1$ . For other versions are major differences between the widths of the beans cultivars,  $V_3$  with 10,93 mm, while the record  $V_4$  average grain width of 12 mm.

• The average thickness of beans on variant  $V_2$  records a value of 16,53 mm, thus representing the smallest beans in the experimental variants studied.  $V_1$  has a mean beans thickness of 19,4 mm, and  $V_3$ ,  $V_4$  respectively have values lower than  $V_1$ .

Table 2

The beans dimensions and the colors, Runner bean (*Phaseolus coccineus* L.), Bucharest, 2013

Variant	Lenght (mm)	Width (mm)	Thickness (mm)	Beans color
$V_1$	20,4	12,83	19,4	black with violet
				points
$V_2$	18,86	9,73	16,53	black
<b>V</b> ₃	19,26	10,93	18,30	violet with black
$V_4$	19,2	12	17,63	white
Average	19,43	11,37	17,96	-

Mean measurements establish that  $V_2$  represents the population with the lowest seed,  $V_1$  is the opposite extreme, and  $V_3$  values slightly superior to the  $V_4$  variant.

Seed color varies from white  $(V_4)$ , to black-violet  $(V_1, V_3)$  and black  $(V_2)$ .

It can be observed an obvious correlation between seed color and flower color: white flowering plants produce seeds white ones with red flowers produce seeds adorned with "drawings" purple and black.

### **Results on plant emergence**

are:

The percentage of germination was determined according to the maximum number of the emerged plants of each experimental variant and the results were summarized in Table 3.

It is noted that the percentage of emergence varies between 80% (V₃) and 86% (V₂).

The number of days required emergence wetted seed which is being recorded mass emergence in 8-10 days. Planting seedlings obtained occurred on 14.05.2013.

Table 3

Variant	Total number of seeds sown	The number of emerged plants	Percentage of germination (%)
$V_1$	50	42	84
<b>V</b> ₂	50	43	86
V ₃	50	40	80
$V_4$	50	41	83

The seed sprouting, Runner bean (Phaseolus coccineus L.), Bucharest, 2013

There were two plants on each nest, and directed on the high trellis of 2,5 m. After planting the plants were watered thoroughly.

# **Results regarding the culture schedule**

Phenological peculiarities of the species can give us information on both the duration of phenological phases and time of occurrence.

This is of particular importance in that conducts certain phenophases correlate with environmental conditions.

For example, the importance of knowing the flowering period lies in the fact that here arises the need to take specific measures to protect plants in flower, especially if it occurs in hot, dry periods.

The period from sowing (24.04.2013) to the emergence of mass was subject to a constant number of 8-10 days for each variant studied. Range from rising at the first leaf was 6-8 days, pointing out the  $V_1$  and  $V_2$ . The period from the east at the first flowering ranged within 30-34 days (beginning of June), which accord with the literature (1).

**Results on plants growth and flowering** 

Plant growth was analyzed in terms of plant height, number of leaves, number of inflorescences and the number of flowers in the inflorescence. Observations were made for each population, May-June 2013, and data (average values) are shown in Table 4 and Figure 1.

Table 4

Date / variant	Plant height (cm)		Leaves number		
	V	VI	V	VI	
<b>V</b> ₁	76,88	86,44	9,25	12,94	
<b>V</b> ₂	72,63	92,13	8,63	13,25	
<b>V</b> ₃	85,63	124,63	7,50	14,63	
$V_4$	64,75	75,25	6,00	10,88	

Vegetative growth parameters, Runner bean (Phaseolus coccineus L.), Bucharest, 2013

The analysis of experimental data it can be seen that the maximum height of the plant occurs in  $V_3$  (124,63 cm), while the minimum is found in  $V_4$  (75,25 cm), other variants having intermediary values in descending order  $V_2$  and  $V_1$ .

The hierarchy is maintained for the values that define the number of leaves, respectively the highest number of leaves was registered at  $V_3$  (14,63), and decreasing from  $V_2$ ,  $V_1$  to the  $V_4$  minimum value (10.88).



Figure 1. The flowering parameters Runner bean (*Phaseolus coccineus* L.), Bucharest, 2013

Regarding the evolution of parameters characterizing the flowering period, it can be seen from figure 1, that  $V_3$  and  $V_2$  share the top positions in both the number of inflorescences (5,5 -V₃; V₂ -5,3) and the number of flowers on one inflorescence (V₂ -6,05 V₃- 5,87).

This time the lowest values are found in the case of V1 that has an average of only 3,18 to only 3,18 inflorescence flowers on one inflorescence.

#### CONCLUSIONS

Populations of Runner bean (*Phaseolus coccineus* L.) used in the experiment behaved in the first growing season generally good, providing some technical information prior to choosing cultivars best adapted to environmental conditions in southern Romania.

The growth of the main stem is higher on the  $V_3$  and  $V_2$  experimental variants in which the plants are more vigorous than that of variants  $V_1$  and  $V_4$ .

Decorative effect due to their foliage (leaf number, and color leaflets) also joined the variants  $V_3$  and  $V_2$ . Foliage color ranging from dark green ( $V_1$ ,  $V_2$ ), to green ( $V_3$ ,  $V_4$ );

The flower color is red  $(V_1, V_2, V_3)$  and white  $(V_4)$ ;

Thanks to these features, the populations mentioned above may be used first in creating high curtains that close perspective to places in the park or garden less attractive. Planting of this species with a high density, can easily mask fences or walls due to runner height and size of leaves;

Decorative effect due to their inflorescences was recorded on  $V_3$  and  $V_2$ , which were evidenced by a greater number of inflorescences. Were revealed in terms of pigmentation, flowers on mentioned cultivars can be recommended to create green curtains with black spots specific local populations that worked.

The height disadvantage of the plants can compensate by pinching works of main stem and shoots and achievement in this compact of a port with a bigger number of shoots and leaves. Due to the high need of water it is demanding that the future researches will be focused on the water consumption, because of its values higher than the lawn and flower species on the landscape development.

We consider it necessary to continue and further research and in terms of the rules of irrigation, the planting distances, and specific works on plants growth and flowering.

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# CONTRIBUTIONS TO PHYTOCOENOLOGICAL STUDIES ON THE CORMOPHYTIC HERBACEOUS VEGETATION FROM THE SUBALPINE LEVEL OF THE LATORIȚA HYDROGRAPHIC BASIN (VÂLCEA COUNTY, ROMANIA) (I)

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Keywords: subalpine level, phytosociological research, herbaceous communities.

### ABSTRACT

In this paper we present our sociological research of herbaceous communities found in the structure of the vegetation cover from the subalpine level of Latorita hydrographic basin. The final set of results is completed by the identification of six associations (Carici dacicae-Plantaginetum gentianoidis Boşcaiu et al. 1972; Eriophoro vaginati-Sphagnetum recurvi Hueck 1925menyanthetosum Sârbu et al. 1997; Arenarietum biflorae Voik 1976; Soldanello pusillae-Plantaginetum gentianoidis Boşcaiu 1971; Orechloo distichae-Juncetum trifidi Szafer et al. 1927; Potentillo chrysocraspedae-Festucetum airoidis Boşcaiu 1971), included in four classes (Scheuchzerio-Caricetea nigrae R. Tüxen 1937; Oxycocco-Sphagnetea Br.-Bl. et R.Tüxen ex Westoff et al. 1946; Salicetea herbaceae Br.-Bl. 1947; Juncetea trifidi Klika et Hadač 1944. Each association is characterized using as criteria the floristic element, life form and geo-element spectra and the values of some ecological indices. Our recorded data formed the basis of a project to improve the protocols of observation and ecological management of the territory of interest.

#### **INTRODUCTION**

In the current paper are presented the results of the coenological research, focused on the diversity of cormophytic herbaceous communities found in the structure of the vegetation layer from the subalpine level of the Latorita hydrographic basin. These link to those that refer to shrub phytocommunities (Anghel 2012) and complete them offering an integrate image on the cormophytic association spectrum from this territory.

INVESTIGATED AREA. The Latorița hydrographic basin was already characterized in a previous paper (Anghel 2011, 2012; Anghel & Toma 2011), from a physical-geographic point of view but also regarding the taxonomic and coenological phytodiversity and in order to avoid pleonasm, here, are inserted only the references. Still, it must be specified that the subalpine level (1,750-2,185 m alt.) occupies ~ 45% of the Latorița's hydrographic basin territory and that it has a bigger extension on the peaks higher than 2,000 m, such as: Galbenu Peak (2,185 m), Cioara Peak (2,146 m), Urda Peak (2,172 m), Nedeia Peak (2,130 m), Negovanu Peak (2,072 m), Muntinu Mic and Mare peaks (2,062 m), Bora Peak (2,055 m), Frătoșteanu Mare Peak (2,053 m) and Ștefanu Peak (2,051 m).

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## MATERIALS AND METHODS

The procedures used in the phytocoenologic research were based on the methodology developed by the Central European Zurich-Montpellier school (Braun-Blanquet 1964), to which some subsequent amendments were added (Barkman et al. 1986; Bohn et al. 2003; Borhidi 1996; Boşcaiu 1971; Mucina 1997).

The syntaxonomical nomenclature of the revisions is in accordance with the *International Code of Phytosociological Nomenclature* (Barkman et al. 1986 and Weber et al. 2000). The taxa's nomenclature is coherent with the one promoted by Ciocârlan 2009. For each species the life form and the geological element were established; their weighting is expressed in a graphical way. Codes and habitats nomenclature follow *Natura 2000*.

## **RESULTS AND DISCUSSIONS**

Processing the data collected from the field during the 104 relevees implementation and the synthesis of the secondary data identified inside the vegetation layer's structure of the subalpine level of Latorița's hydrographic basin, 12 herbaceous cormophytic associations belonging to nine alliances, classified into seven orders and seven classes. In this paper are present only 6 of these classified into 4 classes (Table 1).

Table 1

The conspect of cormophytic associations from subalpine level the Latorita hydrographic basin

Class & order	Alliance	Association
SCHEUCHZERIO-CARICETEA NIGRAE R. Tüxen 1937 CARICETALIA NIGRAE Koch 1926	<i>Caricion nigrae</i> Koch 1926 em. Klika 1934	1. Carici dacicae- Plantaginetum gentianoidis Boșcaiu et al. 1972
<i>OXYCOCCO- SPHAGNETEA</i> BrBl. et R.Tüxen ex Westoff et al. 1946 <i>SPHAGNETALIA MAGELLANICI</i> (Pawłowski 1928) Moore 1968	Sphagnion magellanici Kästner et Flössner 1933	2. Eriophoro vaginati- Sphagnetum recurvi Hueck 1925 - menyanthetosum Sârbu et al. 1997
SALICETEA HERBACEAE BrBl. 1947 SALICETALIA HERBACEAE BrBl. 1926	Salicion herbaceae BrBl. 1926	3. Arenarietum biflorae Voik 1976 4.Soldanello pusillae- Plantaginetum gentianoidis Boșcaiu 1971
JUNCETEA TRIFIDI Klika et Hadač 1944 CARICETALIA CURVULAE BrBl. in BrBl. et Jenny 1926	<i>Caricion curvulae</i> BrBl. in BrBl. et Jenny 1926	<ol> <li>Orechloo distichae-Juncetum trifidi Szafer et al. 1927</li> <li>Potentillo chrysocraspedae- Festucetum airoidis Boşcaiu 1971</li> </ol>

1. As. *Carici dacicae-Plantaginetum gentianoidis* Boşcaiu et al. 1972 (*Caricetum dacicae* Buia et al. 1962) includes phytocoenoses with *Carex nigra* subsp. *dacica* frequently founded in mesohygrophylous swamps, acids (pH = 5-5.8), at altitudes between 1,800 and 2,000 m. This association which has a slight chionophylous character, endemic in the Carpathian of the SE, it has as edifying and characteristic species the *Carex nigra* subsp. *dacica* and the *Plantago gentianoides*. Together with these two, there is a big number of characteristic species to that order and class: *Carex curta*, *Cardamine pratensis* subsp. *rivularis*, *Eriophorum vaginatum* etc. Among these, there were identified species characteristic to the alliance *Cardamine-Montion*: *Caltha palustris*, *Deschampsia cespitosa*, *Carex rostrata*, *Polygonum bistorta* etc.

Place and date of the relevees' executing: Turcinu Peak (21.06.2011); Negovanu (11.07.2007); Nedeia Peak (15.08.2007); Căldarea Igoiu (05.07.2007); Galbenu

(30.07.2010). In the floristic composition of the association the hemicryptophytes prevail (72.73%), followed by geophytes (18.18%) and by phanerophytes (9.09%). In the spectrum of phyto-geological elements, the Circumpolar elements are dominant (36.36%), followed by Carpathian-Balkan elements (27.27%), Eurasian elements (18.18%), Cosmopolitan and Central-European elements (each with 9.09%).

2. As. *Eriophoro vaginati-Sphagnetum recurvi* Hueck 1925 includes phytocoenoses meso-oligotrophic, acidophilic, dominated by *Eriophorum vaginatum* and some bryophytes species from the upper and subalpine level (*Sphagnum angustifolium, Polytrichum communae* etc.), which can be found in the glacial buildings The soil on which the phytocoenoses live and develop themselves, has a hight level of mineral substances (3-5%) and an acid reaction (pH = 4.2-4.6). The phytocoenoses' meso-oligotrophic character it is also influenced by the presence of some elements from the *Caricetalia nigrae*'s order in their floral structure. At the Violeta's Lake level, from Muntinu Mic, at an altitude of 1,545 m, a sub-association *menyanthetosum* Sârbu et al. 1997 was identified and which presents as a differential the specie *Menyanthes trifoliata*.

Place and date of the relevees' executing: Iezerele Muntinului (15.08.2008); Urda (19.08.2008); Iezerul Latoriței (21.08.2009); Violeta Lake (30.08.2009); Cioara Lake (30.07.2010).

In the life form spectrum, beside the hemicryptophytes that dominate (88%), coexist chamaephytes, phanerophytes and geophytes in proportion of 4% each, and in the spectrum of the geological elements, coexist the Eurasian (32%) and Circumpolar elements (28%).

3. As. *Arenarietum biflorae* Voik 1976 – The association's phytocoenoses develop themselves on a siliceous substrate created after crystal schists at altitudes over 1,900 m and that occupies small areas on which the snow lasts longer, in the glacial buildings having a North exposure or North-East. In the weedy layer, beside the builder specie *Arenaria biflora* there are also a series of remarkable species that belong to the *Potentillo-Nardion strictae* alliance (*Agrostis rupestris, Poa alpina, Campanula serrata, Potentilla ternata* etc.), *Caricetalia curvulae* order (*Festuca supina, Carex atrata* etc.)

Place and date of the relevees' executing: Căldarea Galbenu (30.07.2010; Căldarea Bălescu (12.08.2011); Căldarea Cioara (30.07.2010).

From the life form spectrum analysis, the hemicryptophytes result as dominant elements (65.38%), followed by chamaephytes (19.23%), phanerophytes (11.54%) and geophytes (3.85%). The geological elements are represented through Alpine-European species (61.54%), Carpathian-Balkan species (11.54%), Eurasian species (7.69%), Circumpolar and Central-European species (each one with 3.85%). National elements have a big wight (11.54%) which requires a project elaboration regarding an efficient and durable conservation of the territory's phytodiversities.

4. As. Soldanello pusillae-Plantaginetum gentianoidis Boşcaiu 1971 – The association has a thick hygrophyl character, and because of that, it often comes from the priming of peat layer forming process of the association's Soldanello pusillae-Ranunculetum crenati Boşcaiu 1971 advanced stage (Sanda et al. 2008). The Soldanella pusilla and Plantago gentianoides edified phytocoenoses vegetate in the glacial circles with a high humidity of the soil due to the rain and to the stagnant water that comes from the melting of the snow at altitudes between 1,900 and 2,000 m.

Place and date of the relevees' executing: Căldarea Muntinu (25.08.2008); Căldarea Urda (19.08.2008); Iezer Peak (21.08.2009); Căldarea Galbenu (30.07.2010); Dengheru (12.07.2009). In the life form spectrum there are present only the hemicryptophytes and chamaephytes elements, the geological elements are represented through the Alpine-European species (44.44%), Circumpolar species (33.33%), Eurasian and Carpathian-Balkan species (each one with 11.11%).

5. As. **Orechloo distichae-Juncetum trifidi** Szafer et al. 1927 (Juncetum trifidi Buia et al. 1962; Rhododendro-Juncetum trifidi Resmeriță 1975; Junceto trifidi-Vaccinietum Resmeriță 1975). The primary meadows of this association occupies small areas, on acid soil (pH = 4.1-4.5) and oligotrophic, between 1,900 and 2,100 m alt., on slope with inclinations quite big (30-40°), usually on the top, the ridges and abrupt mountainside, exposed to wind. The characteristic and builder species of the association, which covers 50-60% of the area, are Juncus trifidus and Oreochloa disticha; these, sometimes, are codominants in resorts with a North exposure, meanwhile in semi-sunny areas, only Juncus trifidus is dominant, and Oreochloa disticha becomes sporadic. Because of a big surface development of Pinus mugo, on the West and on the North mountainside, the phytocoenoses of this association extend themselves as secondary vegetation.

Place and date of the relevees' executing: Dengheru (12.07.2009); Căldarea Igoiu (5.07.2007); Bălescu (17.08 2008); Coasta Bengăi (14.08.2008); Cărbunele (01.07. 2009).

In the spectrum of the biological forms, hemicryptophytes (76.09%) are preponderant, and in that of the phyto-geological elements prevails the Alpine-European ones (39.13%) and the Circumpolar elements (28.26%).

6. As. *Potentillo chrysocraspedae-Festucetum airoidis* Boşcaiu 1971 (*Potentillo ternatae-Festucetum supinae* Boşcaiu 1971) – It is one of the most spread herbaceous association of the subalpine level, installed on the ridges and on moderate slopes. The soil substrate is not very profound and it is, from the moist surface until the dried one, from very acid till less acid, sometimes rich in humus, formed by crystalline or calcareous substrate. The association is outlined by *Festuca airoides*, or the *Agrostis rupestris* and it gets installed at altitudes that varie between 1,850 and 2,100 m. Because of the intensive pasture this meadow deteriorates and evolves towards *Scorzonero roseae-Festucetum nigricantis*.

Place and date of the relevees' executing: Bora to Miru (10.08.2008); Stâna Miru Peak (28.07.2008); Under Bălescu Peak (12.08.2010); Bălescu Peak (12.08.2010); Ștefanu (06.07.2009); Dosul Micăii (05.07.2009); Micaia (05.07.2008); Cioara (30.07.2010); Coasta Bengăi (21.08.2008); Iezer (21.08.2009)

The life form spectrum is by far dominated by the hemicryptophytes (81.13%), and the geological elements are dominated by the alpine elements (30.19%); the endemic elements have a remarkable presence (3.77%).

The phytosociological method of describing, analyizing and clasifing of the natural vegetation is one of the most preferd and consacrated temathics in the national (Boşcaiu 1971; Drăgulescu 2010; Mihăilescu 2001; Răduțoiu 2008; Sanda et al. 2008) and international research (Borhidi 1996; Dimitrov et al. 2004b; Mucina 1997; Rothmaler 2002; Roussakova 2000; Simon & Pócs 2012). This state of mind is explicable if we consider the fact that is practically impossible to research, monitorize and control the evolution of the ecological sistems in the absence of profound knowledge refering to the origine, development, composition, structure and clasification of the plant communities, defined by their status as main components of the biocoenose of the ecosistems. In this context the interes of the research has considerably risen over the knowledge of the relationships of the phytoassociations and their habitats; the avalance of the scientific papers risen in the last decade (Anghel 2012; Chifu et al. 2006; Devillers et al. 1991; Dimitrov et al. 2004b; Doniță

et al. 2005, 2006; Gafta & Mountford 2008; Hajek et al. 2005, Rodwell et al. 2002) atest to this state of mind.

## CONCLUSIONS

The originality of the study lies in the description (for the first time) of 6 cormophytic herbaceous associations from the subalpine level of the Latorita hydrographic basin. Reporting our existing data from the national (Danci & Cristea 2009, Drăgulescu 2010, Mihăilescu 2001 and Răduțoiu 2008) and international specialty literature (Hajek et al. 2005; Roussakova 2000; Simon & Pócs 2012) it is proven to have multiple structural particularities induced by the context of the ecological factors, biotic and abiotic, of their habitats. The abundant hidrographic network and the presence of the glaciar circles have played a decisive role in this sense. This data brings a significant contribution to the knowledge and understanding of the vegetal layer of the subalpine level of Latorita hydrographic basin.

The coenotaxons described define the habitats with variable conservative value, of which conservation needs to be assigned by the Special Areas of Conservation according to the Habitat Directive, Annex I (Drăgulescu 2010): as. *Orechloo distichae-Juncetum trifidi* – 6150 Siliceous alpine and boreal grassland, habitat with a reduced conservative value; as. *Potentillo chrysocraspedae-Festucetum airoidis* Boşcaiu 1971 – 6150 Siliceous alpine and boreal grassland, endemic habitat with big conservative value; as. *Eriophoro vaginati-Sphagnetum recurvi* – 7110 Active raised bogs, priority habitat, with very hight conservative value; as. *Carici dacicae-Plantaginetum gentianoidis* – southeast Carpathians, eu-mesotrophic swamps with *Carex nigra* subsp. *dacica* and *Plantago gentianoides*: endemic habitat in the Southeast Carpathian, with a big conservative value; recently described by Simon and Pócs from Parâng Mountains, which published 5 relevés from 2,150-2,400 m altitude.

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# CONTRIBUTIONS TO THE STUDY OF THE GENITALIA OF SOME OPILIONIDS (ARACHNIDA, OPILIONES) FROM ROMANIA – PART I NEMASTOMATIDAE

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Keywords: Nemastomatidae, penis, seminal receptacles

#### ABSTRACT

The paper presents the morphology of male and female external genitalia – penis and seminal receptacles of the ovipositor for 5 Opilionid species belonging to Fam. Nemastomatidae collected from Romania.

# **INTRODUCTION**

Being the most important specific taxonomic features, the external genital organs have been thoroughly studied by specialists in taxonomic zoology and for species identification. Since some species of Opilionids have intraspecific variability regarding the form of the seminal receptacles of the ovipositor and the penis form (body and glans), the study of samples obtained from various local populations, is welcome. In Romania, external genitalia of Opilionids were studied by Romanian arachnologists mostly while describing new species and on occasion of faunistical reports.

## MATERIAL AND METHODS

The Opilionid material representing the topic of this paper was collected from the south-west region of Romania, from localities which are to be mentioned for each species.

## **RESULTS AND DISCUSSIONS**

1. Paranemastoma silli (Herman, 1871)

Material examined from: Rânca - Parâng Mountains, leg. det. Anda Babalean

The penis is 2.00 - 2.30 mm. in length, thin and flat. The glans is bifurcated and has spines which form a variable pattern. The thinner branch of the glans has the form of an immobile stylus. The stylus is broad, shovel like. The smaller branch of the glans has almost the form of a triangle. The body of the penis is yellow, the glans is yellow brown.

The intra-population and inter-population variability of the spines pattern of the penis glans (spinulation) in *Paranemastoma silli* has been mostly studied by Avram (Avram, 1973a) on a rich material collected from 11 epigeic and caves stations. Three out from eight spinulation areas described by Avram have been considered in this paper:

• **area I** = the medial part of the short branch of the penis glans;

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• **area II** = the apex of the short branch of the penis glans;

• **area** VI = the long branch of the glans penis – reconsidered by author only the apex of area VI.

As regarding area **VI**, the descriptions of Šilhavý 1966 (Šilhavý, 1966), Šilhavý 1956 (Šilhavý, 1956) and Martens 1978 (Martens, 1978) range within the variability limits of the species as described by Avram (Avram, 1973a) that is without spinulation (but half of Avram (Avram, 1973a) samples have a short excrescence), while the topic sample of this study presented or not one spine on area VI. For area **II** the sample of this study displayed 2 spines, corresponding to Šilhavý 1956 (Šilhavý, 1956) and Martens 1978 (Martens, 1978) and thus ranging within the variability limits of 2 to 4 (rarely 6) spines of Avram (Avram, 1973a). For area **I** Avram gave a large variability: a) lack of spines or protuberances, b) one protuberance, c) one spine and one protuberance, d) 2 spines and 1-2 protuberances. The sample of this study frequently displayed 2 spines without protuberances. The spinulation offered by other authors is: Martens 1978 (Martens, 1978): one spine on a protuberance, Šilhavý, 1956): one spine, Šilhavý 1966 (Šilhavý, 1966): no spine, no protuberance.

*The ovipositor* is short and robust, being 0.80 mm in length. Its upper part is bifurcated and has hardly noticeable rings. The ovipositor has excrescences almost all-over it and they are covered by thin setae. According to Avram, *the seminal receptacles* have the form of a coma- like bag (Avram, 1973a). Within the population from Galbenul – Rânca, the seminal receptacles are fundamentally different from those described by Avram: there are 2 elongated vesicles, on each part of the internal vagina. The variability of the seminal receptacles of the ovipositor in different populations of *Paranemastoma silli* will be the subject of a separate paper.

#### 2. Paranemastoma ancae Avram, 1973

Material examined from: Curechiu – Metaliferi Mountains, leg. det. Anda Babalean

The identification of *Paranemastoma ancae* was done considering the dorsal pattern of the scutum magnum.

The *penis* length is 2.1 mm. The glans of the penis of the studied sample has the following pattern of spines: area I – one spine located on the tip of a protuberance; area II – 6 spines; area VI – with a visible excressence and without spinulation.

The penis is very similar to that of the species *Paranemastoma silli* as regards the presence of spines on the internal part of the smaller glans branch – area I, thus, being different by Avram description for *Paranemastoma ancae* – probably holotype (Avram, 1973b): no spine on area I.

*The ovipositor* looks like that of *Paranemastoma silli*, length -0.5 mm. The length given by Avram is 0.75 mm. (Avram, 1973b). *The seminal receptacles* have the form of a mushroom from which derive 2 oval vesicles crossed in the upper part.

## 3. Nemastoma transsylvanicum Gruber & Martens, 1968

Material examined from: Răchita - Timiș County, leg. det. Anda Babalean

*The penis* is 1.3 mm in length. Viewed frontally, it has a rhomboidal shape. On the glans there are 2 pairs of strong thorns and 2 pairs of more reduced spines. The stylus has also thin spines.

The glans of the penis for the studied sample could be compared only with the profile drawing of the penis of the holotype done by Dumitrescu in 1973 (Dumitrescu,

1973). Because Dumitrescu's drawing and text are confusing - 2 pairs of thorns ("teeths") in the text description and apparently 3 pairs of thorns in the profile drawing, the comparison of the penis sample with Dumitrescu 1973 is inaccurate.

*The ovipositor* is 1.1 mm long. It is covered with longer and denser hairy structures at the level of its bifurcation. The seminal receptacles look like vesicles with a complicated three-plane structure and thus, their arrangement differs from the descriptions of Gruber & Martens (Gruber & Martens, 1968).



Figure 1. Paranemastoma genitalia: a – the penis glans spinulation areas in *Paranemastoma silli* (redrawn after Avram 1973 a); b –penis glans spines pattern in *Paranemastoma silli* of the studied population; c – penis glans spines pattern in *Paranemastoma ancae* of the studied population; d – the seminal receptacles of *Paranemastoma ancae* of the studied population

## 4. Carinostoma elegans (Soerensen, 1894)

Material examined from Tismana - Gorj County, leg. det. Anda Babalean

*The penis* is 1.4 mm length. The glans has on each side six thorns (teeth - like saw teeth or shark teeth). On the tip of the smaller branch of the glans there are 2 spines; the same characteristic at the basis of the stylus.

As resulted from the arachnological available literature the number of the thorns on the penis glans varies: 6 thorns – Starega 1976 (Starega, 1976), 8 thorns – Martens 1978 (Martens, 1978), 8 thorns – Šilhavý 1956 (Šilhavý, 1956) for subspecies *Carinostoma* 



Figure 2. Nemastomatidae genitalia in the studied populations: a – Nemastoma transsylvanycum – the penis glans; b – Nemastoma transsylvanicum – the seminal receptacles; c – Carinostoma elegans – the penis glans; d – Carinostoma elegans – the seminal receptacles; e – Mitostoma chrysomelas – the penis glans; f – Mitostoma chrysomelas – the seminal receptacles.

elegans ornatum. The number of thorns may vary in the three subspecies: Carinostoma elegans (Soerensen, 1894), Carinostoma elegans batorligetiensis (Szalay, 1951), Carinostpma elegans ornatum (Hadzi, 1940).

*The ovipositor* is short - 0.4 mm in length with 2 visible rings having big spines. The edges of the ovipositor are irregular. The seminal receptacles are very complicated, arranged along multiple planes.

## 5. Mitostoma chrysomelas Hermann, 1804)

Material examined from Răchita - Timiș County, leg. det. Anda Babalean

*The penis* measures 1.3 mm. The glans is a little more dilated, thicker than the body and asymmetric, with spines. The stylus has the aspect of a pipe.

The most relevant taxonomic features used in Romanian Mitostoma species identification are the dorsal pattern of squares, the penis and mostly the penis glans and in a lesser extent to none the seminal receptacles. The pattern of the dorsal carrenae (squares

delimitated by denticles) of Romanian Mitostoma species and subspecies: *M. chrysomelas romanicum* (Avram, 1965), *M. dumitrescui*, *M. orghidani* (Avram, 1969), *M. moldavicum*, *M. helenae*, *M. rodnae* (Avram, 1970) is highly variable, thus not recommended to use this character in species delimitation, as Avram herself mentioned in her 1970 paper (Avram, 1970). Also Martens 1978 (Martens, 1978) consider that all Mitostoma species described by Avram should be placed under *Mitostoma chrysomelas* variability. Also Šilhavý 1939 (Šilhavý, 1939) has analyzed the variability on *Nemastoma chrysomelas* group.

As regarding the penis, the form of the penis glans is too much complicated and possible (and apparently) also variable (e.g. is variable in a population of *Mitostoma moldavica* (Avram, 1970). For this reason, in author opinion, the glans of the penis is not solely a very good taxonomic feature. For *Mitostoma dumitrescui* the penis is distinct by the indentations on it body (Avram, 1969) and so, different of *Mitostoma chrysomelas* and all other Avram Mitostoma species.

The ovipositor measures 0.7mm - 0.8mm and it is bifurcated, the fork being brown, whereas the rest of the ovipositor is light-colored. It is covered with small excrescences and setae. On the fork the setae are bigger. The seminal receptacles are placed under a chitin sheath having the form of elongated horseshoes. There are 2 pairs of sheaths, one pair for each branch of the fork. The receptacles have horseshoe shape. The sheaths covering the seminal receptacles originate on the tip of the ovipositor. Unfortunately, the shape of the seminal receptacles varies but they are described only for few species, most probably not on a large number of individuals, thus the variability of the seminal receptacles cannot be discussed.

#### CONCLUSIONS

The variability of the penis spinulation was extended by the presence of one spine on area II for *Paranemastoma silli*.

Considering the spines pattern of the penis glans, *Paranemastoma ancae* perfectly ranges within Avram variability (4) of *Paranemastoma silli*.

The penis of *Nemastoma transsylvanicum* – holotype should be completely and correctly redrawn.

A careful analysis of the 3 varieties (subspecies) of *Carinostoma elegans* is required – searching the dorsal pattern, penis and seminal receptacles of the ovipositor.

A systematic revision of Avram Mitostoma species is required.

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# CONTRIBUTIONS TO THE STUDY OF THE GENITALIA OF SOME OPILIONIDS (ARACHNIDA, OPILIONES) FROM ROMANIA – PART II PHALANGIOIDEA

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Keywords: seminal receptacles, Phalangiinae, Oligolophinae, Leiobuninae

## ABSTRACT

The work is studying the external genitalia of 9 Opilionid species belonging to Suprafam. Phalangioidea collected from Romania. For some species there were noticed differences from other descriptions given in the literature as regarding the form and position of the seminal receptacles. The penis is the most stable taxonomic feature for all studied species.

### **INTRODUCTION**

In Romania, external genitalia of Phalangioidea Opilionids were studied by Avram, mostly while describing new species and by Dumitrescu who offered data regarding the external genitalia of some Opilionids belonging to Gyantinae, Oligolophinae and Phalangiinae subfamilies.

## MATERIAL AND METHODS

The 9 opilionid species were collected from the south-west region of Romania, from localities which are to be mentioned for each species.

#### **RESULTS AND DISCUSSIONS**

1. Phalangium opilio Linnaeus, 1761

Material examined from Vulcan – Hunedoara.

The penis measures 3.0 - 4.2 mm in length and is dumb-bell like. The glans is flat, triangle like. On the superior part of the glans the 2 setae described in the literature by Martens (Martens, 1978) were not observed. The penis is yellow and the broad edges of the body are brown.

*The seminal receptacles* are slightly chitinised. They have the form of a long, slightly curved tube to which is added an almost spherical vesicle, hardly noticeable and sometimes invisible. The smaller vesicle presents minor variations in the descriptions of other authors: with thick walls (Martens 1978), clearly visible (Starega 1976, Šilhavý 1956).

2. Opilio parietinus (De Geer, 1778)

Material examined from Valea Mânastirii - Gorj.

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The penis is 2.7 - 3 mm in length. Its body is flat, wide in the posterior region and narrowing progressively towards its anterior part only to widen again before the glans. Compared to the penis body, the glans is oriented downwards under a sharp angle and is flanked by 2 membranes colored in black. The glans is pyriform, ending in a thin and relatively short stylus. On the glans, on each side, near the stylus there are 2 setae.

The seminal receptacles are hardly noticeable, even after the xilol-fenic acid treatment, because of the dark colour of the ovipositor. On each side of the internal vagina there is a long tube with thickened walls. On the superior part appear to be 2 vesicles partly overlapped. Starega (Starega, 1976) and Šilhavý (Šilhavý, 1956) describe one clear larger vesicle and a smaller triangular vesicle less visible.

3. Opilio saxatilis C. L. Koch, 1839

Material examined from: a) male - Ostroveni - Dolj; b) female - Bistret - Dolj

The penis measures 1.6 - 2 mm, it is flat, shaped like a dumb-bell. Anterior to the region of the glans, the body is dilatated and has 2 holes between which there are 2 lines of hard, sharp setae.

*The seminal receptacles* have the form of a tube with thick walls. In the upper part there is an oval vesicle, while Starega (Starega, 1976) and Šilhavý (Šilhavý, 1956) describe two unequal vesicles.

4. Rilaena triangularis (Herbst, 1799)

Material examined from Piscul Sadovei - Dolj.

*The penis* measures 2.7 mm in length, with the body being light-yellow and the stylus black. The profile of the penis has the form of the figure 7. On the glans there are two setae.

*The seminal receptacles* are made up of a long, thin tube, with very thin walls and with the distal extremity slightly dilated. The length of the tubes is unequal even in the case of the same ovipositor. Some samples present a second inferior vesicle emerging from the vagina interna, much smaller, hardly perceptible, of unknown significance.

5. Lophopilio palpinalis (Herbst, 1799)

Material examined from Mihăilești - Buceș, Hunedoara

*The penis* is 1.4 mm length. The body is flat and wide. The glans has 2 setae on each of its sides. The stylus is black. The penis is light-yellow except for its anterior part which is yellow-brown.

*The seminal receptacles* have the shape of a curved tubes with thick walls in Martens description (Martens, 1978) and thin walls in the studied sample. A second smaller vesicle has been described by Starega (Starega, 1976) and Silhavy (Šilhavý, 1956). The seminal receptacles present a slight variation within the same ovipositor, being asymmetric.

6. Egaenus convexus (C. L. Koch, 1835)

Material examined from Bucovăț - Dolj.

The penis is 4 - 4.5 mm in length and has a flat and wide body which narrows towards the glans. The glans is oval, elongated and small. The stylus is thin. The body of the penis is yellow-brown and the lateral edges colored in dark-brown.

The penis of *Egaenus carpaticus* Avram 1978 (Avram, 1978) has the same aspect as *Egaenus convexus*.

*The seminal receptacles* have the aspect of a short, oval, elongated vesicle on each side of the internal vagina. Silhavy (Šilhavý, 1956) described a spherical organ with a fine granular structure and having an unknown role. This organ couldn't be observed in any of the studied individuals.



Fig. 1 Penis and seminal receptacles in: a – Phalangium opilio penis, b – Phalangium opilio seminal receptacle, c – Opilio parietinus seminal receptacles, d – Lophopilio palpinalis seminal receptacles, e – Rilaena triangularis seminal receptacles, f – Opilio saxatilis seminal receptacles, g – Egaenus convexus seminal receptacles, h – Oligolophus tridens penis, i – Oligolophus tridens seminal receptacles, k, l – Mitopus morio seminal receptacles

7. Oligolophus tridens

The penis is 3.2 - 3.3 mm. On each side of the tip of the glans there are two long setae. The penis varies in different descriptions, the glans being more or less slender, with one or two pairs of setae.

*The seminal receptacles* are tube like with an upper dilatation presenting slight variation of it length and form.

8. Lacinius dentiger (C. L. Koch, 1848)

Material examined from Perişor - Dolj.

*The penis* is 3 mm long. The glans is very flat laterally, triangle shaped, slightly concave in its superior part and very convex in the inferior part.

*The seminal receptacles* are long, tubular, made up each of a long, curved tube with slight sinuosity. A second receptacle, much smaller, triangle or oval vesicular shaped may emerge directly from vagina interna or from the tubular receptacle.

9. Mitopus morio (Fabricius, 1799)

Material examined from Rânca - Parâng Mountains and Vulcan Mountains.

The penis is 2.2 - 2.4 mm in length. The inferior half of the penis body is broader, with parallel edges; it narrows up to the level of the glans where it dilates again. The glans has the shape of a triangle and on its ventral side there is a transparent vesicle. On the glans, near the stylus there can be 2 small setae. The glans is yellow. The penis body up to the glans, the glans tip and the stylus are brown-black.

*The seminal receptacles* are tubuliformes. The arachnological literature describes a long, slightly sinuous tube on each side of the vagina interna and one spherical vesicle (Starega, 1976; Dumitrescu, 1970; Šilhavý, 1956) or two spherical vesicles (Martens, 1978) in the upper region of the tubuliform receptacles. The seminal receptacles are variable in the 2 analysed populations, Vulcan and Rânca: 2 spherical upper vesicles in Parâng sample and only one spherical vesicle in Vulcan sample.

## CONCLUSIONS

- 1. The penis is the most stable taxonomic feature for all studied species.
- 2. Species delimitation should always be done considering the areal and all taxonomic features of intergradante populations.

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Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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## DETERMINATION OF THE FLIGHT CURVE FOR *EURYTOMA SCHREINERI* SCHR. IN CLIMATIC CONDITIONS OF S.C.D.P. VÂLCEA

Bancă Gheorghe¹

Key words: entomofauna, beneficial species, harmful species

#### ABSTRACT

In our country, the wasp seed of plum it is considered as a major pest for the plum orchard. Taking into account the loss of production due to the damage produced by this pest, during 2009-2011, at SCDP Valcea, we made research on the biological cycle of the Eurytoma schreneri species. According to our research the earliest flight has been recorded on 26 April 2010 and the latest on 8 May 2009. This activity was spread over 30 days (in 2009) and 34 days (in 2011).

#### INTRODUCTION

In Romania, plum is the most appreciated and widespread species of fruit growing orchards and remains the leader because he owns share in area under cultivation and production of fruits as well for other attributes.

The plum tree plantation often cannot highlight the genetic potential of production, since many organisms have a restrictive role. Of these, the complex of pathogens and pests has the greatest importance (Seulean, Rodica, 2012).

Worldwide, the plum is attacked by more than 93 species of pests belonging to the following order: *Acari* Order 6 species; *Heteroptera* Order 4 species; *Homoptera* Order 16 species; *Coleoptera* Order 21 species; *Hymenoptera* Order 1 species; *Lepidoptera* Order 33 species; *Diptera* Order 1 species; *Rodentia* Order 2 species (Ţucă O. 2006). Among them is the plum seed wasp *Eurytoma Schreineri* Schr.

Waspy seed plum (*Eurytoma schreineri* Schr.) was first described in Russia by Schreiner (1908) as a pest fruits of plum (*Prunus domestica* L.) in the region Astrachan (T. Georgescu, 2006).

*Eurytoma schreineri* Schr. sistematically framed in *Insecta* Class, *Hymenoptera* order, *Clistogastra* suborder, *Chalcidoidea* suprafamily, *Eurytomidae* family.

Female body is 4-6 mm long, black head and thorax covered by whitish piliferous. First pair of wings is dark. The abdomen is short pedunculate, egg-shaped, smooth and has a yellowish, retractile ovipozitor. The male body is less long with the length 4-5 mm, long and hairy antennae. On mezotorax presents a steeped punctuation. Abdomen is longer than in female, rounded (Perju, Peiu, Nicorici, 1980) (I. Oltean and col., 2001).

The egg is milky white, ovoid, with a long pedicel. The larvae to complete development are 6-7 mm long and legless. Body color is white, capsule cephalic slightly yellow and brown color mandibule. Larvae body is arched and sharp at both ends.

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Pupae are 4-7 mm long. By transforming the adult passes through several phases: white nympha, white nympha with pink eyes, white nympha with pink eyes and feet started, nympha half black with legs and wings initiated, nympha with black legs, wings and antennae released (Georgeta Teodorescu and col., 2003).

In our country, *Eurytoma schreineri* Schr. has one generation per year and wintering in the form of fully developed larvae in infested fruit pits. In spring when the average temperature reaches atmospheric 9-10°C, the larvae turn into pupae, the observations, it was determined that this time corresponds to the time when the forsythia bloom species, respectively mid-March to the first decade of April.

#### MATERIALS AND METHODS

During 2009 - 2011 at the S.C.D.P. Valcea we have made research on the life cycle of the species *Eurytoma schreineri* Schr.

To determine the parameters of the biological cycle of the species *Eurytoma screineri* Schr. in the climatic conditions in S.C.D. P. Valcea we have made research and observations in natural conditions in the orchard.

During observations were taken into account plum phenology and climatic conditions.

After reaching the temperature that constitutes the insect biological threshold  $(10^{\circ} \text{ C})$ , there has been installed the yellow traps. Readings of the traps were made once a week, and captured wasps were removed from the trap.

## **RESULTS AND DISCUSSION**

Taking into account the loss of production due to the damage produced by this pest, during 2009-2011, at SCDP Valcea, we made research on the biological cycle of the *Eurytoma schreneri* Schr. species.

From Table 1 it is clear that adult flight start when has been reach an amount of effective temperature of about  $62.1 \degree \text{C} - 72.6 \degree \text{C}$  (from  $10 \degree \text{C}$  thermal threshold).

According to our research the earliest flight has been recorded on 26 April 2010 and the latest on 8 May 2009. This activity was spread over 30 days (in 2009) and 34 days (in 2011).

During this period were captured 243 adults in 2009, 227 adults in 2009 and 256 adults in 2011.

Maximum flight curve was recorded after 12 to 18 days after the onset of flight (12 days in 2009, 17 days in 2010 and 18 days in 2011), after achieving an amount of active degrees of about 140  $^{\circ}$  C -160  $^{\circ}$  C (Figure 1).

Last adults emerged from infested seeds were reported after achieving an amount of about 246 active degrees ° C -255 ° C, on 26 May 2010, and 6 June in 2009 and 2011.

Knowing this aspect is of particular importance in scheduling chemical control of this pest.

Taking into account the biological cycle it is known that the insecticides were effective only on the adult stage. Given the relatively long duration of *Eurytoma schreineri* Schr. adults flight, that impose applying of two chemical treatments.

Table 1

Date	2009		2010		2011	
	Σ Effective temperature (° C)	Adults	Σ Effective temperature (° C)	Adults	Σ Effective temperature (° C)	Adults
26.04.			72,6	4		
01.05.			92,4	12		
08.05.	65,2	2	111,4	14	89,5	9
15.05.	75,2	14	146,1	25	127,0	18
22.05.	136,2	30	225,8	7	143,8	34
28.05.	179,9	21			187,3	14
06.06.	251,4	1			246,3	3

Quantification of the collected adults of Eurytoma schreineri Schr. species in climatic conditions from S.C. D.P. Valcea (2009 - 2011)



Figure 1. Flight curve of *Eurytoma schreineri* Schr. in climatic conditions from S.C.D.P. Vâlcea (2009 – 2011)

#### CONCLUSIONS

According to our reseach regarding the flight curve of the species *Eurytoma schreneri* Schr. the earliest flight has been recorded on 26 April 2010 and the latest on 8 May 2009. This activity was spread over 30 days (in 2009) and 34 days (in 2011).

During this period were captured 243 adults in 2009, 227 adults in 2009 and 256 adults in 2011.

Maximum flight curve was recorded after 12 to 18 days after the onset of flight (12 days in 2009, 17 days in 2010 and 18 days in 2011), after achieving an amount of active degrees of about 140  $^{\circ}$  C -160  $^{\circ}$  C.

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Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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## HISTO-ANATOMICAL STRUCTURE OF THE ABOVEGROUND STEM AND LEAF OF VITEX AGNUS-CASTUS L. (VERBENACEAE) SPECIES

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Keywords: Vitex agnus-castus L., aboveground stem, leaf, histo-anatomy, structure

### ABSTRACT

This paper presents the histo-anatomical researches on the aboveground stems and leaves of Vitex agnus-castus species, for the reason of pharmacognostic expertise development. It also highlights the peculiar pharmacological importance of Agni casti fructus, a natural product officinal in European Pharmacopoeia.

#### **INTRODUCTION**

*Vitex agnus-castus* L., chaste tree, chasteberry, monk's pepper, *Verbenaceae* family, is a Mediterranean shrub, 1–3 m tall, naturalized elsewhere. In Romania, it is cultivated as an ornamental plant (*Vitex*, 2013).

For therapeutic purposes, flowers and especially fruits harvested at maturity are used. Officinal in the European Pharmacopoeia, *Agni casti fructus* product contains different active principles such as iridoids, *e.g.* aucubin and agnuside (Bruneton, 2009; Högner *et al.*, 2013), essential oil (Bruneton, 2009; Marongiu *et al.*, 2010), flavonoids (Bruneton, 2009; Högner *et al.*, 2013), labdane-type diterpenoids (Ono *et al.*, 2011), lignans (Zhou *et al.*, 2009; Ahmad *et al.*, 2010), fatty acids (Liu *et al.*, 2004), ecdysteroids (Sena Filho *et al.*, 2008; Bruneton, 2009), sterols, catechic tannin, simple carbohydrates, proteins, enzymes, organic acids, vitamins, mineral salts (Bruneton, 2009; Chen *et al.*, 2011).

The medicinal product *Agni casti fructus* has a wide range of pharmacological properties: emmenagogue, galactagogue, sedative, anaphrodisiac, antibacterial (Liu *et al.*, 2004; Bruneton, 2009; Yaghmaei *et al.*, 2012), antioxidant (Sağlam *et al.*, 2007), antitumor, anti-inflammatory, enzyme inhibitory (Zhou *et al.*, 2009; Ahmad *et al.*, 2010; Li *et al.*, 2013). It inhibits prolactin secretion of rat pituitary cells both *in vitro* and *in vivo* (Tamagno *et al.*, 2007).

Agni casti fructus standardized extracts are recommended for the regulation of hormonal balance in premenstrual syndrome (Ambrosini *et al.*, 2013), dysmenorrhea, amenorrhea, oligomenorrhea (van Die *et al.*, 2013), hyperprolactinemia (Tamagno *et al.*, 2007), infertility, acne, menopausal disorders (Danielle *et al.*, 2005). In Africa, India and Southeast Asia, extractive preparations from the flowers of some *Vitex* species are used for their contraceptive effect (Bruneton, 2009).

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Regarding *V. agnus-castus* histo-anatomy, in the specialty papers there are scarce and incomplete data (Rao, 1996). For this reason and because of the medicinal importance of the species, our research is important in the development of pharmacognostic expertise.

#### MATERIAL AND METHODS

The vegetal material was harvested from *V. agnus-castus* plants in blossom, in May 2013, from "Alexandru Buia" Botanical Garden, University of Craiova, Dolj County, Romania.

The fixation and preservation of vegetal material (aboveground stems and leaves) were achieved in 70% alcohol. The cross sections were obtained by manually sectioning using botanical razor.

After washing with distilled water, the cross sections were subjected to the clarification process using 10% sodium hypochlorite (Javel water). Then, the clarifying agent was removed from the cross sections by washing with distilled water. Congo red–chrysoidine mixture was used for the cross sections staining. Depending on the chemical composition of cell membranes, various stains are induced by the reactive: pink to red for cellulose, yellow for suberin, brown for lignified membranes (Andrei & Paraschivoiu, 2003).

Stained and mounted cross sections were analyzed on a Krüss binocular photon microscope at diverse objectives ( $\times$ 4,  $\times$ 10,  $\times$ 40) and then photographed on a Soligor SR 300 system adapted to the microscope.

According to some classical authors (Toma & Rugină, 1998), the description of microscopic cross sections was achieved.

### **RESULTS AND DISCUSSIONS**

# Structure of aboveground stem aged one year

In cross section, the aboveground stem aged one year exhibits a four-rib shape. Following the histological analysis, it was found that the stem has a secondary structure produced by libero-ligneous cambium.

From the outside to the inside of the cross section, the following histological sequence was observed:

Epidermis is made of a single layer of small cells, antero-posterior flattened, with thin radial walls and thickened internal and external tangential walls. Tangential external wall is bulged and covered by a thick cuticle with protruded striations. At the level of epidermis, are found numerous sharp unicellular tector hairs, long bicellular tector hairs, round glandular hairs and stomata.

The bark is organized into two distinct zones: an external area consisting of 4–5 cell layers of angular collenchyma and an internal area of cortical parenchyma with oval cells having various dimensions and accumulating reserve substances.

In the inner part of the cortical parenchyma, arched caps of periphloemic sclerenchyma are found.

The secondary phloem tissue includes sieve tubes, phloem parenchyma and annex cells with reserve substances. Multicellular, uniseriate, cellulosic medullary rays traverse it. The primary phloem tissue is poorly developed with few sieve tubes.

The libero-ligneous cambium is made from anteroposterior-flattened cells with corrugated walls.

The secondary xylem tissue is dominant, consisting of large caliber vessels of secondary xylem stacked radial and accompanied by libriform tissue. At this level, uniseriate medullary rays are lignified.

Primary xylem vessels are few and accompanied by xylem parenchyma and annex cells. At the level of primary xylem, the medullary rays are cellulosic.

In longitudinal sections, it was observed that secondary xylem vessels have spiral, reticular and curly thickenings.

Meatus-type medullary parenchyma is well represented (Figures 1-5).



Figure 1. Cross-section through *V. agnus-castus* aboveground stem. Congo red–chrysoidine staining, ×40. Overview.



Figure 2. Cross-section through V. agnus-castus aboveground stem. Congo red–chrysoidine staining, ×100. 1 – Epidermis, 2 – Glandular hair, 3 – Tector hair, 4 – Angular collenchyma, 5 – Cortical parenchyma, 6 – Sclerenchyma cap, 7 – Phloem tissue.



Figure 3. Cross-section through *V. agnus-castus* aboveground stem. Congo red–chrysoidine staining, ×400. 1 – Secondary xylem vessel, 2 – Libriform tissue, 3 – Primary xylem vessel, 4 – Medullary parenchyma.



Figure 4. Cross-section through *V. agnus-castus* aboveground stem. Congo red–chrysoidine staining, ×400. 1 – Reticular xylem vessel, 2 – Curly xylem vessel, 3 – Spiral xylem vessel.



Figure 5. Cross-section through *V. agnus-castus* aboveground stem. Congo red–chrysoidine staining, ×400. 1 – Bicellular tector hair, 2 – Glandular hair, 3 – Epidermis.

### Leaf's limb structure

In cross section, the leaf's limb shows a plain shape slightly prominent at the median rib's level to the abaxial side.

The upper epidermis is made of a single layer of heterodiametric cells, anterosuperior flattened, with thin radial walls and thickened internal and external tangential walls. Tangential external wall is bulged and covered by a thick cuticle. At this level, there are many sharp, erect unicellular tector hairs.

A single layer of hypodermis is located below the upper epidermis. It is made up of large, heterodiametric cells.

The mesophyll is uniformly, made of 5–6 layers of palisade tissue. The first cell layers consist of large prosenchymatic cells, perpendicularly placed, ordered on the upper epidermis, generating small intercellular spaces. The other layers of palisade cells are smaller, prosenchymatic. At the ribs' level, into the mesophyll, an assimilatory sheath surrounds libero-ligneous conducting fascicles. The plant is of C4 type. Into the secondary ribs, cellular pillars of cellulosic type support the conducting fascicles.

The lower epidermis is made of a single layer of small heterodiametric cells, anterosuperior flattened, with thin radial walls and thickened internal and external tangential walls. At this level, numerous articulated, inflected bicellular tector hairs and stomata are found.

The leaf's limb has equifacial structure from hypostomatic type.

At the level of the median rib, is found one libero-ligneous conducting fascicle supported by a cellular pillar of cellulosic type to the upper epidermis and by an angular collenchyma above the inferior epidermis (Figures 6–9).



Figure 6. Cross-section through *V. agnus-castus* leaf's limb and median rib. Congo redchrysoidine staining, ×100. Overview.



Figure 7. Cross-section through *V. agnus-castus* leaf's limb. Congo red–chrysoidine staining, ×400. 1 – Superior epidermis, 2 – Hypodermis, 3 – Palisade parenchyma, 4 – Libero-ligneous conducting fascicle, 5 – Inferior epidermis, 6 – Bicellular tector hairs.



Figure 8. Cross-section through *V. agnus-castus* leaf's limb. Congo red–chrysoidine staining, ×400. 1 – Palisade parenchyma, 2 – Libero-ligneous conducting fascicle, 3 – Assimilatory sheath, 4 – Inferior epidermis, 5 – Bicellular tector hairs.



Figure 9. Cross-section through *V. agnus-castus* secondary rib. Congo red–chrysoidine staining, ×400. 1 – Superior epidermis, 2 – Palisade parenchyma, 3 – Sustaining pillar, 4 – Libero-ligneous conducting fascicle, 5 – Inferior epidermis, 6 – Bicellular tector hairs.

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#### **Petiole structure**

In cross section, the petiole exhibits a plain shape to the adaxial side and a semicircular shape to the abaxial side.

From outside to the inside of cross section, the following histological sequence is revealed:

The epidermis is made by a single layer of small cells with thickened external and internal tangential walls and thin radial walls. At this level, numerous unicellular sharp and bicellular tector hairs are found.

The angular collenchyma of 3–4 cellular layers is located under the epidermis. Foliar parenchyma is of meatus-type.

Three libero-ligneous conducting tissues are observed. To the adaxial side, two small libero-ligneous fascicles flanking a large arcuate fascicle are found centrally disposed. Two large sclerenchyma caps are evidentiated into the arched opening of the fascicle.

Xylem tissue consists of large xylem vessels radially arranged and separated by slightly sclerified medullary rays.

The phloem tissue consists of sieve tubes, annex cells and phloem parenchyma (Figures 10-12).



Figure 10. Cross-section through *V. agnus-castus* petiole. Congo red–chrysoidine staining, ×40. Overview.



Figure 11. Cross-section through *V. agnus-castus* petiole. Congo red–chrysoidine staining, ×100. 1 – Adaxial conducting fascicle, 2 – Central conducting fascicle, 3 – Sclerenchyma cap, 4 – Foliar parenchyma.



Figure 12. Cross-section through *V. agnus-castus* petiole. Congo red–chrysoidine staining, ×400. 1 – Xylem vessel, 2 – Medullary ray, 3 – Phloem tissue.

### CONCLUSIONS

The histo-anatomical research on one-year aged aboveground stems and leaves of *Vitex agnus-castus* was carried out. The stem exhibits four-rib shape and secondary structure generated by libero-ligneous cambium. The leaf's limb shows a plain shape slightly prominent at the median rib's level to the abaxial side. It has equifacial structure from hypostomatic type. The petiole displays plain shape to the adaxial side and semicircular shape to the abaxial side. In cross section, three libero-ligneous conducting tissues are observed.

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## HISTO-ANATOMICAL RESEARCHES ON PETASITES HYBRIDUS (ASTERACEAE) SPECIES

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Keywords: Petasites hybridus, histo-anatomy, researches

#### ABSTRACT

In this paper, the authors highlight the results of the histo-anatomical researches on the roots, stolons, rhizomes and leaves of Petasites hybridus species, especially for the pharmacognostic and possible toxicological expertise of the medicinal product Petasitidis rhizoma. In addition, other pharmacologically active extracts obtained from P. hybridus have been considered.

#### **INTRODUCTION**

*Petasites hybridus* (L.) P. Gaertn., B. Mey. et Scherb., butterbur, common butterbur, bog rhubarb, *Asteraceae* family, is a perennial herbaceous species, of Eurasian origin, commonly found along watercourses and groves (Ciocârlan, 2000).

From the phytochemical point of view, *P. hybdridus* contains a variety of active principles such as essential oil, eremophilane-type sesquiterpenes like petasin and related derivatives (Istudor, 2001; Chizzola *et al.*, 2006; Aydın & Letzel, 2013), pyrrolizidine alkaloids (Aydın & Letzel, 2013), benzofuran compounds (Khaleghi *et al.*, 2011), polyphenolic acids (Jaiswal *et al.*, 2011), mucilages, pectins, tannin, inulin, simple carbohydrates, proteins, enzymes, amino acids, mineral salts (Istudor, 2001; Aydın *et al.*, 2013).

*P. hybridus* medicinal extracts have different pharmacological actions: spasmolytic in bronchial asthma, asthmatiform bronchitis, allergic rhinitis and some colics (Istudor, 2001; Kälin, 2003; Lee *et al.*, 2004; Dumitru *et al.*, 2011), anti-inflammatory and gastroprotective (Thomet *et al.*, 2001; Fiebich *et al.*, 2005), neuroprotective and sedative for neurovegetative dystonia (Istudor, 2001) and migraine prevention (Oh *et al.*, 2005; Sutherland & Sweet, 2010), vasorelaxant (Wang *et al.*, 2010), cytotoxic and anti-tumor (Bodensieck *et al.*, 2011), anti-androgenic (Lin *et al.*, 2000).

In the specialty papers, there are very few and incomplete data on *P. hybridus* histoanatomy (Haratym & Weryszko-Chmielewska, 2012). This was the first reason of our study. The medicinal properties of the species and its possible toxicological implications, because of pyrrolizidine alkaloids and sesquiterpenes content, represent the second motivation of our research.

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### MATERIAL AND METHODS

The vegetal material was collected in May 2013 from *P. hybridus* plants in blossom, in Lainici village, Gorj County, Romania.

Fixation and preservation of the vegetal material (roots, stolons, rhizomes, leaves) were made in 70% alcohol. Obtained by manually sectioning using botanical razor, the cross sections were washed with distilled water, then undergoing a clarification process for the removal of cell content, employing 10% sodium hypochlorite solution (Javel water). After that, the cross sections were washed with distilled water to eliminate the clarifying agent. The cross sections were stained with Congo red–chrysoidine mixture. The stains induced by the reactive depend on the chemical composition of cell membranes: pink to red for cellulose, yellow for suberin and brown for lignified membranes (Andrei & Paraschivoiu, 2003).

Stained and mounted cross sections were studied on a Krüss binocular photon microscope at different objectives ( $\times 4$ ,  $\times 10$ ,  $\times 40$ ) and then photographed using a Soligor SR 300 system adapted to the microscope.

The explanation of microscopic cross sections was performed in the manner of some classic authors (Toma & Rugină, 1998).

The analysis of stomatal index was made on a Nikon Eclipse 55i binocular photon microscope coupled with a Nikon DS–Fi1 high definition video camera, using ×40 objective (corresponding on 0.037 mm² area). Image acquisition and processing were performed using ImageProPlus ver. 6.0 software package. For each bottom, middle and upper area of the examined leaf's limb, the average of 10 determinations were taken into consideration.

### **RESULTS AND DISCUSSIONS**

**Root structure**. In cross section, the root shows a round shape and a primary structure. From the outside to the inside, the following histological sequence is observed: exodermis formed by 2–3 cell layers with suberin-impregnated walls; exfoliated rhizodermis; cortical parenchyma consisting of 7–8 orderly stacked cell layers; a single layer of endoderm provided with Caspary's thickenings; central cylinder protected by a single pericycle layer; conductive tissues organized into simple xylem and phloem bundles alternative arranged on a circle (Figure 1).



Figure 1. Cross-section through *P. hybridus* root. Congo red–chrysoidine staining,  $\times 400$ . 1 – Cortical parenchyma.

**Stolon structure**. In cross section, the stolon exhibits a round shape and a secondary structure generated by the presence of intrafascicular and interfascicular libero-ligneous cambium.

Epidermis consists of a single layer of small cells, slightly flattened, with slightly thickened internal and external tangential walls and thin and sinuous radial walls. External walls are bulged and covered with a thin cuticle. In some areas, the occurrence of protective suberose tissue is observed.
The bark is organized in a single layer of hypodermis, followed by numerous layers of meatus-type cortical parenchyma interrupted at intervals by aeriferous spaces.

Libero-ligneous conducting fascicles from collateral-opened type are arranged on a circle, with old and new alternating fascicles produced by interfascicular libero-ligneous cambium. They are protected to the outside by groups of cellulose fibers. Cellulosic medullary rays are multicellular and multiseriate. Medullary parenchyma from meatus-type is well represented (Figures 2–4).



Figure 2. Cross-section through *P. hybridus* stolon. Congo red–chrysoidine staining, ×100. 1 – Epidermis, 2 – Hypodermis, 3 – Cortical parenchyma, 4 – Conducting fascicle.



Figure 3. Cross-section through *P. hybridus* stolon. Congo red–chrysoidine staining, ×100. 1 – Epidermis, 2 – Suberose tissue, 3 – Hypodermis, 4 – Cortical parenchyma, 5 – Aeriferous space, 6 – Conducting fascicle.



Figure 4. Cross-section through *P. hybridus* stolon. Congo red–chrysoidine staining, ×100.

1 – Cortical parenchyma, 2 – Phloem tissue, 3 – Libero-ligneous cambium, 4 – Xylem tissue, 5 – Medullary parenchyma.

**Rhizome structure**. In cross section, the rhizome shows a round shape and a secondary structure generated by intra- and inter-fascicular libero-ligneous cambium. From the outside to the inside, the following histological sequence is observed:

Epidermis is made of a single layer of small cells, slightly flattened, with slightly thickened internal and external tangential walls and thin and sinuous radial walls. External walls are bulged and covered by a thin cuticle with protruded striations.

The bark is arranged in a single layer of hypodermis, followed by numerous layers of meatus-type cortical parenchyma.

Libero-ligneous conducting fascicles from collateral-opened type are disposed on a circle. They are protected to the outside by thick sclerenchyma caps. The phloem tissue is made by sieve tubes, phloem parenchyma and fewer annex cells. Secondary xylem vessels are accompanied by libriform tissue and primary vessels by xylem parenchyma. Medullary rays are cellulosic, multicellular, and multiseriate. Medullary parenchyma from meatus-type is well represented (Figures 5–7).



Figure 5. Cross-section through *P. hybridus* rhizome. Congo red–chrysoidine staining, ×100. Overview.



Figure 6. Cross-section through *P. hybridus* rhizome. Congo red–chrysoidine staining, ×400. 1 – Epidermis, 2 – Striated cuticle, 3 – Hypodermis, 4 – Cortical parenchyma.



Figure 7. Cross-section through *P. hybridus* rhizome. Congo red–chrysoidine staining, ×100. 1 – Cortical parenchyma, 2 – Sclerenchyma cap, 3 – Phloem tissue, 4 – Liberoligneous cambium, 5 – Xylem tissue, 6 – Medullary ray, 7 – Medullary parenchyma.

**Structure of leaf's limb**. In cross section, the leaf's limb presents a plain shape slightly prominent at the level of median rib.

The upper epidermis is composed of a single layer of large cells, flattened, with thickened internal and external tangential walls and thin radial walls. External walls are bulged and covered by a thick cuticle.

The mesophyll is uniformly, made of palisade parenchyma. Under the upper epidermis, the palisade parenchyma is composed of three layers of more elongated cells, while the following layers have smaller cell size, but placed in the palisade. At the mesophyll bottom, large aeriferous spaces are found.

Assimilatory sheaths surround libero-ligneous fascicles from mesophyll, so the species is of C4 type.

The lower epidermis consists of a single layer of small cells. At this level, anomocytic stomata and numerous long tector hairs are observed.

The leaf's limb has equifacial structure and hypostomatic, anomocytic type.

In the cross section, the median rib presents a single libero-ligneous conducting fascicle, centrally placed and supported on two pillars of angular collenchyma at the apical and basal pole (Figures 8 and 9).

Compared to medicinal product *Farfarae folium*, which may be confused in certain situation, common butterbur leaf is distinguished both by lower thickness of leaf's limb and pubescent on the both sides, and the stomates morphology and stomatal index, respectively 7.1–7.4 for *Tussilago farfara* against 10.6–10.8 for *P. hybridus* (Istudor, 2001; Ergen Akçin, 2007) (Figure 10).



Figure 8. Cross-section through P. hybridus leaf's limb. Congo red–chrysoidine staining, ×100. 1 – Superior epidermis, 2 – Mesophyll, 3 – Conducting fascicle, 4 – Inferior epidermis, 5 – Angular collenchyma pillar.



Figure 9. Cross-section through *P. hybridus* leaf's limb. Congo red–chrysoidine staining, ×100. 1 – Superior epidermis, 2 – Mesophyll, 3 – Conducting fascicle, 4 – Aeriferous spaces, 5 – Inferior epidermis.



Figure 10. Inferior epidermis of *P. hybridus* leaf's limb. Congo red–chrysoidine staining, ×400. 1 – Anomocytic stomata.

**Petiole structure.** In cross section, the petiole has a semicircular shape with lateral-adaxial wings. From outside to the inside of petiole, the following histological sequence is evidentiated:

A single layer of small cells with thin radial walls makes epidermis; other walls are slightly thickened. The external walls are covered by a thin cuticle.

The angular collenchyma is well developed on the adaxial wings.

The meatus-type fundamental parenchyma is well developed.

Libero-ligneous conducting tissues are organized into 8–10 large bundles placed on a semicircle and each adaxial wing contains other two small bundles. The big bundles exhibit sclerenchyma periphloemic caps (Figures 11–13).



Figure 11. Cross-section through *P. hybridus* petiole. Congo red–chrysoidine staining, ×40. Overview.



Figure 12. Cross-section through *P. hybridus* petiole. Congo red–chrysoidine staining, ×100. 1 – Epidermis, 2 – Angular collenchyma, 3 – Cortical parenchyma, 4 – Sclerenchyma cap, 5 – Conducting fascicle.



Figure 13. Cross-section through *P. hybridus* petiole. Congo red–chrysoidine staining, ×100. 1 – Conducting fascicle, 2 – Medullary parenchyma.

## CONCLUSIONS

Some histo-anatomical researches on *Petasites hybridus* species were performed. The root has a round shape and a primary structure. The stolon and the rhizome show a round shape and a secondary structure generated by the presence of intra- and interfascicular libero-ligneous cambium. The leaf's limb has equifacial structure and hypostomatic, anomocytic type. The petiole has semicircular shape with lateral-adaxial wings and well-developed meatus-type fundamental parenchyma.

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# ECOPHYSIOLOGICAL ASPECTS REGARDING THE ARGINESTI – GURA MOTRULUI FOREST

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*Key words: forest, photosynthesis, transpiration, water content* 

## ABSTRACT

In Arginesti- Gura Motrului forest, the predominant woden species are:. Quercus petrea, Fraxinus angustifolia, Acer campestre, Quercus robur.

Tall trees are making accentuated shading at the level of inferior floors from the forest. Shrubs and herbaceous plants have, because of this reason, a pronounced shadow character. The compensation point of light has low values, from the plants that were studied. The best adapted at low intensities of light shrub is Cornus mas.

## **INTRODUCTION**

The Argineşti-Gura Motrului Forest is located on the right side of the Jiu 's Meadow, on the European Road E 70, between localities Filiaşi and ButoiestI. To the north it borders with Gura Şuşiţa forest, to the east with the Jiu River, to the south with agricultural lands which separates it from Gura Motrului and to the west with agricultural lands which separates it from Jugastru village( Sîrbu Anca et all , 2007).

The south-east of the forest is located at the confluence of the Jiu and Motru rivers. During the period when rains are abundant, low-lying areas are flooded, so that excluding dry periods, almost all year there are puddles and dales where the water persist

In the forest, the predominant woden species are:. Quercus petrea, Fraxinus angustifolia, Acer campestre, Quercus robur.

The research conducted in spring-sumer 2013 in the Arginesti-Gura Motrului forest was aimed to establish eco-physiological particularities of some woody species, particular emphasis was placed on the influence of light and water factors that play an important role in forest ecosystems. It started from the premise that components of forest ecosystems that live in communities are living under different conditions of isolation. Because they coexist in large numbers in limited areas, there were established mutual relations between them in which competition for light, water and minerals plays a decisive rol. Phenomenon occurs when the association is heterogeneous competition better endowed individuals by stealing a larger share of reserves of water or nutrients, all these factors influencing physiological processes going (Parascan D., 1983).

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General information about the vegetation of Motru Valley are known in researches of Grecescu 1898, Enculescu 1923, Borza, Calinescu, Maria Celan, Paşcovschi, Ana Paucă, Pop, Evdokia Puşcaru-Soroceanu 1960 cited by Costache, 2011)

Being located in the southwest of the Getic Plateau, the investigated area is located in the central-european climate regime with sub-mediterraean influences. After Köppen's classification, the investigated area falls into the cold wet climate with specific conditions of oak (after Buia et al., 1961, cited by Costache, 2011).

The research, conducted in April-June 2013, was made of the physiological processes in woody species: *Cornus mas, Crataegus monogyna* and *Acer tataricum*.

In our country, ecophysiological researches over different species from forest ecosystems were realized by Pauca Comanescu M et al. 1980, Tacina Aurica, Pauca Comanescu Mihaela 1992, Murariu A. et al. 2000, 2002, 2006 cited by Stratu, 2011), Stratu Anisoara , 2011. Ecophysiological researches were made over some species from Masaroviskova E, Elias P, 1980, Goryshina, 1983.

## MATERIAL AND METHODS

On the biological material were determined: the intensity of leaf transpiration, the respiration intensity, the compensation point of light, the intensity of photosynthesis, the content of assimilatory pigments and the water content. Transpiration, photosynthesis and respiration in leaves were determined using LCi portable device.

The compensation point of light (light intensity value that photosynthesis is equal with respiration) was after determining photosynthesis at different light intensities. The results were compared with the intensity value of the dark respiration.

The chlorophyll content of leaves was determined with the Minolta portable chlorophyll meter (SPAD units).

The water content and the dry matter were gravimetrically determined by maintaining the biological material to a temperature of 105°C until reaching a constant weight.

## **RESULTS AND DISCUSSIONS**

The diurnal variation of photosynthesis showed one peak: at 2-3 pm hours and a significant decline from 7 pm (Figure 1). The highest value was observed from *Cornus mas*.

Regarding the content of assimilating pigments, measurements were made at different times. The graphical data representing the average of these measurements (Figure 2).

The maximum chlorophyll pigments content have had *Crataegus monogyna* plants and the lower content of chlorophyll occurring in *Cornus mas*.

The diurnal variation of transpiration was determined in May 2013, at different hours of the day. From the data given in Figure 3 shows a maximum process at noon and a minimum in the early morning. The highest diurnal fluctuations of transpiration are found at *Acer tataricum*.

Leaf respiration intensity was determined with the Lci analyzer, covering assimilation chamber with tinfoil paper to prevent light penetration and realization the process of photosynthesis.

It was necessary to know this parameter to be determined then the compensation value of the point of light for each of the species studied.

Measurements were performed in May 2013 at 26 ^oC. From the Figure 4, it appears that *Cornus mas* leaves have the lowest respiration.

In the forest, after the leaves emergence of tall trees, the lower vegetation shading was accented. Because the young trees and shrubs can't accumulate biomass if do not get enough light, was considered important to know the lower limit of growth that must be situated above the compensation point of light (CP).

The knowing of CP value shows a great importance because under this value, plants don't produce organic substances anymore, and they live using their own resources.

For finding the value of the compensation point of light, there were made determinations of photosynthesis at different intensities of light. Obtained values were compared with the values of respiration intensity.



Figure 1. The diurnal variation of photosynthesis ( $\mu$ molCO₂/m²/s)



Figure 2. The content of chlorophyll (SPAD units)

Obtained dates show that all plants that were studied have the CP at low intensities of light, behaving like shadow plants. The highest value of CP was registered at *Acer tataricum* (450lx), and the lowest values at *Cornus mas* (200 lx). *Crataegus monogyna* have the compensation point of light at 400 lx. The dates are consistent with these of Atanasiu L., 1988, who found CP at 300 lx.



Figure 3. The diurnal variation of transpiration (mmol  $H_2O/m^2/s$ )



Figure 4. The intensity of leaf respiration ( $\mu$ molCO₂/m²/s)

The water content is an important indicator of the water regime, the water being necessary for carrying out all the physiological processes. The highest variations of the water content are seen in leaves because the leaves realize the biggest contact surface with the atmosphere. In the leaves, the water content present values between 61, 34 % and 73,28. The highest values of the leaves water content were registered to the *Cornus mas*, and the lowest content to the *Crataegus monogynya* (Figure 6).



Figure 5. The intensity of photosynthesis  $(\mu molCO_2/m^2/s)$ 



Figure 6. The water content (%) of leaves

## CONCLUSIONS

The obtained results reveal specific variation of the physiological indicators for the investigated species.

The highest value of photosynthesis was observed from *Cornus mas* plants. The highest values of the leaves water content were registered to the *Cornus mas*, and the lowest content to the *Crataegus monogynya* 

Obtained dates show that all plants that were studied have the compensation point of light at low intensities of light, behaving like shadow plants.

The leaf respiration intensity has the lowest value at *Cornus mas* leaves, and the highest diurnal fluctuations of transpiration is found at *Acer tataricum*.

The maximum chlorophyll pigments content have had *Crataegus monogyna* plants and the lower content of chlorophyll occurring in *Cornus mas*.

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# STUDY REGARDING THE CONTENT OF CARBON OXIDES IN CRAIOVA MUNICIPALITY

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Keywords: monitoring, sources of pollution, carbon monoxide, urban and rural background

## ABSTRACT

One of the most common pollutants and widely distributed in the atmosphere is the carbon monoxide, which is present both in the atmosphere as well as inside buildings. Global emissions of carbon monoxide in the atmosphere were estimated to be 2600 million tons, of which about 60% are derived from human activities and approximately 40% of natural processes (Dan & Dan, 2002). Ambient concentrations measured in urban areas depend largely on the number of vehicles in traffic (Rudolf, 1994), their capacity, type of fuel used and last but not least the weather and topography. In this context carbon monoxide was monitored in several type stations: urban, industrial, and regional, between December 2012 and January, April 2013. There were observed variable concentrations of CO all week due to heavy traffic in the city. The concentration of CO decreases on weekends when traffic is low and does not exceed the upper limit. If we compare the values recorded at monitoring stations, they are barely visible beyond the permissible CMA (Billa, Işalniţa) and in traffic areas and urban background concentrations recorded approaching limit, compared with Breasta (regional background).

## **INTRODUCTION**

Environmental pollution is one of the most current and most important of mankind. In recent years scientific researches have shown that the chemical structure of the atmosphere is changing due to natural or anthropogenic causes, attention being focused on the impact of human activity on the atmosphere (Fernandez-Bremauntz & Ashmore, 1995). Humankind through their activities contribute to increasing the amount of polluting gases released into the atmosphere, thus contributing to global warming, ozone depletion and many other disorders of the natural environment. The main substances that contribute to air pollution are sulfur and nitrogen oxides, chlorofluorocarbons, carbon dioxide and monoxide, these being only some of the billions of tons of pollutants that generates each year industrial development, which affects aquatic ecosystems and ground when the contaminants are dissolved in water and precipitated in the form of acid rain (Dor et al., 1995).

Carbon monoxide is formed primarily by the incomplete combustion of fossil fuels (coal, oil, etc.). Other sources of carbon monoxide are iron and steel production, petroleum refining, and air traffic (Zhang, 1999). Carbon monoxide can accumulate to dangerous levels especially in the case of calm atmosphere or in enclosed spaces (e.g. underground parking, combustion in enclosed spaces). Carbon monoxide produced from natural sources is quickly dispersed over a large area, without putting human health at risk (Gavrilescu,

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2007). Carbon monoxide is a toxic gas, being lethal in high concentrations by reducing the oxygen carrying capacity of blood due to the permanent connection with hemoglobin in the blood, affecting the respiratory system and cardiovascular system (Bascom, 1996). At relatively low concentrations, carbon monoxide affects the central nervous system, weakens the heart rate, thus reducing blood volume distributed in the body, reduce visual acuity and physical ability, a short exposure can cause acute fatigue, may cause shortness of breath and pain chest of people with cardiovascular disease, cause irritability, headaches, rapid breathing, lack of coordination, nausea, dizziness, confusion, reduced ability to concentrate (Russell et al., 1999). The segment of the population most affected by exposure to carbon monoxide is represented by: children, the elderly, people with respiratory and cardiovascular diseases, anemic people, smokers (Simon, 1999). The total quantity of CO formed all over the world due to human activity is 30% of the total content of CO in the atmosphere. Average residence time of CO in the atmosphere is about 6 months.

#### MATERIAL AND METHODS

In the period 2012-2013, carbon monoxide was monitored in many stations, as follows: Calea Bucuresti (traffic), City Hall (urban background), Billa (industrial background) Isalnita (industrial background), and the town Breasta (rural background). In December 2012, January and April 2013, Billa area was monitored daily, being located near a high-traffic intersection. The measurements were performed using a portable gas detector Oldham (Model MX-Plus Multigas, France) equipped with electrochemical sensors.



Figure 1. Craiova Traffic Map

#### **RESULTS AND DISCUSSIONS**

In Craiova, transport activities are an important source of pollution (65-80%). By analyzing the results there is observed a variation in the carbon monoxide content during the weeks and low levels especially on Saturdays and Sundays. However, in the third and fourth week traffic was intensified, especially small cars. Around the holidays, the traffic was intensified and on holidays it was reduced, which is reflected in Figure 2.



Figure 2. Variation of CO in December 2012

Higher levels of CO were influenced by low temperatures, leading to greater use of thermal heating for homes and social buildings. In the first two weeks of January the concentration of carbon monoxide was reduced due to traffic, being back to normal in the third and fourth week. The first five days of the week are more crowded (higher content of carbon monoxide) than Saturdays and Sundays, when the level is low (Derwent & Middleton, 1995), (Figure 3).



Figure 3. Variation of CO in January 2013

In April, in the first and second week, the concentration of carbon monoxide is lower than the last two weeks, when there are higher concentrations. This can be explained due to heavy traffic on holidays. Saturdays and Sundays are observed approximately lower values. Analyzing monthly charts is observed that the average and maximum values exceeded the permissible CMA every month (figure 4).



Figure 4. Variation of CO in April 2013

As can be seen the majority of values are exceeding the allowed CMA (Billa, Işalnița), and for traffic areas and urban background concentrations recorded approaching limit, compared with Breasta (regional fund) where the concentration of carbon monoxide is 0.3 mg/m (Figure 5, 6.)



Figure 5. Average, maximum and minimum values of CO during the studied period



Figure 6. Average, maximum and minimum values of CO

## CONCLUSIONS

In Craiova Municipality, as in other major Romanian cities, transport activities are an important source of pollution, with a contribution of 65-80%. One of the main pollutants is represented by CO.

Study on the CO in Craiova Municipality reveals variable concentrations of CO all week due to heavy traffic in the city. The concentration of CO decreases on weekends when traffic is low and does not exceed the upper limit. In December, as in April, and near the holidays the traffic is more intense and reduced thereafter.

It was observed that the majority of values are exceeding the allowed CMA (Billa, Işalniţa) and for traffic areas and urban background, concentrations recorded approaching limit, compared with Breasta (regional background).

In compliance with measures set by the Government, to reduce pollution, including CO, is recommended: modernization and increasing public transport infrastructure to improve quality of service, safety and security of transportation, environmental quality meeting pollution standards for cars equipped with hybrid engines using diesel-electric or minimum Euro 5 - Euro 6 for vehicles as it is in many cities of the world.

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# STUDY ON SOIL QUALITY FOLLOWING THE CLOSURE OF A LANDFILL

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Keywords: waste, landfill, soil quality, monitoring.

#### ABSTRACT

In this paper we aimed to establish soil quality following the closure of a landfill without contradicting the law. Soil samples were taken within the landfill and there were monitored the following indicators: pH, conductivity, moisture, TDS and bicarbonate content and heavy metals.

The soil samples analyzed have not been found impressive exceeding the maximum permissible concentrations, but there is noticed the absence of humus, and a weak alkaline soil, sign of the presence of sodium carbonate or sodium exchangeable (absorbed into the soil complex). Without quantifiable effects, the former landfill from Balş can affect the environment, namely vegetation, fauna, landscape, the human factor. The influence may be directly or indirectly, by means of the three vectors: air, water, soil.

## **INTRODUCTION**

In general due to lack of facilities and poor exploitation, landfills are among the recognized generating impact and risk to the environment and public health. Urban landfills are often sources of groundwater pollution by nitrates and nitrites, and other pollutants. Both infiltrations from landfills and water leakage on the slopes influence surrounding soil quality, which is consequential on their usage (Căpăţână & Simonescu, 2006).

By its position, the nature and role, the soil is a component of the biosphere and the product of the interaction between biotic and abiotic environment, representing a specific area of concentration of living organisms, their energy, metabolism and decomposition products. Soil degradation is manifested by the degradation of both quantitative parameters, and the deterioration of the quality parameters. The environment is negatively affected by uncontrolled and irregular deposits and contaminated soils (Rojanschi et al., 1997).

Leachate causes the pollution of the soil, surface water and groundwater. The soil which is an important component of landfill site is a media where polluted materials are deposited. Because of continuous transportation to other media (air, ground and surface water) from this media by evaporation, erosion and infiltration, this component is a natural

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source which is needed to carefully monitor (Banar et al., 2009). In this content, soil characterization (organic matter, moisture content of the soil and pH of the soil, etc.) is very important on the amount and distribution of pollution (Taoeli, 2007; El-Fadel et al., 2002; Daskalopoulos et al., 1997; Tchobanoglous et al., 1993). Since leachates are one of the potential sources of groundwater pollution, monitoring heavy metal content in dumpsite can facilitate to recommend suitable remedial measures (Esakku et al. 2003).

There is a long history association between metals and human development. Heavy metal pollution not only affects the production and quality of crops, but also influences the quality of the atmosphere and water bodies, and threatens the health and life of animals and human being (Kumar, 2008). Almost any material will produce leachate if water is allowed to percolate trough it. The quality of leachate is determined primarily by the composition and solubility of the waste constituents. If waste is changing in composition, for example due to weathering or biodegradation, then leachate quality will change with time. This is particularly the case in municipal waste landfills containing municipal waste (SEPA, 2003).

The main routes of human exposure to soil metals are ingestion, inhalation and skin contact. Since soil is the major sink for airborne metals, the measurement of their levels in this media is useful to establish trends in abundance and their consequences because of natural and anthropogenic changes (Kasassi et al., 2008).

### MATERIAL AND METHODS

The landfill is located in the north - east section of Balş, about 1 km from the first houses, being situated on a swampland. Waste landfill of the city Bals has existed for over 30 years, being opened in 1969. The technology of maintenance and operation of the landfill was relatively simple and consisted of garbage and road leveling and compaction with equipment specific to these types of works.

Bals city is located approx. 25 km from Slatina, on both sides of the river Olteţ, Caracal field. From the morphology, the analyzed geo-morphological unit belongs to the Romanian Plain, at the limit of the Romanian Plain and Oltenia Plain. Soil samples were taken to determine the current state of the environment and the impact of the Balş landfill functioning for over 30 years. Soil samples were taken within the landfill and there were monitored following indicators: pH, conductivity, moisture, TDS and bicarbonate content and heavy metals.

In this regard, the location of sampling points and number of samples were set as follows:

- Sample 1 was collected on the E side of the landfill;

- Sample 2 was collected from the S side of the landfill;

- Sample 3 was collected from the N side of the landfill;

- Sample 4 was collected from the W side of the landfill.

Samples were collected from depths of 0-20 cm and 20-40 cm, and determined indicators were compared with a sample taken from unpolluted soil outside the landfill, 250 m N.

The analyzes were performed in the Laboratory of Quality of the Environment within the Department of Biology and Environmental Engineering of the Faculty of Agriculture and Horticulture. For analyzes there were used the following equipment: Hanna pH meter, conductivity meter Hach, DR2010 spectrophotometer. The heavy metal concentration present in soil was analyzed using atomic absorption spectrophotometer Avanta PM.

## **RESULTS AND DISCUSSIONS**

From the physicochemical characteristics of the soil sample, it was observed that the pH value of the soil sample was slight acid to slightly alkaline in nature with the range from 6.35 to 7.89 ( see figure 1 ).

Found values of pH indicator shows a slightly alkaline soil, sign of the presence of sodium carbonate or exchangeable sodium (complex absorption in the soil).

Reaction soil pH influences the physiological groups of microorganisms, each microorganism and microbial activity showing an optimum activity at certain pH values. Values are observed both weak acid and weak alkaline compared to the control sample which has a neutral value.

Absence implies poor soil humus inappropriate to human activities that are based on its exploitation.



Figure 3. TSS



Regarding the conductivity is observed in 2 of the 4 samples analyzed its values are close to the values determined in control sample, the rest presenting lower values (figure 2). In case of total soluble salts, the values measured in control sample are between 70.24 and 67.24 mg/kg, while at 3 of the samples is observed higher values, and only at sample 4-W, the values measured are lower than control sample (figure 3).

The moisture content of the soil samples ranged from 13, 07 to 23 % with lowest value of 13, 07 % and 14, 3 corresponding to a soil sample location. The results indicated that the moisture content was lower in the first depth (0-20 cm) in all the locations.

Regarding the moisture measured for control sample, it shows higher values compared to the other samples, of 27% for 0-20 cm depth and 28% for the 20-40 cm depth (figure 4.).





Figure 5. Analysis of bicarbonates

Figure 6. Variation of heavy metals concentration in samples

Analysis of bicarbonates in soil reveals that the values determined at the 4 samples are close to the control sample values (figure 5).

In conclusion, at the previously indicators analyzed, conductivity, total salts, moisture and bicarbonates, there were not reported abnormal situations

In the case of heavy metals one can observe a significant increase in samples taken from the inside of the landfill compared with the control sample taken outside, somewhere a normal growth if we considering waste disposal. From the perspective of heavy metals are, there is not a significant exceeding of the maximum concentration (figure 6).

In conclusion it can be appreciated that soil environmental factor, in terms of fertility characteristics there is an imbalance due to household waste storage and not only.

## CONCLUSIONS

At the soil samples analyzed were not found impressive exceedances of the maximum permissible concentrations, but there is no humus, so that once the landfill closed, the soil can not be used in agriculture, but forestry and tree planting, specifically surface rooted tree, bushes or grassing.

Also, there is found a weak alkaline soil, indicative of the presence of sodium carbonate or sodium exchangeable (absorbed into the soil complex). Soil reaction influence physiological groups of microorganisms and nitrifying bacteria that usually operate in soils with neutral or slightly acid reaction.

Without having quantifiable effects Bals city landfill can affect the environment, namely vegetation, fauna, landscape, the human factor. The influence may be directly or indirectly, by means of the three vectors: air, water, soil.

In addition may occur negative influence on the environment in the case of uncertain and future events.

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# PREPARATION OF CELL CULTURES FOR BIOCHEMICAL STUDIES IN GASTRIC ONCOGENESIS

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**Keywords:** *oncogenesis, gastric cancer, cell cultures* 

#### ABSTRACT

In the last 20 years in Europe, gastric cancer mortality reached third place (8.1% of cancer deaths in 2004), after lung and colorectal cancer. In Romania, gastric cancer mortality rate is 2 times higher than in any other EU countries. The aspect of the multifactorial etiology of gastric carcinomatosis is extremely broad and perhaps still incomplete at this time, but many pathological situations, have a precise role in the pathophysiology of gastric adenocarcinosis. Most studies that are currently performed by highly- researchers in laboratories with latest equipment rather have to detect the cause of sickness, its mechanism and of course have the ultimate goal, often unspoken, is to find a way to block the pathological circuit, to remove the trigger factor of the disease for the development of the disease, in other words, finding a cure.

## INTRODUCTION

The progress of medical science in the last 20 to 25 years, in the field of oncology and was not only possible by conducting applied physiology studies, especially for the discovery of new drugs or new therapeutic approaches with potential cytostatic. In Romania (Valean Simona, 2008, Stewart B.W., 2003) gastric cancer mortality rate is 2 times higher than in other EU countries with 16.67 deaths/100 000 inhabitants/year for men and 8.6 deaths /100 000 inhabitants/year for women (with an average of 10.8 % of all cancer deaths). These studies were performed mainly in cell cultures taken from the malignant tissue, which allowed the in vitro study of the mechanism of transport membrane, the receptor responsible for the intercellular exchange of the cycle study of proteins, hormones and enzymes responsible for initiating and maintenance kariokinetic process. The same studies show that since the 70, studies have been conducted on cell lines grown timid on blocking or intensification of differentiation and cell phenotyping. Although the study results were encouraging and the magnitude of cell culture method, only in the 80's some researchers started to "grow cells" derived from human gastric cancer (Christian L. Laboisse, 2008). As we have made more and more scientific observations, more relevant data was gathered through the '90 studies of cell cultures, including human gastric cancer has had a great increase. At this time, they drew some lines on requirement, that there are at least 3 criteria to validate the accuracy of the cell line used in this industry: first, cultured cells must be derived by sampling the original cancer and not metastatic, secondly the cell

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line must be identified unambiguously by immunological methods, cytogenetic and highlighting specific Markers (Christian L. Laboisse, 2008). Such cell cultures could be achieved even in poorly differentiated gastric adenocarcinoma, so with specific histamine H2 receptors in normal cells could be highlighted all other characters of growth, morphology and karyotype of primary gastric tumor cells (Stewart B.W., 2003).

#### MATERIALS AND METHODS

In the current study, we used two commercial cell lines (SW 1353 and K -562) were purchased from American Type Culture Collection, USA, and a human cell line, HGT -1 gastric adenocarinoma ETABL of the according to standard procedures. Working with HGT- 1 cell lines was achieved through direct work performed in the laboratory of the Helsinki University, Haartman Institute, Helsinki Finland, under the guidance of Prof. Univ Dr. Leif Andersson, head of research grup in Pathology Department where the study was conducted in vitro being the subject of this paper. Cells were collected from gastric tumors localized on the posterior wall of the stomach without evidence of metastases intraoperative local or remote. Histological examination highlight the type of cancer was poorly differentiated adenocarcinoma. Culture medium was conducted on an agarose subsequently transferred buffer enriched with 25 mw 4 - (acid 2 - hydroxyethyl) -1- piperazine ethanesulfonic and addition of 10% FBS (fetal bovine serrum) and antibiotics. Tumor samples were disintegrated with 0.07 % collagenase, and the resulting suspension was placed on the agarose in petri dishes of 66 mm diameter at temperature of 50 degrees Celsius, overnight. 10 days later colonies were obtained first; the efficiency of cloning was verified by scoring duplicate plates. A line was left in the incubator for subculture (with environmental DME, inactive FBS, heat- agarose and 10% triptozo -phosphate). After a period of 15 days of incubation we obtained a subculture cell lines. The cell lines thus obtained were pipetted in the container in which cells are able to develop and grow in the liquid medium in which the feeding state for 7 days. After 42-57 passages, we collected cells in culture to undergo HGT- 1 for the determination of the growth curve. Two X- 85 cells were moved to Falcon bottle containing 5 ml of DME, enriched with 10% heatinactive FBS. 3rd and 6th day cells received fresh growth medium and every 24 hours were washed with buffer saline phosphate (Cl Na 8000, Cl K 200, Na2HPO4, 1500; KH2PO4, 200, pH 7.2). Were subsequently removed from the container with 0.25 % trypsin and 0.02 % EDTA. The suspension of cells was then placed in the chamber hemocitometer to be numerate and their viability was assessed by trypan blue exclusion. All procedures were done according to protocols in force (Christian L. Laboisse, 2008, Florence Reyl-Desmars, 1989). For the experiments cells were used at the same passage. HGT -1 is a human cell line of the same physiological characteristics as gastric parietal cells (Christian L. Laboisse, 2008, Florence Reyl-Desmars, 1989). Thus, HGT -1 monolayers of cells were able to secrete H + when they were stimulated with histamine (calculated change in the external pH, delta pH (e) 0.46 + / -0.05), treated with waterproof and fluorescent dye sensitive to pH 8 - hydroxypyrene -1, 3,6- tri-sulphonic acid tri-sodium salt (HPTS). Cell lines were cultured in Modified Minimum Essential medium (MEM) containing 10% fetal bovine serum (FBS), 2 mM glutamine and antibiotics (100UI/ml 100UI/ml penicillin and streptomycin). The cells were cultured in flasks for cell culture and was kept in an incubator wetting at 37 ° C, 95 % O2 and 5 % CO2. In the case of experiments for the present study, the cells were lysed in lysis solution [150 mM NaCl, 20mM Tris - HCl (pH 7.5), 1% NP40, 1 mM EDTA, 1 mM EGTA, 1 mM sodium ortovanadat mixture inhibitor of protease (Boehringer Ingelheim Co.), kept at 4 ° C]. The lysate was centrifuged (12,000 g) for 10 min at 4° C to remove insoluble components. Proteins were quantified using the Bio-Rad kit Dc. Equal amounts of protein were separated on 10% SDS-PAGE gels and then transferred to Immo-bilion-P PVDF membrane (Millipore). The membranes were blocked with 5% milk powder dissolved in Tris saline containing 0.1 % Tween 20 (TBST), then incubated with primary antibody diluted in 1% skimmed milk powder diluted in TBST, will be incubated peroxidase- linked secondary antibody diluted in turn in 1% skimmed milk powder diluted in TBST. Subsequently, the ECL detection system was used for Western blot antibody determination in accordance with manufacturer's instructions. The films obtained were subsequently scanned and densitometry to determine the profile band optical density values . Scanning was performed using 1D LabImage software.

## **RESULTS AND DISCUSSIONS**

From the above, we can say that HGT -1 cells grown are developing the same main physiological characteristics similar to those of parietal cells and could represent an interesting cell culture model to study the mechanisms involved in regulating the secretion of gastric acid. Cell lines thus obtained, are used for various scientific research equipment Westwrn blot method as necessary as the first step (for more complex methods) for the detection and separation of proteins. The Western Blot technique is also referred to as immune-blotting and is a method which is based on three important steps which result in the separation and detection of proteins in a mixture. The first step is the separation of a mixture of proteins on a polyacrylamide gel (PAGE) sodium dodecyl sulphate (SDS). The mixture of proteins is subjected to SDS gel electrophoresis in order to identify all types of protein and reduce the possibility of DNA contamination. Subsequently they are deposited on a thin nitrocellulose membrane, which is able to bind most of the proteins, which is applied to the surface of the gel. Proteins are extracted from the gel using an electric field and then transferred to the surface of the membrane. In the second stage, the membrane is placed in a solution containing detection antibody specific for the protein under study. Only the band containing the protein binds to the antibody, forming a complex with it. The last step is the determination; the membrane is incubated with an enzyme- linked secondary antibody which binds to the primary antibody, thereby forming a complex of the antibody molecule. Detection is accomplished through enhanced chemi-luminescence (ECL, Amersham).

#### CONCLUSIONS

HGT -1 cell lines are particularly important for the study of morphological cells cloned to study chromosomal profile and especially to be appreciated properly the studied cell physiology. Thus, our cells derived from gastric adenocarcinoma as a primary tumor and not metastases gut has a clear origin. We utilised soft agar culture method (Florence Reyl-Desmars, 1989, Sarah B. Frazier, 2008, Wenjing Fang, 1992), that allows cloning of cellular [no fibroblast contamination risk is a major problem especially for type epithelial cell lines. These cells HGT -1 high clone profile in the future will allow us to test the longterm growth of cell lines allowing creation of pure masses similar to the primitive tumor. For this, it is imperative that a tumor to evaluate its stem cell contents of the baggage, before being used as an experimental cell lines in vitro. Many researchers have considered that the key of success is obtaining a high potential of cell lines to be cultured long-term in order to obtain tumor clones, is the systematic comparison of the stability of human cancer cloning efficiency of the cells in culture. Morphological studies have clearly demonstrated that epithelial nature of the cells HGT-1 (Wenjing Fang, 1992, Afratis, N., 2012) is expressed by the fact that mice inoculated can develop sometimes trabecular or glandular structures and even intercellular junctions. As a final conclusion, we can say that in the case of cell lines with long-term growth, particular care should be taken on how to characterize the line. Should avoid contamination with other types of cells, such as HeLa, but this is possible since, as is well known have never been isolated and usually increased in vivo (only rarely, and as discussed above), and other cell lines of human origin than type HGT - 1. So if all steps are followed correctly and applied in a culture of HGT -1 cell lines, will be have clear human tumor origin, specific karyotype for primary cancer, can be clearly differentiated from other cell lines based on distinct chromosomal marker, that have different kariotip than HeLa cells.

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## EDIBLE AND TOXIC MACROMYCETES FROM THE CĂPĂŢÂNII MOUNTAINS

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Keywords: macromycetes, edibility, toxicity, Căpăţânii Mountains, Romania

#### ABSTRACT

The researchs on the Căpăţânii Mountains mycobiota, concerning macromycetes, systematically conducted between 2003-2010, have materialized through the identification of 421 taxa (species, variety, forms) in the territory, belonging to 171 genera, classified in 66 families, 19 orders, 4 subclasses, 5 classes, 2 subphyla, respectively 2 phyla (Ascomycota and Basidiomycota). From the analysis of macromycetes conspectus and its reporting to the current literature it follows that, of the 421 identified taxa, a number of 146 taxa are edible, which is a percentage of 34.67% of all species identified in the investigated area, and 50 macromycetes taxa have more or less serious toxic effects.

#### INTRODUCTION

The Căpățânii Mountains has an area of about 900 km² and is the continuation towards the east of the Parang Mountains.

The Căpăţânii Mountain Flora and vegetation have been intensely investigated (Buia & Păun, 1956; Păun & Popescu, 1970, 1971, 1973; Popescu, 1974  $\rightarrow$  2005; Răduţoiu, 2006; Niculescu, 2006). The published data show that on the territory of these mountains vegetating approximately 900 cormophytic taxa (34 pterodophytic taxa, 9 taxa of gymnospermae and approximately 857 taxa of angyospermae).

Căpăţânii Mountains mycobiota's, especially concerning to macromycetes, has been sporadically studied before 2006, few species of macromycetes being presented in articles (Popescu et al. 2003) or PhD thesis (Răduţoiu, 2005 and Niculescu 2006). Our systematic fungal researches have been carried between 2003-2010 and have led to the identification of 421 taxa [417 species and 4 infraspecific taxa (varieties, forms), belonging to two phyla (*Ascomycota* and *Basidiomycota*), 5 classes, 66 families and 171 fungal genera]. These results have been capitalized through a series of articles (Ciortan 2008a, 2008b, 2009a, 2009b, 2010 and 2013) and from PhD thesis (Simion-Ciortan 2010).

Functional structure of Căpăţânii Mountains, which combines the traditional with modern occupations, based on the exploitation of some resources from major economic importance (coals, oil, gas, mineral waters, limestone, etc.), determines the anthropogenic impact which are strongly felt by exploiting plant resources (forests, grasslands) and of the minerals resources. Among the seasonal occupations hunting and fishing attract many amateurs' sportsmans, both on valleys and on the afforested slopes. They collect for marketing and personal use macromycetes species that, for many times, determines serious

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poisoning of the consumers, caused by the substances contained in the carpophores. When they reach in the digestive tract, produce the gastrointestinal disturbances, which prevent their absorption. Other things, much more dangerous, enters in the blood circulation and deteriorates the viscera (the liver and kidney) through a process that can take several hours and without symptoms, leading ultimately, to death. The accidents are caused by the incorrect identification of the species, but also due to the ability of ascoma and basidioma of edible fungi to accumulate toxic substances. Industrialization, and use of pesticides, has induced the transformation of many edible species into the toxic species. *Agaricus* sp., *Marasmius oreades* or *Macrolepiota procera* species and all macromycetes who come into contact with these substances are generally toxic. Toxic are becoming, also, the species which grow in the vicinity of roads with heavy traffic, industrial areas with emissions of the harmful products and contaminants into the atmosphere.

The theme of poisonous mushrooms was debated in numerous treaties and monographs (Heim, 1978; Bresinsky & Besl, 1985; Baier & Vančura, 1991; Teuscher & Lindequist, 1994; Stamets 1996; Flammer & Horak, 2003) [in Kreisel, 2005]. In Romania the theme was less addressed. The works as *Plante toxice din România* (Zanoschi et al., 1981) contains chapters or subchapters in which are presented and some toxic macromycetes. Şesan and Tănase (2004) have published a *Ghid de recunoaştere a ciupercilor comestibile şi toxice* into which, besides the description of the principals' toxic syndromes, shall be made comparative descriptions of toxic species, emphasizing the similarities and differences that might create confusion in the species recognition. Reference to the toxicity of the toxic macromycetes is also made in various papers, in Eliade and Toma (1977) atlas, in Sălăgeanu and Sălăgeanu (1985) identification manual, in *Ghid practic de micologie* (Pârvu 2007).

#### MATERIAL AND METHODS

The proper identification of taxa was done using papers (identification manuals, albums, atlases) developed by the Romanian mycologists (Eliade & Toma 1977; Sălăgeanu & Sălăgeanu 1985; Pârvu 1999, 2007; Şesan & Tănase 2004) and foreigners mycologists (Thiers 1975; Kühner & Romagnesi 1978; Demoulin & Marriott 1981; Orton 1984; Lotina Benguria 1985; Moser & Jülich 1990; Bon 1990, 1991, 1993, 1997, 1999; Canduso & Lanzoni 1990; Lannoy & Estades 2001; Laskibar & Palacios 2001, 2005; Gerault 2005; Palazón Lozano 2006).

To assess the macromycetes edibility from the territory we used data taken from the specialty literature: Heim 1969; Eliade & Toma 1977; Sălăgeanu & Sălăgeanu 1985; Lotina Benguria 1985; Fischer & Bessette 1992; Rothmaler 1994; Keizer 1998; Pegler & Spooner 1998; Laskibar & Palacios 2001, 2005; Dickson 2003; Fergus & Fergus 2003; Şesan & Tănase 2004; Kreisel 2005; Palazón Lozano 2006; Hall et al. 2007; Pârvu 2007; Tagliviani & Tagliviani 2009.

Nomenclature - the one used by Kirk (2010) in *Index fungorum - Species fungorum* and Kirk, Cannon, David & Stalpers (eds) (2008) in *Dictionary of the Fungi*, 10^{-th} edition.

## **RESULTS AND DISCUSSIONS**

**Edible species.** From the analysis of macromycetes conspectus and the current literature, from 421 taxa, 146 taxa are edible, which is a percentage of 34, 67% of the taxa alive identified in the investigated area. They nutritional value is, however, different. According to the classification system with three-stage developed by Sălăgeanu & Sălăgeanu (1985), they are thus distributed: 83 taxa (56, 84% of the total edible species)

shows mediocre edibility, 34 taxa (23, 28% of the total edible species) shows good edibility, and 29 taxa (19, 86% of the total edible species) shows very good edibility (table 1).

# Table 1

Species	Edible macromycetes ta:		es taxa	a from Capaçanii Mountain		S			
Species		Eulointy		species	Edibility				
Miterest and a sub-	X	XX	XXX		X	XX	XXX		
Mitrophora semilibera	•		-	Stropharia coronilia	•				
Morchella escuenta	-		•	Clitocybe costata	•				
Verpa bonemica	•				•				
Verpa digitaliformis	•			Clitocybe odora	•				
Peziza badia	•			Infundibulicybe geotropa	_	•			
Peziza varia	•			Leucocortinarius bulbiger	•				
Agaricus arvensis			•	Melanoleuca evenosa		•			
Agaricus bisporus			•	Melanoleuca grammopodia	•				
Agaricus campestris			•	Lenista flaccida	•				
Agaricus langei		•	•	Lepista juda	-		•		
Agaricus sulvaticus		•	•	Tricholoma myomyces		•	-		
Agaricus silvicola			•	Tricholoma vaccinum		•			
Rovista nigroscons			•	Rolatus apandiculatus	•		•		
Bovista nlumbea				Boletus chrysenteron			•		
Calvatia gigantea				Boletus edulis	-		•		
Chlorophyllum rhacodas	-			Boletus fachtnari			•		
Continus comatus				Boletus geotineri		•			
Custodarma amianthynum			•	Boletus luridus					
Laugagagiaus laugathitas				Polotus maging	•		-		
Leucouguncus ieucointies				Polotus regius			•		
Lycoperdon parlatum	•			Boletus reliculdius			•		
Lycoperdon pertatum	•			Boletus rubellus	•				
Lycoperaon pratense	•			Loopinum noou doooghmun	•				
Lycoperaon uirijorme	•	_		Leccinum pseudoscabrum	•				
Macrolepiota excortata	<u> </u>	•		Leccinum scabrum	•				
Macrolepiota mastolaea		•	_	Strobilomyces strobilaceus	•				
Macrolepiota procera			•	Chroogomphus helveticus	•	-			
Phaeolepiota aurea			•	Gomphidius glutinosus		•			
Amanita excelsa var. spissa	•			Gomphidius viscidus		•			
Amanita rubescens var. annulosulphurea		•		Gyroporus castaneus			•		
Amanita rubescens var rubescens		•		Hygrophoropsis	•				
		-		aurantiaca	-				
Amanita strobiliformis		•		Suillus granulatus			•		
Amanita vaginata	1	•		Suillus grevillei	•		-		
Amanita vittadinii	•			Suillus luteus	1		•		
Limacella guttata	-	•		Ramaria botrytis		•	-		
Pluteus cervinus var cervinus	•	-		Ramaria flava		•			
Pluteus petasatus				Auricularia auricula-	•				
1 mens perusanas	-			judae	1				
Volvariella bombvcina	1		•	Pseudohydnum	•				
				gelatinosum	1				
Volvariella gloiocephala	٠			Guepinia helvelloides	٠				
Cortinarius caperatus	1		•	Cantharellus cibarius	1		•		

Edible macromycetes taxa from Căpătânii Mountains

							1
Cortinarius multiformis	٠			Cantharelus cinereus		•	
Clitopilus prunulus			•	Cantharelus tubaeformis	•		
Entoloma clypeatum f. clypeatum		•		Craterellus	•		
				cornucopioides			
Fistulina hepatica	٠			Clavulina amethystina	•		
Laccaria amethystina		•		Clavulina cinerea	•		
Laccaria laccata	•			Clavulina coralloides		•	
Hygrocybe chlorophana	٠			Hydnum repandum		•	
Hygrocybe conica	٠			Laetiporus sulphureus	•		
Hygrophorus eburneus	•			Meripilus giganteus	•		
Hygrophorus lindtneri	•			Polyporus squamosus	•		
Calocybe gambosa			٠	Polyporus umbellatus	٠		
Lyophyllum connatum	٠			Sparassis crispa	٠		
Gymnopus dryophilus	٠			Albatrellus ovinus		•	
Marasmius oreades			٠	Lactarius aurantiacus		•	
Megacollybia platyphylla	٠			Lactarius deliciosus	•		
Rhodocollybia butyracea f.	٠			Lactarius lignyotus	٠		
butyracea							
Rhodocollybia butyracea f. asema	•			Lactarius piperatus	•		
Armillaria mellea		•		Lactarius rufus	•		
Flammulina velutipes	•			Lactarius salmonicolor		•	
Strobilurus esculentus	٠			Lactarius semisanguifluus		•	
Xerula longipes		•		Lactarius subdulcis	•		
Xerula radicata	•			Lactarius volemus	•		
Pleurotus cornucopiae		•		Russula aeruginea		•	
Pleurotus eryngii			٠	Russula alutacea	•		
Pleurotus ostreatus			٠	Russula aurea	•		
Pleurotus pulmonarius			٠	Russula atropurpurea	•		
Lacrymaria lacrymabunda	٠			Russula cyanoxantha		٠	
Psathyrella candolleana	٠			Russula delica		٠	
Agrocybe cylindracea			•	Russula mustelina		٠	
Agrocybe pediades	٠			Russula ochroleuca	٠		
Hypholoma capnoides	٠			Russula olivacea	٠		
Kuehneromyces mutabilis			•	Russula rosea	•		
Pholiota adiposa	•			Russula vesca		•	
Pholiota aurivella	٠			Russula virescens		•	

**Toxic species.** In Căpăţânii Mountains were identified 50 macromycetes species of toxic effects, more or less severe, that we present below according to the type of intoxication caused (table 2).

Has hypothesized, and circulating in the medical information and in the mass media from Romania, that the macromycetes may be hybridized in nature and so the poisoning may be explained at consumption of edible macromycetes species. However, this is an aberration, due to the fact that their sexual reproduction is extremely sophisticated. The hybridization involve the union of individuals that are distinguished by one, two or more pairs of allele genes (which determines one, two or more characters), understanding here specimens of edible species with specimens of the toxic species. The sexual reproduction of the macromycetes (somatogamia involve the union of two somatic haploid nuclei, with different sexual potentiality (+/-), components of the primary mycelium of some strains of the same species. This means that all cells of mycelium are sexualized, able to supplement the function of gametocyst and undifferentiated gametes. In this way, only through of the dykarion (following the merger of two compatible monocarions of the same primary mycelium) forms the ascoma and basidioma. It means that we can not speak of the combination of two cells with different potentials which come from two different primary myceliums, ie from the two different species. Cell hybridization to macromycetes is only performed *in vitro*, by mixed cultures of two types of strains.

However, the polluted, contaminated environment can help transform the edible mushrooms of some poisonous, due to the capacity of carpophores to absorb and accumulate a large quantity of harmful substances. In Romania, research on the involvement of fungi in the absorption of heavy metals were performed by Agoroaei et al. 2008; Butnaru et al. 2008; Tănase et al. 2008a, 2008b).

Table 2

Toxic macromycetes species from Căpățânii Mountains				
Syndromes	Species			
Phaloidian	Amanita phalloides and Galerina marginata			
Syndrome				
Gyromitrian	Gyromitra infula, Helvella crispa, H. elastica and H. lacunosa			
Syndrome				
Orellanian	Cortinarius orellanus, C. cinnamomeus and C. cinnabarinus, Lepiota			
Syndrome	castanea			
Muscarian	Amanita muscaria, Inocybe rimosa, I. erubescens, C. candicans and			
Syndrome	Mycena pura.			
Coprinian	Ampulloclitocybe clavipes, Coprinopsis atramentaria and			
Syndrome	Coprinellus micaceus			
Resinoidian Syndrome	Agaricus xanthodermus, Amanita gemmata, Boletus satanas, , H. sinapizans, Hypholoma fasciculare, H. sublateritium, Lactarius blennius, L. scrobiculatus, L. torminosus, L. turpis, Lepiota clypeolaria, L. cristata, Ramaria formosa, Russula emetica, R. fellea, R. foetens, R. queletii, R. fragilis, R. maculata, R. pectinata, Ramaria aurea, R. formosa, R. pallida			
Psihotropic-	Panaeolus papilionaceus var. papilionaceus, Panaeolina foenisecii,			
Narcoidian	Stropharia aeruginosa, Mycena pura, Pluteus salicinus			
Syndrome				
Pantherinian	Amanita pantherina, A. citrina, A. muscaria			
Syndrome				
Hemolytic	Amanita vaginata, A. rubescens, A. crocea, Gyromitra esculenta,			
Syndrome	Scleroderma bovista, S. cepa, S. citrinum, S. verrucosum			

There is no valid principle which would demonstrate that a macromycetes species is edible or poisonous. The only way to know if a mushroom is edible or poisonous is by the knowledge of the following set of characters of the carpophores, the only one able to decide:

➤ morphological features:

- macroscopic: type (ascoma, basidioma), shape, color, stipe, pileus, hymenophore (gills – lamellae, lamellulae, tubes, pores, folds), ring, volva, cortina, viscosity, scale, latex, etc;
- microscopic: spore (ascospore, basidiospore), asca, basidia, trama, cutis, cystidia, hypha, etc;

organoleptic features: smell, taste, tactile sensation;

> macrochimic features (trama or cuticle response to various chemical reagents and air).

The intoxications produced by fungi can be classified, according to the time of incubation (according to the time that elapses from mushroom ingestion to onset of the first clinical signs), into two main groups: severe and easy.

A. Sever intoxications have a large latency time; clinical signs appear between 6 and 24 hours after ingestion (generally between 9-15 hours). In this category are included the following syndromes: phaloidian (cyclopeptic), gyromitrian (hydrazinic) and orellanian.

1. Phaloidian Syndrome (hepatic, late coleriform, ciclopeptic intoxication, mycotism holeriform). The species that determine it: *Amanita phalloides, A. verna, A. virosa, Galerina marginata* and other (Lotina Benguria 1985) (See Table 2 for species found within area).

2. Gyromitrian Syndrome (helvellian, monomethyl hydrazine intoxication, mycetismus sanguinareus). The species that determine it: *Gyromitra esculenta*, *G. infula*, *Sarcosphaera crassa*, *Helvella crispa*, *H. elastica* (the recommended, however, to be edible in young stage after prolonged boiling), *H. lacunosa* (the recommended, however, to be edible in young stage after prolonged boiling) (Lotina Benguria 1985). (See Table 2 for species found within area).

3. Orellanian Syndrome (with renal manifestation) [called paraphalloidian intoxication by Heim (1978) due to the similarity of symptoms to those produced by the ingestion of species *Amanita phalloides*]. The species that determines it: *Cortinarius orellanus*, *C. orellanoides*, *C. splendens*, *C. vitellinus*, *C. sanguineus*, *C. semisanguineus*, *C. cinnabarinus*, *Lepiota helveolla*, *L. castanea* (Lotina Benguria 1985). (See Table 2 for species found within area).

B. Easy intoxications have a short latency time, clinical signs appear between  $\frac{1}{2}$  hour and 6 hours; are the most common. In this category are included the following syndromes: muscarian, coprinian, resinoidian, psihotropic), pantherian and hemolytic.

1. Muscarian Syndrome (sudorian, muscarinian, mycocolinergic). The species that determine it: *Amanita muscaria, Inocybe (I. adequata, I. rimosa, I. erubescens, I. argilacea), Clitocybe phyllophyla, C. candicans, C. ericetorum, Mycena pura* (Lotina Benguria 1985). (See Table 2 for species found within area).

2. Coprinian Syndrome (acetaldehydic, nitroidian or vascular/pseudoantabus). The species that determine it: *Ampulloclitocybe clavipes*, *Coprinopsis atramentaria* şi *Coprinellus micaceus* (last two indicated as edible in the early stages by Fergus & Fergus 2003), all present in the investigated area.

3. Resinoidian Syndrome (gastric intolerance syndrome (gastroenteryc/ acroresinic). The species that determine it: Agaricus xanthodermus, Amanita gemmata, Boletus satanas, Entoloma lividum, Hebeloma crustuliniforme, H. sinapizans, Hypholoma fasciculare, H. sublateritium, Lactarius blennius, L. scrobiculatus, L. torminosus, L. turpis, Lepiota clypeolaria, L. cristata, Omphalotus olearius, Ramaria formosa, Russula emetica, R. fellea, R. foetens, R. queletii, R. fragilis, R. maculata, R. pectinata, Ramaria aurea, R. formosa, R. pallida, Tricholoma pardinum (Lotina Benguria 1985). Excepting Entoloma lividum, Hebeloma crustuliniforme, Tricholoma pardinum and Omphalotus olearius, all other species were identified of Căpăţânii Mountains area.

4. Psihotropic-Narcoidian Syndrome (hallucinogen, cholinergic, PSL – Perspiration Salivation Lacrimation, mycetismus nervosus), produced by species forming psilocybin and psilocin. The species that determine it: the mushrooms of the genera

Panaeolus, Panaeolina, Stropharia (S. aeruginosa), Mycena pura [ingested in certain amounts can cause narcotic effects, tremor and temporary amnesia, perhaps hallucinatory effects (Pârvu 2007)] and Pluteus salicinus. In the territory of Căpățânii Mountain were identified: Panaeolus papilionaceus var. papilionaceus, Panaeolina foenisecii [after Stamets (1996) are not indicated as mycotoxin-forming], Stropharia aeruginosa [after Stamets (1996) suspect, but not known to be toxic-active] and Mycena pura (Stijve & Bonnard (1986) have not detected psilocybin or psilocyn in this species [Stamets 1996]). After Stamets (1996) species Pluteus salicinus is slightly too moderately active in terms of toxic activity. For this species Kuyper (1985) report a content of 0,05% psilocybin, without psilocyn and  $0 \rightarrow 0,008$  beocystin, and Christiansen et al. (1984) report 0, 35% psilocybin and 0,011% psilocyn (Stamets 1996).

5. Pantherinian Syndrome, atropinian syndrome, is also called because the symptoms that precede it are identical, generally, as those appearing after ingestion of solanine from *Atropa bella-donna*. The species that determine it: *Amanita pantherina*, *A. citrina*, *A. muscaria*, *A. regalis* (Lotina Benguria 1985). Excepting *A. regalis*, all other species were identified of Căpăţânii Mountains area.

6. Hemolytic Syndrome. The species that determine it: Amanita vaginata, A. rubescens, A. lividopallescens, A. crocea, Gyromitra esculenta, Scleroderma bovista, S. cepa, S. citrinum, S. verrucosum. A. crocea (Lotina Benguria 1985). Excepting Amanita lividopallescens and A. crocea, all other species were identified of Căpăţânii Mountains area.

#### CONCLUSIONS

Of the 421 macromycetes taxa identified in the Căpățânii Mountains, 146 are edible.

50 macromycetes species identified in the Căpățânii Mountains determine toxic effects.

Toxicity of macromycetes is genetic features.

Cell hybridization to macromycetes is only performed *in vitro*, by mixed cultures of two types of strains.

The polluted, contaminated environment can help transform the edible mushrooms of some poisonous, due to the capacity of carpophores to absorb and accumulate a large quantity of harmful substances.

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# UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# THE MICROBIAL LOAD OF THE AIR IN EDUCATIONAL SPACES

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Key words: air microflora, Penicilium spp., Cladosporium spp., Aspergillus spp

#### ABSTRACT

Air microflora is composed of very different organisms from soil, water, and living organisms belonging to all systematic categories: bacteria and bacterial spores, yeasts, mold spores, algae and protozoa.

In 2011 during October and November were made a study regarding the indoor air microbiological contamination in three rooms of University of Craiova. The research has been repeated in the same period October and November in 2012.

The Penicillium genra was the most frequently predominant followed by Cladosporium, Aspergillus, and Alternaria. Alternaria and Cladosporium spp are considered to be the most important allergenic fungi in outdoor air, while Aspergillus spp and Penicillium spp were recently recognized as important allergenic fungi in indoor air and generally from external sources.

### **INTRODUCTION**

Most people spend over 90% of their lives indoors: in houses, offices, and schools, where they are exposed to some indoor environmental factors (bioaerosol) that influence their health and physical condition (Abdel Hameed, A.A., 1999).

Therefore there has been a growing interest in indoor microbe studies in recent years. The aim of those studies is not only estimation of the airborne microorganisms but also their identification and the determination of factors influencing bioaerosol composition inside the rooms (Stryakowska-Sekulska, et al., 2007).

Biological contamination of indoor air is mostly caused by bacteria, moulds and yeast. They can be dangerous as pathogenic living cells but they can also secrete some substances harmful for health. These are different kinds of toxic metabolism products, for example mycotoxins (Flannigan, B., 2001, Aisey, D. et al., 2003, Pieckovae, K., 2002).

It is supposed that about 30% of health problems relevant to the indoor air quality is the result of a human organisms reaction to moulds (Gutarowska, B. and Jakubobowska, A., 2002). However, it can be noticed that during the last 20 years opinions concerning innocuous fungal spore amounts in the indoor air of various kinds of rooms have varied.

According to Berk et al., in 1979 exposure of 20 cfu/m3 to over 700 cfu/m³ has no harmful effect.

In recent years, a dramatic increase in the number of allergic reactions to fungal spores has been observed.

Young people, including students, constitute a large group of allergy sufferers; they experience the above-mentioned allergic symptoms throughout the year, but the

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symptoms intensify during spring and summer months (Jain, A.K., 2000). For that reason, regular monitoring of the indoor air quality in public buildings such as libraries, lecture halls, schools etc. is fully justified (Agnieszka Kalwasinska, et. al, 2012).

## MATERIAL AND METHODS

During October – November 2011 and October – November 2012, in three rooms belonging to the University of Craiova (C 005, C 263 and Computer Lab) has been made a study in order to microbiological assessment of microclimate conditions.

Locations chosen to collect the samples needed for air microbiota composition are located in the University of Craiova Craiova, Rooms C 005, C 263, Department of Computer Science.

For collecting microorganisms it has been used the sedimentation method, a method widely used to measure the degree of microbial contamination of the atmosphere in inhabited spaces.

Sedimentation method does not permit exact quantitative determination, some earlier observations reported that results of sedimentation method are usually higher than numbers obtained with the use of air samplers.

However, data collected by sedimentation method allow the drawing of correct conclusions on types of microorganisms present in the air and can give a rough approximation of bacterial and fungal concentration.

The number of microorganisms expressed as  $CFU/m^3$  has been estimated using the Omelianski formula, the results expression has been expressed in colony forming units UFC/m3 air.

Where:

UFC/m3 aer=100x100xn/SxK

UFC - the number of colony forming units;

n - the number of colonies resulting from the sedimentation of the cells in the surface of the medium;

S – surface of the Petri plate, [cm²];

K - coefficient that depends on the time of exposure:

K= $\tau/5$ ;  $\tau$  = time of exposure, [min].

The method involved the opening and exposure of Petri dishes with sterile culture medium for 5 minutes in different locations of the studied area, the number of cards displayed varies depending on the size of the room.

The plates were placed at distance of 1-1.5 m from the walls, maintaining a distance of approximately 2 m from each other, so that the entire surface is studded with these. During the exposion of Petri dishes windows and doors were closed, and the movements of any kind are prohibited. After exposure, the plates were seeded and incubated in the thermostat at a temperature of 28  $^{\circ}$  C.

Subsequently the colonies counted, on the assumption that each colony has grown from a microorganism. We used the PDA (potato-dextrose-agar) and PGA (peptone-dextrose-agar) media both in classical and variant mixed with streptomycin (35 mg/1) and pink bengal (33 mg/1).The determination of the number of mycelia colonies was done after 5 days. Determination of fungal genera was made using a piece of material mounted in a drop of lactofenol between the blades.

## **RESULTS AND DISCUSSIONS**

During October – November 2011 and October – November 2012 from rooms belonging to the University of Craiova (C 005, C 263 and Computer Lab), 90 samples were collected for microbiological assessment of microclimate conditions and the learning conditions of students.

There has been isolated a total of 333 colonies of fungi on 90 Petri dishes, quantified (to determine the frequency of occurrence) and then identified.

For room C 005 of the University of Craiova were revealed the following fungal genera: *Penicillium, Alternaria, Cladosporium, Aspergillus* and *Fusarium*.

Of these, the highest concentration of bacteria per m³ of air has been recorded at the *Penicillium* (153 UFC/m³), *Cladosporium* (64,7 UFC/m³) and *Alternaria* (25 UFC/m³). For room C 263, University of Craiova, were found only 3 types of fungal: *Penicillium*, *Cladosporium* and *Alternaria*, each with UFC/m3 air concentrations of: 412.3 (*Penicillium* spp), 45.9 (*Cladosporium* spp ) and 25.4 (*Alternaria* spp.)

For the Computer Lab of the University of Craiova have been identified seven types of fungi, as follows: *Cladosporium*, *Penicillium*, *Alternaria*, *Aspergillus*, and *Rhizopus Stemphyllium*, the highest concentrations of spores in the air has been recorded for *Cladosporium* spp (321, 4 UFC/m³), Penicillium spp (304.5 UFC/m³) and *Alternaria* spp (68.6 UFC / m³).

With reference to the above we can say that of the studied rooms, in the computer lab of the University of Craiova, there is a maximum air infestation that may cause allergic reactions, infections and inflammation.

In the other two locations considered in the study were recorded values below the normal value permissible limit (rooms C 005 and C 263) so there is no risk that indoor air can affect human health.

The *Penicillium* genra was the most frequently predominant followed by *Cladosporium*, *Aspergillus*, and *Alternaria*. *Alternaria* and *Cladosporium* spp are considered to be the most important allergenic fungi in outdoor air, while *Aspergillus* spp and *Penicillium* spp were recently recognized as important allergenic fungi in indoor air and generally from external sources.

## CONCLUSIONS

Observations on the microbial load of the air in the studied rooms revealed the following:

In the case of Computer Lab of the University of Craiova is a high infestation of air (> 700 UFC/m³) that may cause allergic reactions, infections and inflammation.

In the case of C 005 and C263 rooms of the University of Craiova there was recorded values below the normal limit allowed (<550 CFU/m³), so there is no risk that indoor air can affect human health.

The *Penicillium* genra was the most frequently predominant followed by *Cladosporium*, *Aspergillus*, and *Alternaria*. *Alternaria* and *Cladosporium* spp are considered to be the most important allergenic fungi in outdoor air, while *Aspergillus* spp and *Penicillium* spp were recently recognized as important allergenic fungi in indoor air and generally from external sources.

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# Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# THE QUANTITATIVE ANALYSIS OF SOME FLAVONOIDS IN THE SPECIES ACHILLEA MILLEFOLIUM, ANETHUM GRAVEOLENS, ORIGANUM MAJORANA AND ROSMARINUS OFFICINALIS.

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Key words: flavonoids, HPLC, ethyl alcohol, medicinal plants.

## ABSTRACT

The paper consists of the quantitative analysis of some flavonoids (kaempferol, myricetin) from the ethanolic extracts obtained from the fresh vegetal material on which a study was carried out. The quantitative determination of the kaempferol and myricetin was made through the chromatography of liquids (HPLC) to which a spectophometer UV-VIS VARIAN MODEL 345 was

coupled.

The researched species were : Achillea millefolium – the common yarrow, Anethum graveolens – the dill, Origanum majorana – the majoram; Rosmarinus officinalis – the rosemary. From the research carried out, the highest kaempferol (0.0086 mg/mL) and myricetin (0.0428 mg/mL) content resulted in the ethanolic extract of Origanum majorana.

## **INTRODUCTION**

Dill (Anethum graveolens) is an annual herb with short life, native to the South-West and centre of Asia. The leaves are thin, long and arranged in feather shape. The yellow flowers are clustered like an umbrella on top of the plant and they appear in the middle of the summer.

Yarrow (Achillea millefolium) is a herbaceous perennial species, spread in Europe and Asia. The plant is 20-80 cm tall, it has finely divided leaves and rich, white terminal inflorescence. The parts used in treatments are the flowers and leaves.

Marjoram (Origanum majorana) is a medicinal plant which is considered a natural remedy, originary from the Eastern part of the Mediterranean Sea from where it spread on all continents (Pârvu C., 2002).

The marjoram grows 20-60cm, with a relatively weak radicular system, with thin, fine stems and hairy, ovoid leaves. The stems ramify very much, even from the base, having a bush-like aspect. The flowers of the marjoram are velvety grey or white-pinkish. The entire plant has a velvet-like aspect, being covered with fine, short and thick hairs. The used part is the aerial part. (Mocanu Ş. & Răducanu D., 1983; Sava D., 2008)

Rosemary (Rosmarinus officinalis) is a plant originary from the Mediterranean Sea (Mocanu Ş., Răducanu D., 1983). Its stem is 60-150 tall, with arched branches. At the base

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of the stem the cortex exfoliates and in the superior part it is hairy and grey-coloured. The leaves have a lineal shape and remain green over the winter. The flowers are light bluish, slightly purple, rarely white, offering an aspect of ear. The fruit is a smooth, brown, ovoid-shaped nutlet. The used part is the aerial part (Sava D., 2008).

The flavonoids represent a class of compounds which by their structure have antioxidant properties which gives them the capacity to intercept and inactivate the free radicals ( $O_2^{\bullet}$ , HO $\bullet$ , ROO $\bullet$ , etc) (Condrat D., 2010).

The flavonoids have either a direct action of inhibiting the free radicals or an indirect one by protecting and regenerating the primary antioxidants.

The kaempferol and myricetin are representative terms for flavonoids. From a structural point of view they contain in their molecule a benzene nucleus (nucleus A) condensed to a pyranic nucleus (nucleus B) to which in position 2 a second benzene nucleus (nucleus C), which like nucleus A contains hydroxyl groups, is linked. These hydroxyl groups determine polyphenol properties (Cacig S., 2007).

The moderate and well controlled supplementation in flavonoids combined with other natural antioxidants (anthocyanidins, ascorbic acid, etc) may reduce the frequency of occurrence of some illnesses related to the antioxidant aggression.

The aim of the research carried out is to establish the quantity of kaempferol and myricetin (mg/ml of vegetal extract) from the analysed hydro-alcoholic extracts.

The specialty literature does not offer information regarding the quantities of compounds with antioxidant character which are presented in the chemical composition of the studied species of plants, although their pharmacological actions are well known (Istudor V., 1998) and this is why a closer study is necessary.

## MATERIAL AND METHODS

Calibration lines were drawn with a view to carrying out the quantitative analysis of the kaempferol and myricetin through chromatography of high-performance liquids. Based on these lines the two flavonoids were determined quantitatively.

Vegetal material: Dill - Anethum graveolens, Yarrow - Achillea millefolium, Marjoram-Origanum majorana, Rosemary -Rosmarinus officinalis

Obtaining the extracts:

5 g dry and finely ground vegetal material undergoes the static extraction (maceration) with 45 ml solution of ethyl alcohol (96%), for 10 days, at the room temperature, stirring 3-4 times/day, the samples being kept in dark (Farmacopeea Română, 1993).

The solutions were filtered through 4 strata of gauze, the obtained filtrate being used for the quantitative determination of the kaempferol and myricetin.

Vegetal extracts with a clear aspect and specific colour were obtained.

Reactives: kaempferol ( $\geq$ 99%) – Roth, myricetin ( $\geq$ 98%) – Roth, ethanol p.a. – Merck, methanol HPLC – Merck, phosphoric acid HPLC – Merck.

The standard solutions of kaempferol and myricetin

The standard substance (0.15 mg/mL) weighed with the analytical scales with a precision of 0.00001 g was transferred in a graduated balloon flask of 10 mL and dissolved with ethanol and thus obtaining the stock solution. By successive dilutions, solutions of different concentrations were obtained from the standard solutions. Based on the solutions with various concentrations, calibration lines were drawn.

In order to determine the wavelength at which the absorption of the standards is maximum, the UV analysis was performed. High-performance liquid chromatography (Condrat D., 2010).

The used chromatograph was of the type HPLC Varian Pro Star model 240, being composed of a ternary pump, automate injector, thermostat set at the room temperature and a UV-VIS (UV-VIS VARIAN MODEL 345) detector. The chromatographic separation was performed on a chromatographic column of the type Omnispher 5-C18, 150 x 4.6 mm.

The mobile phase consisted of an acetonitrile mixture: phosphate buffer pH = 2.5, in the volume ratio 40:60, for kaempferol, and for myricetin methanol mixture: phosphate buffer in the volume ratio 50:50.

The detection was achieved at 360 nm for kaempferol, and for myricetin at 300 nm. The beginning of the mobile phase was 1 mL/minute.

The injection volume was of 5µL.

# **RESULTS AND DISCUSSIONS**

In table 1 there are presented the ranges which are characteristic in the UV fields of the standards of kaempferol and myricetin.

Table 1

Standard	λ	Absorbance	$\lambda$ HPLC detection
	[nm]		[nm]
Myricetin	205.1; 209.9; 297	0.858; 0.846; 0.405	300
Kaempferol	268; 295; 364	0.418;0.413; 0.299	360

The ranges characteristic to the UV field of kaempferol and myricetin

The quantitative determination of the kaempferol and myricetin was based on the retention time and the wavelengths selected from the bands characteristic to the UV field of the analysed standards.

The calibration lines had the following correlation coefficients and equations:

1) $r^2 = 0.9998 - kaempferol;$	$y = 2.7411 \cdot 10^7 x - 5.1313 \cdot 10^4.$
2) $r^2 = 0.9998 - myricetin;$	$y = 8.4418 \cdot 10^{6} x - 1.5277 \cdot 10^{4}.$

The retention times and concentrations of the kaempferol and myricetin from the ethanol extracts are presented in table 2.

#### Table 2

Name of plant/Species	Analysed	Retention time	[mg /mL]
	compound	t _r [min.]	
Dill /Anethum graveolens	kaempferol	$4.316\pm0.097$	0.0050
Yarrow/ Achillea millefolium	kaempferol	$4.120\pm0.099$	0.0026
Majoran /Origanum majorana	kaempferol	$4.407\pm0.188$	0.0086
	myricetin	$3.885\pm0.035$	0.0428
Rosemary/Rosmarinus	kaempferol	$4.269\pm0.050$	0.0040
officinalis	myricetin	$3.836\pm0.084$	0.0298

The content of kaempferol and myricetin in the researched ethanol extracts.

The presence of myricetin and kaempferol at the plant species presented in the paper was studied both under qualitative aspect (spectroscopy UV/VIS) and quantitative aspect (high-performance liquid chromatography).

As regards myricetin, one can notice that as compared to the kaempferol, it is found in the hydro-alcoholic vegetal extracts in which the highest quantity could be identified.

The marjoram extract has a content which is two times higher in kaempferol (0.0086 mg/mL) as compared to Basil extract (0.0044 mg/mL). The myricetin content of the Rosemary extract is higher (0.0298 mg/mL) than in the Thyme extract (0.0108 mg/mL), but lower than in the Basil extract (0.0603 mg/mL) (Condrat D. et al., 2011). The lowest kaempferol content was determined in the Yarrow extract and the highest in the marjoram extract.

The experimental results obtained from the chromatographic analysis were in a good concordance with those obtained using the method of the external standard of finding again some known quantities of pure flavonoids.

# CONCLUSIONS

The HPLC method, coupled with the electronic spectroscopy (UV-VIS) proves an optimal efficiency for the determination of the kaempferol and myricetin concentration in the hydro-alcoholic extracts of the analysed species of plants.

The therapeutic properties of the studied plants in raw state or under the form of tea, can be explained in a certain degree by the significant content of flavonoids present in their chemical composition.

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# STUDIES CONCERNING THE INCIDENCE OF ESBL PRODUCING BACTERIA IN VARIOUS INFECTIONS

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Keywords: antibiotic resistance, Enterobacteriaceae, extended spectrum beta-lactamases

#### ABSTRACT

One of the greatest threats to the public health is the emergence of antimicrobial resistant strains of pathogenic bacteria. The selective pressure of uncontrolled antibiotic usage in human therapy of diseases is the main risk factor for spreading of resistant bacteria. Some bacterial species have multidrug resistance to antibiotics by extended spectrum beta lactamases (ESBLs) synthesis, under plasmidial genes or transposable elements control. In this study 1625 urine samples and 265 samples of stool from patients with suspicion of urinary tract infections, respectively enteric diseases were tested for presence of ESBLs producing bacteria. Among all E. coli strains, 24% from strains implicated in urinary tract infections and 35.18% from strains implicated in enteric infections were ESBLs positive.

#### **INTRODUCTION**

The antibiotic resistance of pathogenic bacteria is a main concern for medical world and a constant problem for treatment of bacterial infections. Antimicrobial resistant bacteria occur both in community and in hospital environment (DebMandal et al., 2011). The main risk factor for spreading of resistant bacteria is the easiness of plasmidic transfer between various bacteria, especially in the terms of selective pressure of intense and inadequate antibiotic treatment of diseases, both in human and animal therapy.

It was estimated that the antibiotic market worldwide consumes between 100 and  $200 \times 10^6$  kg. Because the resistant bacteria occur in different circumstances, not only in hospitals, is imperative to explore the antibiotic pressure in the environment (Wise, 2002).

The urinary tract infections may be considered as common infections. The most frequent pathogen, *E. coli*, developed varied survival strategies to grow in nutrient deficient environment like urinary tract. Like other Enterobacteriaceae, *E. coli* have multidrug resistance to antibiotics by extended spectrum beta lactamases (ESBLs) synthesis and other mechanisms, including mutations (Nica et al., 2007). This kind of antimicrobial resistance quickly spread because the plasmidic genes (and molecular characterization of plasmids is epidemiologically useful) or the transposable elements (Khadgi et al., 2013). The best known variants of ESBLs are TEM (Temoneira), SHV (Sulphhydryl variable) and CTX-M (Cefotaximase - Munich) (Burduniuc et al., 2012).

The *E. coli* strains could be gastrointestinal pathogens, too. They are classified in enteric pathotypes on the basis of serological and virulence characteristics. Some of enteric *E. coli* strains synthesize extended spectrum beta lactamases and resist to antibiotic treatment. In healthy people ESBLs bacteria is usually not a problem (is called colonisation

or carriage), but extended spectrum beta lactamases synthesis in *E. coli* strains limit the therapeutic options for patients with various infections, because bacteria concomitant have resistance to other classes of antibiotics.

## MATERIAL AND METHODS

In our study 1625 urine samples and 265 stool samples were collected, during 8 months (January - August 2012), in the laboratory of MEDAS Medical Centre. The urine samples were gathered in sterile containers, from patients with suspicion of urinary tract infections, and the stool samples were collected from patients with suspicion of gastrointestinal infections, children and adult patients, male and female.

For urine samples the macroscopic and microscopic observations were made, after that the quantitative and qualitative urine culture was made.

For *quantitative urine culture* we used the serial dilution method. Serial dilutions from 1/10 to 1/10000 were inoculated on the surface of two media: blood agar and AABTL (Bromothymol-blue Lactose Agar) or blood agar and MacConkey medium. After 24 hours at 37°C, the plates were used to establish the number of colony forming units per ml (CFU/ml), according to dilution of sample. The level of bacteriuria was estimated, to establish the result of investigation; significant bacteriuria was considered for samples with 10⁵ (or more) CFU/ml.

The *qualitative urine culture* was performed by Gram reaction and biochemical features. Some growth media were used: MIU (motility - indole - urea), TSI (triple - sugar - iron agar), MILF (motility - indole - lysine decarboxylase - phenylalanine deaminase), malonate broth and Simmons citrate medium. The pathogenic bacterial strains were identified and tested for antimicrobial susceptibility.

The antimicrobial susceptibility of bacterial strains was determined by standard disk diffusion method (Bauer et al., 1996). The Mueller-Hinton agar was used to inoculate the bacterial suspensions and standard antibiotic disks (Oxoid) were placed on the medium surface. After minimum 18 hours at 35 - 37°C, the growth inhibition zones were measured and the susceptibility level of bacteria was established. The double - disc synergy test was used to detect one of the antimicrobial resistance mechanisms of E. coli, the presence of extended spectrum beta lactamases (ESBLs). This test used a beta lactamases inhibitor (usually Clavulanic Acid, 10µg/disc) in association with Cephalosporine (for example Cefotaxime 30µg/disc). Disc with Cefotaxime and disc with Amoxicillin + Clavulanic Acid combination were placed on the inoculated surface of medium at 25 - 30 mm away. After incubation period the growth inhibition zones were observed. If the bacterial strain was ESBL producer, the zone of either cephalosporin is expanded by the clavulanate and are obvious synergy images between two discs as "champagne cork" (Burduniuc et al., 2012). Because the variation of ESBLs, the double – disc synergy test cannot be standardized. For instance, for TEM and SHV variants the synergy is more obvious with Ceftazidime and for CTX-M types the synergy is more obvious with Cefotaxime (Livermore and Brown, 2001).

The double – disc synergy test was used because the common disk diffusion method cannot furnish enough data about the resistance mechanisms. Although most ESBLs confer resistance to one or more beta lactam antibiotics, bacterial enzymes does not always increase enough the minimum inhibitory concentration to be called resistant by standards (Bradford, 2001).

For stool samples the bacterial culture was made. The stool samples were used to obtain suspensions in sterile saline solution. The suspension was inoculated on selective media, for instance Mac Conkey and EMB (Eosin Methylene Blue or Levine medium). The inoculated plates were incubated overnight at 37°C and the colonies of lactose non-

fermenters or lactose fermenters were selected. The identification of bacterial strains was done on the basis of biochemical properties. The MIU, TSI, MILF and Simmons citrate medium were used.

The identified enteric pathogenic strains were tested for antibiotic susceptibility by standard disk diffusion method and double – disc synergy test, to determine the mechanisms of antibiotic resistance.

# **RESULTS AND DISCUSSIONS**

Among 1625 urine samples and 265 stool samples, from children and adult patients, male and female, 525 (32%) were positive for urinary tract infections, respectively 96 (36.22%) were positive for gastrointestinal infections (Figure 1).



Figure 1. Incidence of urinary tract infections and gastrointestinal infections

The main pathogen in uncomplicated urinary tract infections is *E. coli* (Nielubowicz and Mobley, 2010; Kashef et al., 2010); 71.42% of confirmed urinary infection cases were determined by *E. coli* strains. Other pathogens were *Enterococcus sp.*, *Streptococcus sp.*, *Klebsiella sp.*, *Proteus sp.*, *Enterobacter sp.*, *Morganella sp.*, *Acinetobacter sp.*, *Staphylococcus sp.* (Figure 2).

In gastrointestinal tract infections, the predominant bacteria were *E. coli* (56.25% from all infections), but other bacteria were implicated, too: *Proteus sp., Klebsiella sp., Citrobacter sp., Pseudomonas sp., Enterobacter sp., Hafnia sp., Salmonella sp.* (Figure 2).

The female subjects with urinary tract infections and gastrointestinal tract infections were more than male ones (70.09% from all urinary infections, respectively 54.16% from all digestive infections, Figure 3). Other studies about the incidence of urinary tract infections reveal that women are more susceptible than men for these infections (Kashef et al., 2010).

The strains of *Escherichia coli* isolated from urinary tract infections and gastrointestinal infections were tested to cephalosporin (Cefotaxime) and combination beta lactamic antibiotic + beta lactamases inhibitor (Amoxicillin + Clavulanic acid).

The presence of ESBLs was established as mechanism of antibiotic resistance of bacteria. Among *E. coli* strains isolated from urinary tract infections 24% were ESBL producers, and 35.18% of *E. coli* strains isolated from gastrointestinal tract infections were ESBLs positive (Figure 4).



Figure 2. Incidence of E. coli and other bacteria in urine and stool samples



Figure 3. Incidence of urinary tract infections and gastrointestinal infections depend on gender of patients



Figure 4. Incidence of ESBLs producers *E. coli* strains in urinary tract infections and gastrointestinal infections

The incidence of ESBLs producers is not the same for all bacteria and for all regions of the world. Our study revealed a higher incidence than other references.

The results of our study revealed different percentages for incidence of ESBL producers *E. coli* strains in male and female patients. Hence, for 90 strains of ESBL producers *E. coli* isolated from urinary tract infections, the incidence was 20.14% from *E. coli* strain in women and 33.64% from *E. coli* strain in men. From gastrointestinal tract infections 19 strains of ESBL producers *E. coli* were isolated; the incidence of ESBLs producers *E. coli* was 44.82% in women and 24% in men. These differences could be related to various incidences of infections in women and men.

Certain studies indicate the incidence of ESBLs producers *E. coli* strains is just 5.3% for urinary tract infections and 15% for intestinal carriage (Burduniuc et al., 2012). Other studies indicate 30% percentage of ESBL producers from Enterobacteriaceae family (Jabeen et al., 2005). It is a fact the frequency of ESBLs bacteria varies by geographic region; some studies revealed that in Europe, North America and Latin America being about 1-8% for *E. coli* (Jabeen et al., 2005), but in other region being much higher.

## CONCLUSIONS

The most common pathogen of urinary tract infection and gastrointestinal infections was *E. coli* (with 71.42%, respectively 56.25% incidence). The women were more susceptible than men to these infections, because of their anatomy and physiological conditions.

Among all *E. coli* isolated strains 24% were ESBL producers in urinary tract infections and 35.18% in gastrointestinal infections, most probably CTX-M types. The percentages were different for infections in women and men.

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# RESEARCH REGARDING LIQUID AND SOLID RUNOFF, SOIL AND NUTRIENT LOSSES AT PREAJBA-GORJ

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Keywords: losses of nutrients, erosion, soil, liquid runoffs.

#### ABSTRACT

The present study was conducted in the period 2010-2012 and shows the runoff volume of soil and nutrients from stagnic albic luvosoil from Preajba – Gorj Experimental Field on pasture crops with a slope of 6%. Amounts of soil lost by surface erosion averaged over the three years of experimentation were different depending on crops (sown pasture, natural pasture, corn).

At the natural and sown pasture is recorded the lowest amount of soil loss (0.45 to 0.68 t soil/ha) because these crops both through the root system and the total degree of ground cover, retain very good soil from phenomenon erosion.

Amounts of humus, nitrogen, phosphorus and potassium are lost per hectare in direct correlation with the amounts of soil loss, higher at corn and lower for natural and sown pasture. Micro process also suffers a loss on the slope lands but in very small quantities.

# **INTRODUCTION**

Researches on nutrient losses are strictly related to erosion, as the largest quantities of nutrients are lost with the solid material transported as a result of the action of erosive agents.

Soil erosion affects 1.7 billion hectares worldwide of which 1,1 billion ha are affected by water and 0.6 billion ha by wind (Pierzynsky, 1994). This has adverse consequences from the ecological point of view, because it contributes not only to the decrease of soil's thickness, but also to main changes of the physical, chemical and biological properties. Thus, it reduces the water holding capacity and nutrients vital to plant growth. The movement of soil particles and their accumulation in downstream located areas destabilizes ecosystems in the sense that with sediment accumulates and large amounts of nutrient that can get into waterways, causing pollution and their eutrophication (Merot, 2008; Behrendt, 2008). Ways in which these elements are lost:

- loss through the eroded soil;

- loss of elements once the water is drained from the soil surface;

- the movement of these elements in the profile of the soil by means of water infiltration into the soil.

The role of nutrients in the eutrophication of surface water and groundwater contamination has long been unknown, it is due to the primarily nitrogen and phosphorus

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from fertilizers applied to the soil, industrial and municipal wastewater activities (Ullen, 2005).

#### MATERIAL AND METHODS

In the experimental field of Crops and Pasture Research Center Preajba, Gorj County, belonging to the University of Craiova, experiences were placed on stagnic albic luvosoil with a slope of 6%, including nine variants in five repetitions. Each experimental plot had dimensions 4x25 m and a surface of 100 sqm.

In order to avoid the influence of runoffs from a plot to the other, each parcel was delimited on all of its sides by means of plastic boards with dimensions of 5000 x 250 x 50 mm, which were inserted into the soil to a depth of 100 mm. In the downstream part of the plot was build a system for collection of runoffs in each plot, consisting of a triangle of concrete, finished with a tube that drains into a collecting basin. At the end of the collecting tube is installed a splitter plate with a row of seven holes, each hole being then separated in 7 parts, in such a way that the volume of runoff collected in collector basin to be only the 49th part of the total amount of plot.

- The 9 experimental plots included the following crops and treatments:
- Plot 1 = natural pasture unfertilized,
- Plot 2 = natural pasture fertilized with N138,
- Plot 3 = natural pasture fertilized with N162 P81 K100,
- Plot 4 = sown pasture unfertilized,
- Plot 5 = sown pasture fertilized with N138,
- Plot 6 = sown pasture fertilized with N162 P81 K100,
- Plot 7 = unfertilized corn,
- Plot 8 = corn fertilized with N138,
- Plot 9 = corn fertilized with N162 P81 K100.

In this experiments were pursued in 2010-2012, the average volume of liquid and solid runoffs, loss of soil and nutrients averaged over the three years of research.

In the laboratory, the following determinations were made:

- establishing the total volume of runoffs water + soil;
- determining runoff and soil eroded by filtration;
- analysis of the eroded soil analysis to determine runoff requiring:
  - content of humus kg/ha;
  - nitrogen content kg/ha;
  - content of phosphorus kg/ha;
  - potassium content kg/ha;

- content of microelements kg/ha;

- analysis of leaking water to determine runoff requiring:
  - content of nitrogen kg/ha;
  - content of phosphorus kg/ha;
  - potassium content kg/ha;
  - content of microelements kg/ha;

# **RESULTS AND DISCUSSIONS**

Crops and Pasture Research Center Preajba - Gorj, is located on the territory of Târgu-Jiu, Balotesti hill 12 km from Targu-Jiu,  $45^{0}00'17$ , 1" longitude and,  $23^{0}21'40$ , 3" latitude.

Preajba experimental center is located on the highest terrace of Jiu (the 5th terrace), at an altitude of 355 meters It is spread over an area represented by two forms of mezorelief:

- the platform of the 5th terrace, which generally has a general plan appearance, slightly inclined towards W-SV, and the slope of 0-2%;

- Preajba western side of the Preajba stream which is a slope stabilized with slightly wavy appearance, and with a slope of 5 to 45%.

Topography of the area is the result of time over the river Jiu (terraces) and within the terraces, actions of stream Preajba (valley with its slopes).

According to the climate station Tg-Jiu, the climate is temperate continental with evident Mediterranean influences. Average yearly temperature is 10.2°C and precipitation regime is 770 mm.

Rainfall regime is percolative, distributed unevenly, causing drought in July-August-September.

Following the determination of the medium volume of liquid and solid runoff, the loss of soil and nutrients averaged over the three years of research (2010-2012) are shown in Tables 1 and 2.

From the data analysis contained in these tables we can conclude the following:

Discharge of liquid or loss of water from Preajba luvosoil were between 518.4 and 821.5 m³/ha representing 9.6 to 13.2% of rainfall. The lowest water runoff occurred in natural pasture fertilized with N162 P81 K100, 518.4 m³/ha, and respectively the highest 821.5 m³/ha were recorded at unfertilized corn. Therefore, for the weeding crops, the amount of water drained on the soil from rainfall is much higher.

In direct correlation with water loss were also the soil loss at ha or erosion. It recorded the lowest value 0.45 t/ha at natural pasture fertilized with N162 P81 K100, evidence that plants and roots grew vigorously and held more ground. The largest amount of eroded soil was recorded at unfertilized corn 5.26 t/ha.

The loss of humus were higher at unfertilized corn and lower at natural pasture fertilized with NPK 0.45 t/ha.

Nutrient losses were calculated by analyzing the leaked water and soil, after which the two values were summed. Thus, the nitrogen losses amounted values between 0.82 and 4.16 kg/ha, the biggest losses occurring in unfertilized corn and the lower losses at the natural pasture fertilized with NPK due to its vigorous growth.

Phosphorus losses are much lower compared to losses of nitrogen 0.029 to 0.126 kg/ha/year, lower values occurring in natural and sown pasture after NPK fertilization, because of vigorous plant growth and root system.

Potassium loss have a value slightly higher that those of the phosphorus, but also is less than the loss of nitrogen with values ranging from 0.21 kg/ha at natural pasture unfertilized and 0.74 kg/ha at corn fertilized with NPK.

Microelements loss of Fe, Mn, Cu, Zn have lower values than those of the macro elements, being of the order of grammes per hectare, the highest values being registered to the Fe³+ and Mn⁴+ and the lowest at the Cu and Zn. We notice the presence of phosphorus in fertilizers applied contributing to reduce the loss of Zn, Fe, Mn, due to formation of sparingly soluble phosphates.

Variant	Liquid	Eroded	Humus	Nitrogen kg/ha Phosphorus kg/ha			g/ha	Pot	assium k	g/ha		
	runoff	soil	Kg/ha	Water	Soil	Total	Water	Soil	Total	Water	Soil	Total
	m ³ /ha	t/ha										
Natural pasture Mt	571,3	0,62	13,2	0,12	0,76	0,88	0,015	0,02	0,03	0,09	0,12	0,21
Natural pasture N138	533,2	0,51	12,5	0,10	0,72	0,82	0,011	0,018	0,029	0,11	0,17	0,28
Natural pasture N162P81K100	518,4	0,45	10,8	0,10	0,70	0,80	0,009	0,02	0,029	0,11	0,19	0,30
Sown pasture Mt	611,5	0,68	15,4	0,14	0,92	1,06	0,011	0,020	0,031	0,08	0,17	0,25
Sown pasture N138	582,7	0,60	14,1	0,12	0,84	0,96	0,017	0,026	0,043	0,10	0,22	0,32
Sown pasture N162P81K100	552,8	0,55	12,3	0,11	0,80	0,91	0,014	0,022	0,036	0,14	0,22	0,36
Corn Mt	821,5	5,26	176,5	1,72	2,44	4,16	0,017	0,021	0,038	0,21	0,31	0,50
Corn N138	794,3	5,01	157,9	1,70	2,01	3,71	0,054	0,072	0,126	0,24	0,46	0,68
Corn N162P81K100	756,5	4,72	144,3	1,51	1,85	3,36	0,041	0,058	0,099	0,31	0,43	0,74

Soil and nutrient losses at Preajba-Gorj during the 3 experimental years

Table 1

Variant	Liquid	Eroded		Fe g	/ha		Mn g	/ha		Cu	ı g/ha		Zn g	/ha
	runoff	soil	Water	Soil	Total	Water	Soil	Total	Water	Soil	Total	Water	Soil	Total
	m ³ /ha	t/ha												
Natural pasture Mt	571,3	0,62	2,8	8,5	11,3	0,35	18,0	18,35	0,67	0,78	1,45	1,03	2,31	3,34
Natural pasture N138	533,2	0,51	3,2	13,6	16,8	0,47	19,6	20,07	0,82	1,44	2,26	1,44	2,86	4,30
Natural pasture N162P81K100	518,4	0,45	2,2	9,5	11,7	0,29	16,2	16,49	1,01	1,60	2,61	1,72	3,11	4,83
Sown pasture Mt	611,5	0,68	1,7	12,5	14,2	0,42	17,2	17,62	0,79	1,11	1,90	1,33	2,07	3,40
Sown pasture N138	582,7	0,60	2,8	21,6	24,4	0,56	20,3	20,86	1,21	3,42	4,63	1,84	2,78	4,62
Sown pasture N162P81K100	552,8	0,55	2,0	18,3	20,3	0,35	15,6	15,95	1,56	4,01	5,57	1,88	3,39	5,27
Corn Mt	821,5	5,26	11,2	64,5	75,7	0,73	116,4	117,13	2,61	5,23	7,84	2,61	13,14	15,75
Corn N138	794,3	5,01	12,6	83,8	96,4	1,09	134,3	135,39	2,01	5,01	7,02	2,22	12,01	14,23
Corn N162P81K100	756,5	4,72	9,3	56,4	65,7	0,66	105,2	105,86	2,25	4,54	6,69	1,84	10,58	12,42

Microelements losses, Fe, Mn, Cu, Zn, at Preajba, Gorj during the 3 experimental years

Table 2

#### CONCLUSIONS

The amounts of soil lost by surface erosion averaged over the three years of experimentation (2010-2012) were different depending on the culture experiment.

The amount of soil loss at natural pasture was reduced by 0.45 t soil/ha at N162 P81 K100 dose and 0.62 t/ha at unfertilized who had a lower growth of plants. Similar value was obtained in the sown pasture as from 0.55 to 0.68 t soil/ha.

Quantities of humus loss per hectare are in direct correlation with higher amounts of soil loss at corn crop, from 144.3 to 176.5 kg/ha and lower at natural and sown pasture by 10.8 to 15.4 kg/ha.

Nitrogen lost by surface runoff is also higher at corn crops (3.36 to 4.16 kg/ha) and lower, of 0.80 to 1.06 kg/ha at natural and sown pasture ;

The amount of phosphorus lost from the soil in slope from Preajba - Gorj are smaller than the nitrogen, however, they are higher in corn crops, of 0.038 to 0.126 kg/ha and smaller at natural and sown pasture of 0,029 - 0.043 kg/ha;

The amount of potassium lost on soils on slope are lower than nitrogen, but larger than those of phosphorus, which are based on experimental cultures, being lower at natural and sown pasture, 0.21 - 0.32 kg/ha compared to 0.50 to 0.74 kg/ha at corn crops;

Microelements also suffer a loss process on soils, but in very small quantities, of the order of grammes per hectare.

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# ASSESS THE BIOENERGETIC POTENTIAL FROM PRIMARY AND SECONDARY PRODUCTION OF COWPEAS CULTURE IN SANDY SOIL CONDITIONS

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Keywords: genotype, leaf area index, biomass, energy

## ABSTRACT

Together with crop rotation, plant density and sowing period, the variety is among the factors which contribute to increased production without requiring an additional energy. Research conducted at Research - Development Centre for Field Crops on Sandy Soils, Dabuleni, Romania in 2012-2013 on 15 genotypes of cowpeas, in terms of energy content of secondary production (root, stem + leaf, pods without seeds) shows that it originates largely from stems + leaf mass (61-89 %). The percentage of grains in pods, expressed on weight of the pods, varies between 75 to 80.7%, with the highest values at the cowpea genotypes that have been less biomass, but higher grain production. The leaf area index (L.A.I.) was positively correlated with total production of bioenergy (r = 0.681**) and negative with grain yield obtained in cowpeas (r = -0.634 *). The correct choice of genotype may help to reduce energy consumption per kilogram by up to 41.7%.

# **INTRODUCTION**

Intraspecific competition between plants takes place during the foliar and root system development, and the results show that higher growth is achieved as energy biomass plant is cultivated in an area as similar to origin area (Dadson, R. B., 2005, Draghici Reta, 2012). Bioenergy is energy from biomass, because as you grow plants absorb the solar energy and carbon dioxide with which forming the organic matter in which the stored energy. The studies show that the use of bioenergy in the EU could double or triple, without harming the environment and without causing reduced production of food, feed or raw materials (Gorun Marian, 2013). In the concept of bioenergy are identified following types: biomass, bioethanol, biodiesel, and biogas. There are many potential sources of bio-energy which are currently underexploited, such as the biomass of certain plants that incorporated in soil can help to reduce of agricultural inputs. Increasing bioenergetic yield of plants by choice of appropriate product in a given area could have a substantial contribution to reducing problems of energy security generated by global climate change (Karp Angela, Ian Shield, 2008).

## MATERIAL AND METHODS

Researches were carried out between 2012 -2013, at CCDCPN Dabuleni in the irrigation conditions, on a sandy soil with a low nitrogen content (from 0.035 to 0.19%), well stocked in phosphorus (73.8 to 101.4 ppm), low to medium stocked in potassium (63 -

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87 ppm) with a low content of organic carbon (0.08% - 0.34%) and a low pH acid to neutral (5.6 to 6.8). Were experimented 15 cowpea genotypes, in order to assess the potential of bio-energy plant. During the period of vegetation were carried out determinations of leaf area and plant productivity. Has been determined on components biomass, resulting from the harvest plant (roots, stems + leaves, pods without seeds). Was calculated then the energy content of production, using for grain yield energy equivalent of 3.905 Mcal / kg and for biomass reference was made 1 kg haulm soybeans = 3.684 Mcal / kg on the 89% dry matter (Tesu Ion, Vasile Baghinschi, 1984). Because at harvested of cowpea crop the dry matter percentage was 46.75% (averaged over the two years of study), has resulted an energy equivalent of 1.93 Mcal for 1 kg haulm of cowpeas. The results were calculated and interpreted using variance analysis and mathematical functions.

# **RESULTS AND DISCUSSIONS**

Analyzing the climatic conditions of during the study period 2012-2013, it highlights the suitability of the sandy soils area for the cowpeas crop. Havinhigh requirements as against temperatures all cowpea genotypes have carried out their biological cycle in May-August period, when in air has accumulated a sum of about 2801°C and 1571°C active degrees. All of the processes of plant growth were held at an average temperature in air higher than 18 °C and a relative humidity, range within the from 60.7 to 73.9% (Figure 1)



Figure 1. Climatic conditions prevailing in the period 2012-2013

Size of leaf area and weight components of cowpeas plant emphasizes significant differences between genotypes experienced (Table 1). Under the conditions in southern Oltenia the varieties of cowpeas have developed a luxuriant vegetation biomass with a leaf area index during flowering phase between 4.36 to 6.94, based on which can be selected genotypes for the destination biomass incorporated into soil or used in animal feeding. High values of leaf area index were recorded at genotypes: Jiana, D2-b/93, D3/93, D12/2001 and D12/2000. Analyzing the results shown in Table 1, is observed that leaf area is positively correlated with the weight of cowpeas haulm composed of stems and leaves and negatively with grain weight. Production results obtained from harvesting differentiates the destination of cowpeas genotypes. Thus, were noted by a high grain production (2334-

2698 kg / ha) genotypes: Ofelia, D2-3a, Aura, D2-b/93, D4-4, D3/93 and D8/2000, which were registered distinct and very significant differences in production to control variety (Table 2). The genotypes of cowpeas were registered an energy consumption per kg

grain from 35.3 to 44% less, compared with the control variety, Jiana. The amount of bioenergy resulted from the secondary production obtained at harvest was between 31455-65702 Mcal / ha. Were detached by productions over 45000 Mcal / ha the following genotypes: D12/2001, D12/2000, Jiana, D5-3, D2-b/93, and D4-4.

Table1

Variability of biometric measurements carried out cowpeas genotypes tested in sandy so	ils
conditions from RDCFCSS, Dabuleni	

			Biometric measurements at harvesting plant								
No.	Genotypes	Leaf area	Total plant weight at harvest	C	omponent	s weight / p -g-	lant	Components in pods (%)			
		maex		Root	Stems + leaves	Sheaths	Grains	Grains	Sheath s		
1	Jiana	6.94	159	17.5	126.5	3.9	14.1	78.4	21.6		
2	Aura	4.96	135.5	17	82	7.6	28.9	79.5	20.5		
3	Ofelia	5.35	129	14.5	84.1	5.6	24.8	80.7	19.3		
4	D2-3a	5.28	97	10	61.3	6.4	19.3	75.3	24.7		
5	D3-5	4.64	106	11.1	72	5.1	17.9	78	22		
6	D4-1	5.79	103	9.4	68.7	5.3	19.6	78.7	21.3		
7	D4-4	5.55	134.5	13.5	95	6.5	19.5	75	25		
8	D5-3	5.83	139	12.5	107	4.9	14.6	75,1	24.9		
9	D2-b/93	6.67	142	18.8	94.4	5.6	23.3	80.3	19.7		
10	D3/93	6.09	128	14.5	89.9	5.3	18.3	77.4	22.6		
11	D9/2001	6.1	115	11.6	71	4.3	17.4	80.2	19.8		
12	D12/2001	6.89	178.5	13.8	144.3	4,9	15.5	76	24		
13	D14/2001	5.36	122	13.8	83.5	5.5	19.2	77.5	22.5		
14	D8/2000	4.36	122	14.4	79.8	5.7	22.2	79.3	20.7		
15	D12/2000	6.49	169	18.5	131.5	4.8	14.3	75	25		

		Total production of bioenergy (Mcal / ha)	Grain Yield		•		Production of haulms *			
No.	Genotypes		Kg/ha	Mcal/ha	Energy spor (Mcal/ha)	Energy consumption (%)	Kg/ha	Mcal/ha	Energy spor (Mcal/ha)	Consum de energie (%)
1	Jiana	64594	1512	5905	Control	100	30409	58689	Control	100
2	Aura	52738	2472**	9653	3748	61.7	22324	43085	-15604	136.2
3	Ofelia	52635	2698***	10536	4631	56.0	21813	42099	-16590	139.4
4	D2-3a	41573	2591***	10118	4213	58.3	16298	31455	-27234	186.5
5	D3-5	44271	2198*	8583	2678	68.7	18491	35688	-23001	164.4
6	D4-1	41802	2014	7865	1960	75.1	17584	33937	-24752	172.9
7	D4-4	56150	2401**	9376	3471	62.9	24235	46774	-11915	125.5
8	D5-3	58693	2222*	8677	2772	68.1	25915	50016	-8673	117.3
9	D2-b/93	57335	2411**	9415	3510	62.7	24829	47920	-10769	122.4
10	D3/93	53423	2334**	9114	3209	64.7	22958	44309	-14380	132.5
11	D9/2001	46128	1916	7474	1569	79.0	20546	38654	-20035	151.8
12	D12/2001	72543	1752	6841	936	86.3	34042	65702	7013	89.3
13	D14/2001	50092	2208*	8622	2717	68.5	21487	41470	-17219	141.5
14	D8/2000	49501	2336**	9122	3217	64.7	20922	40379	-18310	145.3
15	D12/2000	69484	1700	6639	734	88.9	32562	62845	4156	93.4`
Specification			LSD $5\% = 553$ LSD $0,1\% = 9$ 1 kg cowpea s	1 kg cowpea haulm (s.u. – 46.75%) = 1.93 Mcal/kg *root+ stems + leaves + pods without seeds						

Production results obtained at the genotypes of cowpeas tested in the sandy soil conditions from RDCFCSS, Dabuleni

Table 2



Figure 2. The correlation between the number pods per plant and grain yield obtained from 15 genotypes of

Figure 3. Correlations between leaf area index and energy production and grain yield obtained from 15 genotypes of cowpeas

Analyzing the functional connection between the number of pods formed per plant and grain yield obtained from the 15 genotypes of cowpeas is observed a positive correlation that shows that once with increasing the number of pods, is recorded growth of grain production, a phenomenon highlighted very well using linear regression, whose coefficient = 0.907 ** emphasizes a significant distinct correlation between cause and effect (Figure 2).

The increasing of leaf area index resulted in a positive linear regression at the total production of bioenergy (r = 0.681 **) and a negative regression of grain production (r = -0.634 *), registered at genotypes of cowpeas (Figure 3). The vegetative growth is detrimental to fructification of plant cowpeas, because the rich vegetation prevents the penetration sunlight from the bottom of flower vexille, an essential condition in the process of the pollen fecundation and in the uniformity of maturation the pods.

## CONCLUSIONS

In the pedoclimatic conditions from southern Oltenia the cowpeas varieties tested have developed a luxuriant vegetation, with a leaf area index during flowering phase between 4.36 to 6.94

Were highlighted by high grain yields (2334-2698 kg / ha) genotypes: Ofelia, D2-3, Aura, D2-b/93, D4-4, D3/93 and D8/2000, recording energy consumption per kilogram of grain from 35.3 to 44% less compared with the control variety Jiana.

The amount of bioenergy resulting from secondary production obtained at harvest was between 31455-65702 Mcal / ha, emphasizing through high levels genotypes: D12/2001, D12/2000, Jiana, D5-3, D2-b/93, and D4-4.

The leaf area index, resulted in the flowering phase of the plant at cowpeas genotypes, was positively correlated with total production of bioenergy (r = 0.681 **) and negatively with grain yield (r = -0.634 *).

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# PRELIMINARY RESULTS ON SWEET POTATO (IPOMOEA BATATAS) ON SANDY SOILS

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Keywords: variety, photosynthesis, transpiration, yield, quality

#### ABSTRACT

The extension arid areas with visible accents of desertification, determines the orientation in using sandy soils towards new species of plants and establish their cultivation technology in order to mitigate negative effects of climate change. In this regard, during 2012 -2013 was initiated research at the Research - Development Center for Field Crops on Sandy Soils, Dabuleni, Romania on testing of some Korean genotypes of sweet potatoes (Ipomoea batatas) using different planting methods and periods. The results obtained emphasize the capitalizing with good results the ecopedological conditions of sandy soils. Physiological measurements made in the completion of growth the shoots phase highlights the maximum photosynthesis in protected version (30.21 µmol  $CO_2 m^2 s^2$  at 9 o'clock, when the area was a leaf temperature from 33.6 to 34.8 ° C¹). In terms of production of tubers, the best results were recorded in PE mulch protected version (20427 kg / ha).

## INTRODUCTION

Sweet potato is originally from Central America and is cultivated on large areas in China, India, Japan, Africa, USA and Mediterranean areas of Europe. Sweet potato constitutes an important foodstuff in a eating healthy.Compared with ordinary potato, cooked in the same way, eating sweet potato increases blood sugar levels by 30% less than normal potato. Rich in nutrients and fiber (of which 40% soluble fiber that helps lower the blood sugar levels and cholesterol), sweet potato is the ideal food for diabetics, children and pregnant women (Betty J. Burri, 2011 Mihaela Cioloca et al., 2013). Through its qualities, sweet potato varieties with yellow and orange pulp are a valuable source of vitamin A and vitamin B6. Also, sweet potatoes provide the a considerable amount of vitamin C and vitamin D, which are essential in the formation of bones and teeth, for good digestion, for wound healing and strengthening the immune system. Sweet potato also contains iron, that helps to metabolize protein, but also magnesium, a antistress mineral.

Preoccupations for sweet potato cultivation in Romania took place in Bucharest USAMV (Ciofu Ruxandra, 2005 Cosmin Musat, 2010), and were resulting in the creation of two varieties (Victoria IANB in 1991and Crux in 1997), for fresh or preservatives in consumption in order to ensure of good nutrition from terms of nutrition. Research conducted in Nigeria by Ladokum O.A., et al., 2007, underlines the importance sweet

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potato cultivation, as the main nutrition resource for the African population. The variability of climate, especially the lack of rainfall and low fertility of sandy soils, make in the surface pretty large the production obtained from some crops to be much diminished. To obtain high yields, stable and secure, is a necessity the choice assortment of plant varieties with high adaptability to unfavorable climatic and soil conditions. In this context, at

RDCFCSS Dabuleni were initiated investigations in 2012-2013 on the testing of some Korean genotypes of sweet potatoes (Ipomoea batatas), using different methods and planting epochs, in order to develop the culture technology and promote in the area of this plant.

## MATERIAL AND METHODS

The research was conducted in 2012-2013 at RDCFCSS Dabuleni, on a sandy soil with low natural fertility (0.42 to 0.77% humus) and pH (H2O) = 5.9 to 6.8, in the bilateral Cooperation Protocol between Institute of Agricultural Science and Technology of Kyungpook National University from South Korea and Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu SISESTI" Bucharest. In 2012 were studied three varieties of sweet potato: Pum, Hwang and Bam, planted in two soil protection: with transparency PE mulch and unmulched. Varieties were received from Korea in the form of shoots rooted and were planted in field research on 8 May 2012. In 2013 Year were studied, at Korean sweet potato Pumpkin variety, two planting epochs: 30 May and 20 June. The tubers received from Korea have been planted in the greenhouse, on April 19, in order to obtain the shoot, necessary setting up culture in field. Planting was carried out in a greenhouse in shaped layer, protected in tunnel with transparency PE mulch and shoots were harvested at the stage of 30 cm, with 6-7 nodes in two stages, a day before planting in the field. After harvest, shoots were shaped by creating a slanted cut, at a distance of 1 cm from the first node, being kept by for 24 hours at a temperature of 20 °C and, then, planted the second day after the 17-18⁰⁰ hours. At planting and during the growing season was assured moisture content in the soil at a minimum threshold of 80% of active moisture interval. The determinations of plant physiology were performed by measuring with the device LCpro + Photosynthesis Portable System and quality analysis were carried out in the laboratory, using standard methods of analytical chemistry.

# **RESULTS AND DISCUSSIONS**

The climatic conditions in 2012-2013 period, reveals an accentuation of atmospheric drought, highlighting an increased by approx. 2 ^oC of the average monthly temperatures in the vegetation period of sweet potato and a lower precipitations of 55 mm than the average multiannual (1956-2013), (Figure 1). Analyzing the average temperature of the air in 2012, there is a limit to variation in temperature, between 17.2 ^oC in May, and 26.4 ^oC, in July, with an average of 22.3 ^oC. In 2012 Year sweet potato he held biological cycle in conditions accumulation of the 2066.4 degrees biologically active (Table 1). Precipitations and relative air humidity recorded during this period was insufficient for plant growth and development, it is necessary to provide soil with water, through irrigation until the minimum threshold of 70% of active moisture interval.

The determinations regarding the process of photosynthesis in sweet potato plant (Figure 2) highlight the importance of genotype and method of cultivation. We notice an diurnal variation of photosynthesis, in relation to the environmental conditions at the surface of the leaves (temperature, atmospheric pressure). The photosynthesis rate has registered a maximum of 30.21 micromol  $CO_2 \text{ m}^2\text{s}^{-1}$  at 9 o'clock, when the leaf area there was a temperature from 33.6 to 34.8 °C and a photosynthetic active radiation = 1204-1400

micromol. Increase the temperature, to 42.6 to 44.7 ° C, generated the closing of stomata and inhibition of photosynthesis and, simultaneously, increasing the plant transpiration (Figure 3). Pum variety used of best the water lost through transpiration, recording the highest rate of accumulation of  $CO_2$ . The environmental conditions created by mulching with transparency PE mulch, have been better utilized at lower temperatures, so that the temperature increase at 16⁰⁰ hour the photosynthesis rate has registered higher values in unmulched variants. The physiological determinations during for the intensive rearing shoots of the three sweet potato varieties, planted in different versions, highlights a significant reduction in the rate of photosynthesis, together with intensification of transpiration (Figure 4). The correlation between the two physiological processes are significantly negative (r = -0.570 *). Between the three genotypes of sweet potato grown in sandy soil conditions, the best production results are obtained at Pum genotype (Table 2), with an average of the two methods of 19697.5 kg / ha tubers and 17043 kg / ha haulm. The climatic conditions of the study period, with high temperatures throughout the summer and very little precipitations, sweet potato roots were well developed as protective mulch, which has preserved soil moisture; the production obtained is of 20427 kg / ha. All genotypes studied had yellow pulp.



Figure 1 Climatic conditions prevailing at weather station at RDCFCSS Dabuleni

Table 1

Climatic conditions during the growing season of sweet polatoes, 2012											
Climatic element / month			Mo	nths		Period 08.V. 2012 -10. X. 2012					
	V	VI	VII	VIII	IX	Х	Average	Sum	∑>10 ( ⁰ C)		
Average ( ⁰ C)	17.2	23.3	26.4	24.3	19.9	22.9	22.3	3508.3	2066.4		
Maximum ( ⁰ C)	31.8	37.8	41.4	42.6	32		42.6		-		
Minima ( ⁰ C)	7.9	9.8	12.4	6.5	4.4		4.4				
Precipitations (mm)	93.8	32.4	8.2	21.8	8	0	-	160.6			
Relative air humidity (%)	76.8	65	47.7	52.8	58.5	51.6	58.7	-			

1. . .





Figure 2 Daily variation of photosynthesis, on some sweet potato genotypes, in the growth phase of shoots

Figure 3 Daily variation of transpiration, on some sweet potato genotypes, in the growth phase of shoots





Figure 4 The correlation between plant transpiration and photosynthesis in sweet potato planted in sandy soil conditions

Table	2
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riouucu	Froduction of tubers and biomass registered in sweet potato, planted on sandy son										
Experimental variant		No. plants / m ² existing	No. of	Productio	Pulp						
		at the harvesting	plant	Tubers	Haulm	color					
	Pum	2.89	39	20427	17817	Yellow					
Mulch	Hwang	1.33	32	17301	11508	Yellow					
	Bam	0	-	0	0	-					
	Pum	2.68	22	18968	16269	Yellow					
Unmulched	Hwang	1.33	47	13172	6468	Yellow					
	Bam	0.33	40	5436	3095	Yellow					

Production of tubers and biomass registered in sweet potato, planted on sandy soil

Of the two planting epochs experienced in 2013, was evidenced by a higher clamping shoots and intense rhythm of the plant growth to planting on 30 May (Table 3). Determinations the dynamic of biomass accumulation in tubers, highlights an increase of the weight tubers and of the length haulm, depending on the number of days after planting (Table 4). The measurements performed at 19 September 2013, highlights the formation of

1070.5 g tubers/ plant, at sweet potatoes planted on 30 May and 880.3 g tubers / plant, at sweet potatoes planted on 20 June, with an average weight of a tuber 118.9 g and 88 g.

## Table 3

Specification / calendar date of	Pla	nted at May,	30	Planted at June, 20			
determinations	22.08	10.09	19.09	22.08	10.09	19.09	
Number of days after planting	84	103	112	63	82	91	
Length haulm	170,2	203,8	210	123,5	163,1	187,3	
No. tubers / plant	13	10	9	3	9	9	
Tuber weight / plant (g)	975	990	1070	153	660	850	

Influence of planting epoch on the behavior of sweet potato in sandy soil conditions

Table 4

Determination in dynamics regarding productivity of sweet potatoes planted in sandy soil conditions

Planting epoch	% of clamping plants	Determinations carried out to July 27					
		Number of days after planting	The average temperature in air (C ⁰ )	Length haulm (cm)	Growth rhythm (cm/day)		
May, 30	95.5	58	22.6	146	2.51		
June, 20	70	37	23.01	88.8	2.39		

Biochemical composition of sweet potato tubers is largely influenced by the characteristics of varieties and growing conditions. The research results obtained, presented in Table 5, show that in the variants mulch has accumulated a larger amount of soluble solids, as a result of the reduction in the intensity of the respiration process, emphasizing in this sens Hwang variety (13.6% of soluble solids). The acidity of sweet potato tubers was very low (0.23 to 0.26%), without being influenced by the method of cultivation. Under the aspect of the glucides content in tuber, the highest values were obtained for the genotype Pum and Hwang, in mulch culture (10.12% and 11.2%). Vitamin C content showed values within the 13.2 to 18.48 mg/100g fresh substance, being influenced mainly by cultivated variety.

# Table 5

method										
	Method of cultivation	Water	Total drv	Soluble drv	Titrable acidity	Glucides	Vitamin C			
Variety		%	matter %	substance %	%	%	mg/100g fresh substance			
Pum	Mulch	62	38	12.2	0.26	10.12	17.6			
	Unmulched	73.16	26.84	11.3	0.26	9.38	13.2			
Hwang	Mulch	67.21	32.79	13.6	0.23	11.2	13.2			
	Unmulched	70.51	29.49	12.7	0.26	10.54	16.72			
Bam	Mulch	-	-	-	-	-	-			
	Unmulched	65.78	34.22	11.5	0.23	9.55	18.48			

Biochemical composition of sweet potato tubers in relation to genotype and cultivation method

# CONCLUSIONS

Under the conditions of sandy soils, sweet potato developed biological cycle by the accumulation of 2066.4 degrees biologically active. The Pum variety, has harnessed the best water lost through transpiration, recording the highest rate of accumulation of CO2.

In sandy soil conditions from Dabuleni the best production results were obtained from genotype Pum, in the protected version with transparency PE mulch (20427 kg / ha).

Planting as early of sweet potato, revealed a higher percentage clamping shoots an intense rhythm of plant growth and higher productivity. Biochemical composition of sweet potato tubers was significantly influenced by the characteristics of the varieties and growing conditions.

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# THE ECO-TOXICOLOGICAL INFLUENCE OF THE CHLOROTHALONIL PESTICIDE / FUNGICIDES IN SOME VARIETIES OF CARASSIUS AURATUS GIBELIO BLOCH SPECIES

Fodor Marioara^{*1}

Keywords: pesticide, insecticide, fishes

## ABSTRACT

The laboratory experiment was focused on highlighting the toxic effect of Chlorothlonil, pesticide/fungicide, over some specimens of Carassius auratus geibelio Bloch fish species, circumstance which generated metabolic changes, behavioural, but also "swelling" of the investigated organism`s body.

The excess use of pesticides in agriculture generate a significant debate as a point of view of their utility problems, due to the toxicity and the big degree of retain, even bio-amplification, reason for which the general accepted option is to replace them with ecological pesticides. As a result, the topic of this paper is extremely important nowadays, which is concentrated with the ecotoxicological impact of some pollutant substances on living organisms with particular accent to fish.

## **INTRODUCTION**

The unreasonable application of pesticides constituted an important source of soil pollution and by irrigations or rain all these toxic substances have reached the level of water, affecting all living organisms. (Brezeanu, Gh, Simon - Gruița A., 2002).

It is appreciated, without a mistake, that the health of human beings can strongly be affected by ingesting some fish species contaminated with such unhealthy substances.

Xenobiotic substances (pesticides) have been known to affect physiological processes of living organisms (fish) as well as influencing the growth and development. (Mălăcea I., 1969, Petre M., 2003).

Therefore, those tests performed with Chlorothalonil pesticide might give answers related to toxicity, bio-accumulation, and metabolic changes of living organisms.

# MATERIAL AND METHODS

The biological material used for this scientific research is represented by young specimens of *Carassius auratus gibelio Bloch* species, which are part of the class Actinopterygii, *subclass: Teleostei, order: Cypriniformes, family: Cyprinidae, kind: Carassius.* 

These specimens have a large body (important aspect for investigations) and adapt easily to laboratory conditions (closed environment – aquariums, artificial food).

As a standpoint of life conditions, these fish are not particularly affected by certain environmental surroundings, therefore poor oxygen habitats from lakes or even running waters are the main places in which this species are found.

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Moreover, due to the fact that this fish can live in waters which are extremely poor in oxygen with a different range of pH, they can resist to air conditions if taken out from water (several hours), and also to extreme temperatures of up to 40°C in which the quantity of oxygen is relatively low. They usually feed with larvae, crustaceans, plants, molluscs, eggs etc (http://www.*crap.ro*/pagina/74/305/carasul.html).

## Used method:

► Winkler – for the determination of the oxygen dissolved in water and the calculus of the respiration metabolism, two types of fishes were subjected to analyse (*Carassius auratus gibelio Bloch*) Mălăcea I. 1969, Strungaru, Gr., Pop, M., Hefco, V., 1983 Marinescu, Al. G., 2000).

# DissectionsDirect observations

## **RESULTS AND DISCUSSIONS**

The biological experiment was based on making a lot composed of seven fishes belonging to *Carassius auratus gibelio* Bloch species, with an average of 17.5 g which were exposed to the action of Chlorothalonil, in a concentration of 0.0002 ml/l water.

All laboratory studies were conducted in a specific environment, with temperatures between 17-20  0 C and all fish have not been fed throughout the duration of the examination, the main aim of the experiment being to determine the levels of oxygen consumed.

In addition, the calculations in order to find out the levels of used oxygen were performed using the *Winkler method* for each fish independently. As a result, each specimen is represented with a separate line on the following common graph.



Figure 1. This graph shows the level of oxygen consumed by all seven fish separately, belonging to Carassius auratus gibelio Bloch species which were intoxicated with Chlorothalonil in a concentration of 0.0002 ml/l water.

Following the eco-toxicological investigations related to the action of Chlorothalonil fungicide in a concentration of 0.0002 ml/l water performed on fish belonging to *Carassius auratus gibelio Bloch* species, the following statements and points of view can be formulated.
The respiratory metabolism decreases sharply, energetic, and is highlighted by a decrease of the oxygen consumption over the first two days after the pesticide has been added to water (Fig 1).

Therefore, when the first day is being considered after the intoxication, a decrease of oxygen consumption has been identified with a percentage of 55.02% compared to the control value (M) which is 190.1 ml  $O_2/Kg/h$  and the maximal value of oxygen usage has been recognized after two days, this being 75.93 ml  $O_2/Kg/h$ .

After two days, the oxygen consumption value remains low until the end of the experiment (Fig 1), and before death, the specimens have been found in a latency state with slow reactions even to other stimulus.

As a result, it is appreciated that throughout all this last period, there are no variations of oxygen consumption and therefore nor the energetic/respiratory metabolism undergo changes, which denotes how good the experiment was carried out.

The decrease of oxygen usage is amplified also by the lack of food throughout the investigation and the extremely low doses of pesticides used have a minor influence, without producing multiple and rapid symptoms.

In other words, a minimal adaptation of specimens happened in relation to small doses of toxic substances as a defence reaction which includes the physiologic plan. Within water, no other substances were found to amplify or annihilate the pesticide and therefore there were no metabolic changes.

The species used for the investigation was fairly resistant to toxic substances and as a result, there is a significant period of latency until the fish die (in other words, the specimens have a latent death).

Additionally, it is appreciated that the respiratory metabolism (as a standpoint of oxygen consumption) of all specimens tested is relatively similar. Also, a similar ethologic behaviour was found for all fish.

Intoxication with big doses of Chlorithalonil (pesticide/fungicide), respectively 10ml/l water, of some specimens of *Carassius auratus gibelio* Bloch fish, in order to induce a fast death. The aim of the experimental procedure was to intoxicate the biological material under a short period and performing some disections to observe the difference between the tested specimens and the control (M), but also to prelevate biological samples to show the level of intoxications, the spread of the toxic substance (pesticide/fungicide) within blood, muscle tissue and internal organs.

The doses of pesticide used (10ml/l water) were bigger to induce death and also to observe how fast the substance spreads to blood and to smooth or striated tissues.

After all specimens died, the fishes were left another 1 hour and 30 minutes in water with pesticide (Chlorothalonil), to investigate the degree of impregnation of tissues with the active substance.

This second fish (Figure 3) appears to be inflated/"swollen" in an antero-posterior or ventral plane which dennotes intoxication. Figures 3 and 4 highlight the intoxicated fish after approximately 1 hour and 30 minutes after death, and suggest the inflated/"swollen" appearance, fact which was already confirmed by the literature (Mălăcea I., 1969), this being a typical case for all intoxicated fish.



Figure 2. First fish (1) belonging to *Carassius auratus gibelio Bloch* species – no toxins/ control (M) (original picture). This fish has a length of 17.5 cm and represents the control with no toxins



Figure 3. This fish (2) belonging to *Carassius auratus gibelio Bloch* species was intoxicated with Chlorothalonil pesticide/fungicide in a concentration of 10ml/l water (original picture). The length of this fish is 22 cm



Figure 4. This fish (2) belonging to Carassius auratus gibelio Bloch species was intoxicated with Chlorothalonil pesticide/fungicide in a concentration of 10ml/l water (original picture)



Figure 5. This fish (1) belonging to Carassius auratus gibelio Bloch species – no toxins/control (M) – dissection (original photo)



Figure 6. This fish (2) ) belonging to Carassius auratus gibelio Bloch species was intoxicated with Chlorothalonil pesticide/fungicide in a concentration of 10ml/l water / disection (original picture)



Figure 7. This second intoxicated fish is a female (due to the presence of roe) and after the dissection a slight darker colour of striated musculature was seen, as Figures 6 and 7 suggest.

This fish which has not been intoxicated (Figure 5) is a male (due to the presence of milt) and following the dissection there were no changes of colour of the muscles: striated, smooth-internal. There is no inflated/"swollen" apperance as well.

#### CONCLUSIONS

All fish belonging to *Carassius auratus gibelio* Bloch species intoxicated with Chlorothalonil in a concentration of 0.0002 ml/l water suggest the presence of a sharp decrease of oxygen consumption for the first day.

The maximal decrease of oxygen usage has been recorded over the first and second days after intoxication.

After the third day of intoxication, the oxygen consumption remains still low.

The decrease of oxygen usage is underlined not only by the fact that all fish have not been fed over the period of the experiment, but also by the fact that all doses of pesticide were extremely low and the time needed for the action of those substances is relatively lent.

Moreover, a minimal adaptation of those specimens has happened to extremely low doses of toxic substances as a defence reaction, including the physiological one.

The species subjected to the experiment is relatively resistant to pesticides, in other words those fish die in a longer period.

When bigger doses of Chlorithalonil pesticide (fungicide) are used to intoxicate *Carassius auratus gibelio* Bloch species, respectively 10 ml/l water, they have a relatively faster death.

If those specimens intoxicated are being kept for an extra 1 hour and 30 minutes within the toxic substance, this phenomenon leads to the inflated /"swollen" appearance of fish.

After dissections, not only the swelling phenomenon of specimens, tissues, organs was observed, but also the change in colour of striated and internal musculature in comparison with the control.

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# THE METABOLISM OF CARASSIUS AURATUS GIBELIO BLOCH SPECIES INTOXICATED WITH XENOBIOTICS (PESTICIDE, THIAMETHOXAM)

Keywords: pesticide, insecticide, fishes

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#### ABSTRACT

The toxicological effects of pesticides expressed on specimens such as Carassius auratus gibelio Bloch are extremely complex, being in a directly proportional relationship with the explosive evolution of chemical compounds on sale.

As a result of xenobiotic intoxications, (pesticides, Thiametroxam), in a dose of 30 mg/l water on Carassius auratus gibelio Bloch species, major metabolic changes are produced and further dissections performed on overdosed specimens with the same pesticide, in a lethal dose of 30 mg/100 ml water, can lead to direct observations and comparative, interpretative analyses.

## **INTRODUCTION**

The pollution of water with xenobiotic substances (pesticides) generates multiple modifications at the level of fish species, one of the most important being the eco-toxicological nature, physiological (including metabolic), ethological, bio-chemical, sanguine and other.

Interestingly, the main effect is recognized as being the toxicological consequence, but also the remaining substances within those fish subjected to examination or the transmissibility to humans in the case of ingestion of those species intoxicated with pesticides (Botnariuc N. & Vădineanu A., 1982).

Furthermore, in order to protect the quality of water and the fish stock, it is important to know the toxicological action of organic substances on fish, in this case xenobiotics, pesticides (UE, 2006).

Thus, the existence of organisms is determined by the nature and the conditions of life, even if sometimes the appearance or disappearance of different species is considered to be an adaptive necessity which makes possible the development of increased resistance specimens.

In addition, one of the main purposes of hydrobiology (as an interdisciplinary scientific side) is a detailed study of the potential or the state of stability of ecosystems. This case referring to those sweet aquatic (Brezeanu, Gh & Simon - Gruița A., 2002).

Moreover, one of the principal topics of investigation, analysis and scientific research is represented by the study of the eco-toxicological impact of polluting substances (*pesticides*) on specific species of fish, with direct approach to freshwater specimens.

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Therefore, their adaptive limits to contaminated xenobiotic hydric medium conditions is relatively interesting, and, as a result, it is appreciated how crucial the science contribution is to solve the problem liked to the stability of aquatic ecosystems.

As a specific measure of defence in relationship with any aggressive-polluting forms, living organisms had developed specific response self-mechanisms to any toxic actions. Some of them are associated to a physiological nature e.g. metabolic, ethologic or dynamic as a standpoint of sensorial movement and migration towards less polluted aquatic zones with different temperatures etc.

All of these have the main aim to make organisms resistant to different pollution factors as a point of view of adaptation and reproduction of species.

Due to obvious reasons, specific specimens which are extremely sensible or less adapted usually disappear, being replaced with others with a better resistance in a direct specific relationship of maintaining the ecological equilibrium with trophic adequate chains and links. (Brezeanu, Gh & Simon - Gruița A., 2002).

#### MATERIAL AND METHODS

The biological material used for scientific research is represented by young specimens of *Carassius auratus gibelio Bloch* species, which are part of the class Actinopterygii, *subclass: Teleostei, order: Cypriniformes, family: Cyprinidae, kind: Carassius.* 

Winkler – for the determination of the oxygen dissolved in water and the calculus of the respiration metabolism, two types of fishes were subjected to analyse (*Carassius auratus gibelio Bloch*) Mălăcea I. 1969, Strungaru, Gr., Pop, M., Hefco, V., 1983 Marinescu, Al. G., 2000).

The method of dissection, direct observation, comparison between intoxicated species in relation with the control.

## **RESULTS AND DISCUSSIONS**

Extremely interesting are the toxic-dynamic forms of study, representing the way and the mechanisms of action of toxic substances, resorption processes, remanence, as well as generated effects for example: catabolic and anabolic modifications of enzymatic systems, the metabolism of toxic substances in this case pesticides at different concentrations, adaptation to increased substance concentration, accumulation of toxic material within tissues (tegument, muscles and bones) as well as detection using sensing organs in relation with an alteration of aquatic medium, the possibility of mucus secretion.

Highly important are all modifications appeared when the intoxication of all specimens subjected to analyses was taking place, some of them visible with the naked eye for example tegument lesions similar to those behavioural such as: agitation, anxiety, a change in the position of body or even aggressiveness of one against another.

Also important are all observations based on the dissected specimens, which were intoxicated with a bigger dose of pesticides leading to an achievable visual comparison of tissue colours and internal organs in relationship with the control.

In addition, another part of the research can be the detailed investigation of absorption way and the specific action of xenobiotic (pesticide), intra- and intercellular diffusion, elimination or non-elimination of that certain substances as well as the repair of toxic elements.

Thus, with this conditions, *adaptive capacity* of living organisms is suppressed with specific defence reactions. Therefore, sometimes, these reactions are represented by

*physiologic reactions, implicit adaptive metabolic, respiratory, secretory* (of mucus for fish instances), *ethologic* (to avoid the zone where the toxic is found) etc.

Additionally, there is the possibility of generation of adaptive reactions which can denote survival within different conditions, unfavourable and to give rise to specific methods of saving the species. However, this idea does not have to lead us to save organisms by themselves, but to the possibility of condensing all our human beings forces in order to prevent and counteract any forms of pollution even those anthropic.

One of the experiments performed to highlight the toxicity of pesticides was focused on intoxications of some specimens of *Carassius auratus gibelio* Bloch with a bigger dose of pesticides, to underline better the modifications of metabolic order.

Thus, seven young specimens, healthy, non-parasite were used as an experimental lot.

All data and metabolic results (expressed using oxygen consumption), but also the behaviour of intoxicated specimens with 30 mg/l pesticide/ insecticide Thiamethoxam, lead to different values of metabolism expressed by the oxygen consumption. For each intoxicated specimen there is a separate graphical representation, and added together can be showed within a single graph with 7 diagrams (Fig. 1)





Therefore, the interpretive analysis of all seven diagrams – Fig.1 (*which underlines the oxygen consumption for each specimen separately Carassius auratus gibelio Bloch, including the level of respiratory metabolism*), indicates evolutions and representations of a similar behaviour, suggesting that the experiment was valid.

For all seven intoxicated specimens each separate line showed on the graph has the same characteristics.

Beginning with a control (M) from the first day, the general tendency of all seven specimens was a decrease of oxygen consumption until the third day, inclusively.

The same pattern of all the seven samples (graph) denotes how good the experiment was performed as a standpoint of toxicology and physiology.

The samples of the lot belonging to species *Carassius auratus gibelio* Bloch had an average weight of 12.5 g, were subjected to the toxicological action of pesticide (insecticide) Thiamethoxam, with a concentration of 30 mg/l water, had a same behaviour (with small different tendencies) in comparison with the control (M) on all graphic points.

As a result, within the first day of the intoxication (24 hours), a decrease of oxygen consumption was highlighted with an increased percentage of 27.23%.

This data underlines the immediate eco-toxicological influence of pesticide (insecticide) Thiamethoxam on young organisms subjected to examination and the sudden influence as a standpoint of decreased metabolism in comparison with the oxygen consumption.

Additionally, another idea that can be deducted from the Figure 1 can be the immediate physiological action of self-regulation, as well as multiple biochemical reactions of adaptation to toxic environment, regardless of the nature. Furthermore, adaptive reactions are immediate and there is a tendency of maintaining them over all duration of the toxicological test, on a descendant pattern.

The decrease of the level of consumed oxygen is significant for the following days, continuing with the second day (with a percentage of 25.85%).

In other words, according to the diagram (common graph), for the first two days (48 hours) there are tendencies for all specimens to decrease the level of oxygen consumption.

After that, for the following days, until the 14th day, the tendency was similar to a small fluctuation or oscillation, either in an ascendant way for example from the second to the third day with a percentage of 2.74%, or in a descendant way from the third to the fourth day with a percentage of 5.29%.

This inclination of both increasing and decreasing was maintained until the end of the experiment.

Finally, after 14 days from intoxication, all fish subjected to examination had a decrease of oxygen consumption of 46.22% in comparison with the control (M), which had the value of  $186.5 \text{ ml O}_2/\text{Kg/h}$ .

Therefore, it is appreciated that, with the same dose of toxic substance, the behaviour is similar for all specimens used for analyses.

Dissections and comparative examination of overdosed specimens with 30 mg/100 mL water with the same pesticide.

The second specimen dissected (Figure 2, second fish), was intoxicated with Thiamethoxam, in a concentration of 30mg/100ml water, for 30 minutes, in order to demonstrate the lethal action after the fish has been introduced in water.

For a good visual interpretation of the differences between the two fish, the intoxicated one has been immersed for 30 minutes in the toxic solution, to allow the muscles and internal organs to impregnate with the toxic substance and to change the colour.

From this picture, the first dissected fish - no toxins (Fig. 2 - first one) has a normal colour (pink to beige) of striated muscles, and in the interior, similarly, the red colour of internal organs can be seen.



Figure 2. The disection of two Carassius auratus gibelio Bloch fish of which one was intoxicated with pesticide Thiamethoxam, with a dose of 30mg/100ml water (the second one) – (original picture)

The method of direct observation (with naked eye) highlights the difference of colour between the striated muscular tissues and the smooth muscular (abdominal, internal organs), intoxicated, in comparison with the normal.

The second fish – intoxicated, underlines the beige to orange colour of striated muscular tissue and brown of internal organs. Similarly, another observation can be stated as after only 30 minutes, the internal organs have a bigger volume in comparison with the normality, fact which is confirmed by theory (Mălacea I., 1969), the process being named « bloating » or inflation.

#### CONCLUSIONS

Ethologic, all samples of the experimental lot had same behaviour, for the duration of all intoxications with pesticides. At the beginning of the experiment, a lightly aggressive situation has been identified when all tested fish were examined.

This experiment underlines the toxicological effect of pesticide / insecticide Thiamethoxam, in a concentration of 30mg/l water, on specific specimens from the species *Carassius auratus gibelio* Bloch, as a standpoint of a change in the oxygen consumption, respectively, respiratory metabolism (energetic).

After the third day, until the end of the test, a decrease of oxygen has been identified, which is relatively slow, respectively, with slight alternations or fluctuations.

It is appreciated that a steady decrease of oxygen consumption until death occurs, represents a physiological action of self-regulation, which induces multiple biochemical reactions of adaptation to toxicity, no matter of the nature.

Furthermore, adaptive reactions are immediate which tend to be maintained for all duration of the toxicological test on a descendant pattern.

The fact that after the second and respectively the third experimental day, all specimens attempted to adapt in the same way by a small decrease of oxygen, this denotes a common idea that all species have the same characteristics for any type of toxic substance.

In the case of intoxications with Thiamethoxam, in a concentration of 30mg/100ml water (lethal dose) for some specimens of *Carassius auratus gibelio* Bloch, significant observations can be observed, as a point of view of a change in the colour of the striated and smooth muscles and internal organs, but also the process of « bloating » or inflation in comparison with control M.

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## INFLUENCE OF THE CONSTRUCTION MATERIALS INDUSTRY ON THE SOIL

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Keywords: building materials, cement, soil pollution, calcium carbonate, pH.

#### ABSTRACT

Construction materials has at its background the processing of natural rocks such as silicates, clays, magnesite, limestone, gypsum and so on, the most polluting being the industry of cement, asbestos, and gypsum. The cement industry is one of the most important in terms of air pollution (Dhir, 2011) often giving a typical appearance to the surrounding lands (Rojanschi, 1997).

Cement production increased atmospheric protection issues and local geography, although there have been taken measure, both in terms of raw materials used and the implementation of advanced processing technologies (Lazaroiu, 2006).

In the present paper, there were determined the spreading distances of cement dust and how it affects the soil. Cement dust causes pH changes and accumulation of calcium carbonate, determinations being performed on 0-20 cm depth. The soil types analyzed shows a variable pH, which dropped to the same depth. Cement powder is inversely related to the level difference and the distance from the source.

## **INTRODUCTION**

Air pollution through its impact on the biosphere, but also on water, is one of the major problems of human society at this end of the millennium. The air surrounds us everywhere; the quality of our existence depends on air quality, especially in the context of industrialization and urbanization, which have changed the basic structure of the environment (Damtoft et al., 2008). The atmospheric air is one of uncontrollable environmental factors, (Moldoveanu, 2005) as pollutants once in the atmosphere; it dissipates quickly, and cannot feasibly be captured to undergo remediation-treatment operations (Lăzăroiu, 2006).

There are known the primary air pollutants, the adverse effects on plants, animals and human reactions occurring in the body (Costache &Modrogan, 2006) and sources of its origin. Therefore, the fight for clean air is now a cause for global concern.

Particulate materials that pollute the atmosphere in the county have a different character according to the sources that generate those (Gavrilescu & Popescu, 2012) namely: fossil fuel power plants, mining pits, deposits of slag and ash dumps, traffic, building materials manufacturers and residential heating for the cold season.

Thus for the area Bârsești the potential polluters are: SC SIMCOR VAR SA Oradea - Tg. Jiu Workstation, SC MACOFIL SA, SC LAFARGE CEMENT ROMANIA

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SA - Tg. Jiu Plant and traffic. Lafarge Cement produces nine common types of cement. They are divided into two categories: Portland cement and Portland cement composites. Portland cement CEM I, CEM I 52.5R, CEM I 42.5R, have a high resistance (Xiaocun et al., 2012) and have a wide range of use: it is used in the prefabricated concrete industry which requires high class concrete, as well as concrete/mortar (Cheung et al., 2011) used for short-term repairs, injections and special pasta, which can be used in road pavements for roads, highways and airports, prefabricated industries, autoclaved aerated concrete and adhesives. Composite Portland cement (CEM II) with high initial resistance contains additives (Dhir, 2005) which can be: inorganic mineral materials or derived from the manufacture of clinker as slag, ash, limestone, ultrafine silica etc.

#### MATERIAL AND METHODS

The study was performed in 2012 in Gorj county, city or Barsesti, at SC Lafarge Cement SA. The soils from which there have been collected samples are eutricambosoil on clay and mezocalcaric type. Sampling depth was 0-20 cm for agrochemicals samples, and for pedological soils up to 50 cm. Directions of sampling were EW and NS, depending on the direction and velocity of prevailing winds. Measurements were made using equipment of Laboratory Quality of environmental factors from our faculty; using a pH meter Hanna and calcium carbonate was determined according to the methodology ICPA.

### **RESULTS AND DISCUSSIONS**

Due to its geographical position, Targu Jiu area benefits from a temperate continental climate moderated by hills, with Mediterranean influences. Climate elements that characterize are specific to Targu Jiu Depression - Campu Mare. Data were taken from A.N.P.M. Gorj.

The value of annual average air temperature in the city of Târgu Jiu is  $11.04^{\circ}$ C. The average annual temperature varies between  $10.2^{\circ}$ C and  $11.9^{\circ}$ C, over the period analyzed and shows one more time the continental climate.

Continental climate is manifested by an uneven distribution of rainfall during the year. The monthly distribution of rainfall varies depending on the frequency and direction of baric systems of air masses and fronts. Rainfall has the same influence as the influence of continental air, which are mostly in the form of rain, but inconsistent across Targu Jiu. Precipitations are characterized by a high variability in time and space. The trend in annual average monthly precipitation amounts recorded in Targu Jiu, a continuous increase in February (41.1 mm) to July (108.9 mm) and a constant decrease until December (60.1 mm).

The main winds are Western winds and foehn phenomena (Vântu Mare). Analysis of this climatic parameter ranged from 1.7 m/s in August and 3.8 m/s in March. Analyzing annual wind rose, we find that the highest frequency have winds from the north-east (11.8%) and south west (11.4%) and the lowest frequency has south-east wind sector (2.3%) (Figure 1).

Within the industrial dusts (powders) a special place is occupied by pollution due to cement factories. It is estimated that the dry percentage at grinding stage removes dust in the atmosphere as 1-3% of processed raw material (limestone + clay). It is dispersed over large distances resulting in deposition of 500-1000  $t/km^2/year$  in the surrounding areas, reducing the transparency of the atmosphere and reducing the processes of photosynthesis, leading to reduced agricultural production and, last but not least, environmental degradation and the appearance of respiratory diseases.

One of the properties of the dust particles is sedimentation, which characterize the relapse of dust on the soil. Factors governing sedimentation are turbulence state of the

atmosphere, wind speed, altitude emission, speed vertical climb emissions, mass and particle size. The inert particles may be adsorbed onto the surface of toxic substances.



Figure 1. Wind rose (after A.N.P.M. Gorj)

The residence time of cement dust into the atmosphere is 3-7 days. As can be seen in Figure 2, the soil pH decreased from alkaline to neutral, weak acid together with distance from the source, regardless of the direction of the wind. As can be seen in Figure 3, CaCO₃ decreases with distance from the source, from 3.7% at 250 m to 0.6% at 1000 m in the direction of SW-NE. If wind direction is NE-SW, CaCO₃ accumulation is not observed only up to a distance of 500 m. Both the CaCO₃ and pH were determined by the depth of 0-20 cm.



Figure 2. Variation of soil pH according to emission source (cement dust) and the predominant wind direction



Figure 3. Soil CaCO₃ concentration according to emission source (cement dust) and the predominant wind direction







Figure 5. Variation of pH according to the soil depth (eutricambosoil mezocalcaric)

Soil types analyzed (Figure 4-5) have a variable pH, which decreases in the case of eutricambosoil on clay from 8.2 to 5.9 (30 cm), with an increase on C horizon, and for the eutricambosoil mezocalcaric from 7.9 to 5.4 on same depth.

Calcium carbonate is present in the first two horizons in the case of eutricambosoil on clay and only in the first horizon in the case of eutricambosoil mezocalcaric. Value of 2.4% CaCO₃ registered on horizon C is characteristic of the soil type (Figure 6, 7).



Figure 6. CaCO₃ concentration according to depth of the soil (eutricambosoil on clay)



Figure 7. CaCO₃ concentration according to soil depth (eutricambosoil mezocalcaric)

#### CONCLUSIONS

Cement dust dispersion cause environmental degradation and health problems of the population in the area bordering source pollution such as S.C. Lafarge SA. Wind is characteristic of cement dust spread into the environment (wind direction has the highest frequency and intensity EW). Cement dust accumulation in the soil (in the first 20 cm) is as follows: up to 500 m there are accumulations in both directions (EW, NS); after 2500 meters cement dust is no longer accumulating.

The depth to which the cement dust enters is related to the level difference. Calcium carbonate decreases with distance from the source. The pH value decreases from alkaline to neutral and weakly acidic with distance from the source.

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# STUDY OF GROUNDWATER QUALITY FROM RURAL AREAS OF OLTENIA

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Keywords: groundwater, pollution, rural areas, chemical indicators.

#### ABSTRACT

Limited amount of underground water sources, progressively increasing water requirements, the emergence of pollutants produced very stable, currently require a radical improvement in all categories of water management in order to ensure a balance between availability and consumer requirements. The purpose of this paper is apparent from the necessity of knowledge as opportunities for exploitation of groundwater smallholders and in order to supply their level of knowledge and vulnerability to nitrates.

In 2013 there were analyzed waters from wells in four villages in the rural area, located on different soil types. There have been found exceeding the maximum permissible concentration especially for nitrates, nitrites and ammonium ions, as well as significant differences between the waters of hilly area and along the valleys on groundwater.

#### **INTRODUCTION**

Pollution sources may have a higher or lower effect on groundwater. Due to the specific conditions of formation and movement of groundwater in the aquifers, their quality is determined by the geological structure of the layer crossed by hydrodynamic factors and certain anthropogenic factors.

In particular situations, through infiltration or directly, different pollutants can get in contact with groundwater, causing contamination and their depreciation. Accidents of this type are especially common in the low depth groundwater. (Sarala C, Ravi Babu 2013)

Intrusion of pollutants in these areas triggers a series of complex physical phenomena (adsorption, capillary retention, ion exchange), chemical (precipitation of various salts and the formation of gels) and biological manifested by biodegradation processes (Rojanschi & Bran, 1997).

Salt loading is accomplished by physical dissolution of the soluble salts: chlorides, sulfates, nitrates, etc. from the layer of soil above the groundwater layer, the solubility of mineral compounds of the aquifer, from the chemical reactions taking place at this stage, in the presence of water (Savin, 2008).

During infiltration through the soil some chemical components of the water undergoes significant changes that affect the quality of groundwater. Such modifications

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are well known: dissolved oxygen, nitrate, ammonium ion, sulphates and organic matter (Hua et al., 2012).

Industrial units polluting the aquifer in the area where they operate, typically build a network of observation wells, both on the platform of units as well as in the adjacent area. Even though in time there was a decreasing trend of the influence of pollution sources on groundwater aquifers due to the significant reduction in recent years in the volume of industrial production and agro-livestock production, but also by implementing measures wastewater, groundwater quality was still improper because of their slow rate of selfpurification (Cârţână, 2005).

From the most recent comprehensive evaluation, most intense forms of multiple depreciation remain in the quality of groundwater in some areas, especially in rural areas, because: the irrational application of organic and mineral fertilizers, liquid waste mismanagement (Peck et al., 2013) that reach the ground, both directly (through waterproof latrines, ditches and drainage channels etc.) and indirectly through slow infiltration (from manure storage, improvised household waste pits, etc.).

## MATERIAL AND METHODS

In this paper there were studied 4 villages located in Dolj: Castranova, Argetoaia, Orodel and Cornu. There were identified a number of wells on land within 500 m, water samples were collected, determining the physico-chemical parameters.

Samples were taken in 2013 using a portable kit for water sampling; determinations were executed on the spot using the Hanna pH meter, Hach titration and the DR 2010 spectrophotometer. With this device it is possible to analyze a number of elements in the water, such as nitrate, nitrite, ammonium ion, chlorine, phosphorous, iron, chloride, sodium, potassium.

The purpose of this paper consists of the need to know both the possibilities of exploitation of ground water by smallholders for both household and agricultural activities. Accordingly, any assessments, and forecasts that are not based on a thorough knowledge of these indicators would lead to false conclusions with direct implications for human health.

## **RESULTS AND DISCUSSIONS**

In 2013 water samples were taken from several wells located in four representative locations of Dolj County.

Castranova village is located in the south-eastern of Dolj County, among the Leu – Vişina road, bounded on the north by the municipality of Leu, Apele Vii village at east, village Mîrşani at south and village Bratovoieşti at west. Dominant soils are represented by degraded chernozem (north-east and west), reddish brown forest soils (southeast), sand, loess deluvial, with remnants of red clay sands and gravels.

We studied water of the village Castranova because of the history of this area. In 1900 Craiova started to modernize water network capture. The source was discovered at 35 km from Craiova, in the village of Castranova by french engineer Lydel.

Water was peculiarity both then as now. In the locality water samples were taken on an area of 500 m, from 5 wells. Average results are presented graphically. Groundwater presents relatively constant parameters. They are characterized by high mineralization, rich content in carbon dioxide and low oxygen concentration. As can be seen from the average values, analyzes characterized as being clean water and enter this in standards for drinking water (Figure 1, 2).

Argetoaia village is located on the stream Argetoaia, 45 km from the city of Craiova, Dolj County in the North West of Dolj County. The relief is of inter-piedmont

plain and valleys and is crossed by the river Urdinița. Soils are brown and brown podzolic, clay-alluvial soils, moderate podzolic.

Groundwater may contain elements whose concentration exceeds permissible standards for drinking or industrial use. A sample was collected from a borehole at a depth of 300 m, along the valleys of groundwater.



Figure 1. Average values of nitrogen content, chlorides, iron, sodium and sulfur (mg/l)



Figure 2. Average values of indicators: nitrogen, phosphorus, ammonia, chemical oxygen demand, pH, potassium and hardness (mg/l)



Figure 3. Average values of nitrogen content, chlorides, iron, sodium and sulfur (mg/l)

The quality of water depends on the composition of the field and usually refers to the content of iron, carbonates, bicarbonates,

ammonia, nitrates, and nitrites. In the case of Argetoaia village, there have been exceedances for nitrates, chloride, iron, and sulfate and ammonium ion. Water tastes muddy and brackish. Water flows from springs located on the hill slope, with a very slow flow in the valley, which gives rise to a marshy area, which produces water puddles. The water seeps into the ground from the valley to groundwater, leading to the appearance of this phenomenon (see Figures 3, 4).



Figure 4. The average values of indicators: nitrogen, phosphorus, ammonia, chemical oxygen demand, pH, potassium and hardness (mg/l)

Orodel village has the following neighbors: to the north - village Carpen, south - village Caraula, west – village Verbita and Plenita and east - Vârtop and Vela village. Orodel village is situated in the High Plain Bălăcița, at the southern edge of the Getic Plateau. Specific soils and leached chernozem soils are reddish brown (including transition). The villages which are part of the Orodel commune are located on land with different landforms. Some of them on the ground and others on lowland hills plateau from the north-west of the village. Wells from which water samples were taken are located on the plateau. From analyzes is observed maximum concentration exceedances of sodium, potassium, sulfur and chemical oxygen demand (Figure 5, 6).



Figure 5. Average values of nitrogen content, chlorides, iron, sodium and sulfur (mg/l)

At the same time samples were taken from the valley of Orodel villages (village Cornu part of Orodel) (Figure 7, 8).



Figure 6. Average values of indicators: nitrogen, phosphorus, ammonia, chemical oxygen demand, pH, potassium and hardness (mg/l)



Figure 7. Average values of nitrogen content, chlorides, iron, sodium and sulfur (mg/l)



Figure 8. Average values of indicators: nitrogen, phosphorus, ammonia, chemical oxygen demand, pH, potassium and hardness (mg/l)

Compared to the hilly area, water samples from wells located on valley presents average values exceeding the maximum permissible concentration at the following indicators: nitrates, nitrites, sodium, ammonia, chemical oxygen, potassium and sulphate. It may be considered that permissible values for waters of the fountains are exceeded for oxidability and ammonium ion concentration and this is because those waters are polluted due to infiltrations from livestock manure.

Also increased nitrogen content is due to leachate. As a result of this in the village Orodel, village Calugarei, there were conducted drilling for water collection and treatment plant (accessing European funds) for the purpose of providing inhabitants, safe drinking water.

## CONCLUSIONS

This paper presents studies on groundwater quality monitoring of 4 regions of Dolj County. Waters were taken from five wells within a radius of 500 m from each village.

There were exceedances of maximum permissible concentrations for chloride, sodium, potassium for Argetoaia and Castranova localities and for the samples collected from the village Orodel, the differences are significant in areas located in the hilly area compared to the valley. There are observed exceedances in the case of nitrates, ammonium ion, oxidability, potassium, sodium and sulfates.

It is required rigorous waste management and the construction, extension or upgrading of wastewater treatment plants, as the groundwater pollution, in particular, is an almost irreversible phenomenon and has serious consequences for the use of underground reservoirs supplying drinking water. Remediation of the groundwater sources is extremely difficult, if not impossible.

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# CONCEPTUAL SUBSTANTIATION ON INNOVATIVE PHOTOVOLTAIC PANEL WITH ADJUSTABLE DIRECTION IN THE THERMOPANE GLASS

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Keywords: solar energy, photovoltaic panels, microcellular system

#### ABSTRACT

Solar energy can be converted directly into electricity using the photoelectric effect, photovoltaic respectively. The purpose of this paper is to present an innovative model of double glazed windows with shutters photovoltaic panels. This model was build up in the Renewable Energy Laboratory of the University of Pitesti. It is a double glazing consists of two panes of glass of the same size - evenly spaced and isothermally bonded (with special silicone chit) and a system of slats made of photovoltaic microcellular, mounted in the space between the two glass sheets. For the patented solar panel were monitored parameters such as: room air temperature, temperatures of the primary and secondary plant heat flow coefficients, the temperature difference. Flow rates were measured punctual. Simultaneously, measurements and recordings were made of the meteorological parameters (temperature, humidity, solar radiation, wind) that allow the performing of thermal and global balance for building system – installation.

## **INTRODUCTION**

Solar energy source is inexhaustible, and is also one of the cleanest forms of energy. Huge quantities of this energy underlie all natural processes on Earth. Capture, conversion and storage of solar energy in some form (heat or electricity) are specific technological approaches (Paulescu M., 2005), Solar energy can be used with maximum efficiency and moderate investment for domestic hot water or in residential space heating (Asenjo S., 2010).

In the face of foreseeable major shortcomings, such as: the depletion of fossil fuels and the destruction of the ecological balance, solar energy appear as the most probable solution to replace the currently exploited energy sources (Marinescu, N. C., 2010).

The advantages of using solar installations are found in situations such as:

- Solar panels are easy to install;
- Solar systems have a degree of zero pollution;
- A solar system has a long service life (of 20 years)
- high security in installation and maintenance (no danger of fire, explosion);
- Provide utility hot water year-round, (in winter in combination with other heat source, reducing in this way the cost of consumed energy - approximately 65%);
- Installation and maintenance costs are minimal.

The main objective of this paper is to investigate a new concept of photovoltaic panel which use solar energy, in accordance with the structure and the characteristics of the tire, to obtain improved thermal regimes.

#### MATERIAL AND METHODS

During the course of the research was designed and studied a new solar panel system, patented by the author. The parameters such as: room air temperature, temperatures of the primary and secondary agents for heat plant, the flow coefficients, the differences in temperature were monitored for this system.

Simultaneous, were made and records measurements of meteorological parameters (temperature, humidity, solar radiation, wind) for performing real and global thermal balance in system building - installation.

The comfort parameters for civil buildings are: temperatures between 18-20  $^{\circ}$  C, 35-70% relative humidity and 0.15-0.25 m/s air velocity. The differences in temperature for wall should be less than 4.5°C and for terrace less than 3.5°C (SR EN ISO 13790/2005).

The photovoltaic panel with adjustable direction in thermopane glass is designed to capture sunlight and transform it into electricity. It is composed of two sheets of glass, having the same dimensions, separated from each other by a aluminium spacer, typically for thermopane glass, provided with granules of silica gel inside, bonded together with double-sided tape. The windows sheets are connected at their ends by a plastic bracket, according to the technology of manufacture and sealed with mastic, to achieve hermetic space between the two glass panes (figure 1).



Figure 1: The photovoltaic panel type curtain

#### **RESULTS AND DISCUSSIONS**

One of the results of this paper was the construction of the photovoltaic panel with adjustable direction in thermopane glass (figure 1), system designed and breveted by the author (Cicerone Marinescu, 2012). The novelty consists in the (construction) of a system of adjustable levers and rods in the space between the two windows. Rods and levers have dual role, namely: mechanical and electrical. The mechanical role is highlighted by the mechanical support of photovoltaic cells and angle configuration of cells. The electric role is highlighted by the electrical connection of cells and the reconnection of obtained electric current and forwarded by levers. Therefore, the concept aims to build a double glazing manufacturer of clean energy, higher efficiency, having low manufacturing cost. Designed system can be adapted to fixed construction (curtain walls, doors, windows) or used in combination with conventional double glazed windows or solar cell thermal panes.

In the second part, our study consists in the verifying of obtained parameters by using this new system of photovoltaic system. The experimental measurements were made throughout the day, starting at 8.30 until 18.30. To measure voltage it was used a multimeter and for measuring light intensity was used a light meter. It was five time repetition and the average value for the parameters has been noted in tables. The environmental parameters (wind speed, radiation and temperature) were taken from the database of the automatic weather station (located on the building of the Faculty of Science). The measurements were made from 30 to 30 minutes and the observations about the state of the atmosphere (clear or cloudy) have been noted. In figure 2 is presented the evolution of voltage according to the light intensity, throughout a day.



Figure 2. The voltage and the light intensity for the new photovoltaic system

There is a direct correlation between obtained light intensity and the intensity of solar radiation, as expected, with maximum values at the middle of the day (1105 lux at 12.30).

The panel type curtain produces an average voltage of 16.31 V. We want to know how many Wh would produce the curtain wall if it instead of normal windows will install windows with photovoltaic panels type curtain, incorporated. Making calculations for a curtain wall of 75 m2, the photovoltaic panel type curtain produces, in an hour, an electrical energy of 3.12 kWh. This energy can be used without requiring any cost, in addition to the investment. The described concept shows that the ordinary glass windows are turning into photovoltaic panel with low price in comparison to the known solar panels; furthermore, the production of high-efficiency electric power is done cheaper, without environmental pollution.

### CONCLUSIONS

It was design and realised a new type of system to promote technical solution in the area of new solar panels in installations which use combined renewable sources and/or substitute of energy.

The great advantage of this type of system is the architectural and technical adaptation to any construction. Furthermore, the presented system has a high degree of thermal isolation by reducing the glass flow inside the glazing.

The using of this new concept allows the transformation of consuming energy building into a passive/ generator of energy building.

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## COMPARISON OF THE MONTHLY AND ANNUAL TRENDS OF EXTREME AIR TEMPERATURES AT PITESTI AND MARACINENI IN THE PERIOD 1982-2011, VERSUS ROMANIA

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*Keywords:* climatic changes, fruit trees, phenological dynamics

#### ABSTRACT

In our study were analyzed climate changes, both in Romania by the average of 30 representative weather stations, uniformly distributed on the whole territory of the country, as well as the ones from Maracineni and Pitesti stations between the years 1982-2011 (30), by comparing the trends between them. We have tried so, to establish also, the representativeness for Romania of climate change recorded at Maracineni as well as to Pitesti. We have described for each climatic parameter, only annual and monthly average linear trends for 10 years, which were statistically assured ( $P \le 0.05$ ). Comparing the mean linear trends in 10-years of maximum air temperatures, it was observed that the range of the year with statistical insurance is located only in the summer period for Romania (0.88°C in June, 0.82°C in July and 0.70°C in August) and only in June and July for the two stations of Arges County analyzed. Regarding trends in minimum temperatures in all three summer months, the increasing trend was better statistically assured and greater in station analyzed, compared with the average for Romania (0.61°C in June, 0.67°C in July and 0.75°C in August).

#### **INTRODUCTION**

Global warming and associated regional climate changes have been intensively studied across the globe in recent years. Most of the scientific researchers agree that significant increases in global temperature ('temperature' in this paper represents surface air temperature at 2 m from the soil, unless otherwise indicated) has occurred in the past century and this will continue into the foreseeable future.

According to IPCC 2012 report 'Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation' (A Special Report of Working Groups I and II of the IPCC), was noted that there is medium confidence that droughts will intensify in the 21st century in some seasons and areas, due to reduced precipitation and/or increased evapotranspiration. This applies to regions including southern Europe and the Mediterranean region, central Europe, central North America, Central America and Mexico, northeast Brazil, and southern Africa. Sandu et al. (2010) showed that at the level of 1901-2008 period, analysis of the values of the annual mean air temperature from a total of 17 meteorological stations with consecutive series of observations over 100 years, shows

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that the annual mean temperature increased by  $0.5^{\circ}$ C between 1988-2008 (10.1°C) over the whole period analyzed (9.6°C), a value which is below the global average warming of 0.6°C. The main results of Croitoru and Piticar (2012) has been identified that both extreme daily maximum and minimum temperatures have increased in the analyzed areas. The aim of our study was to determine if climate change trends recorded in Pitesti and Maracineni, are representative for the general trend reported in the entire country in the last thirty years (1982-2011).

#### MATERIALS AND METHODS

In this study, we used a database collected by the National Meteorological Administration Bucharest and Research Institute for Fruit Growing Pitesti, which consists of daily minimum and maximum temperatures of the air (2 m from the ground) during 1982-2011, from 30 stations, evenly distributed throughout Romania. To highlight the temporal thermal variability over the past 30 years, represented by annual and monthly averages, but also its spatial deviations, the data was statistically processed using specialized programs SPSS 14.0. To determine the intensity, duration, form and character of thermal variation, we chose as a time for analysis, year and month. To do this, daily database was condensed into 2 datasets as follows: 1) for Romania 30 annual values (average of 30 stations) compared with Pitesti and Maracineni, and 2) 360 monthly values (30 years x 12 months) from the same meteorological stations and comparisons. Each of the two data sets have undergone the same steps of analysis: for each simple strings of values (maximum temperature (MAXT), minimum temperature (MINT) and mean daily temperature amplitude (MDTA)) were considered: the central tendency by average, median and mode and dispersion indicators were represented by extreme values, maximum amplitude of variation, histogram and normality of data distribution was checked with Shapiro-Wilk statistical test. In the case of two-dimensional strings (temperature - year of study), simple correlation coefficients and linear regression of datasets were determined (Y = a + bx equation, where 'Y' was successively MAXT, MINT and MDTA and 'x' year of study) and were compared with the non-linear (curvilinear) equations. Linear trends of average temperatures increasing over 10 years, were computed from the regression coefficient (b) of the linear equation, multiplied by 10 (years).

## **RESULTS AND DISCUSSIONS**

The dynamics of the annual mean temperatures in Romania versus Pitesti and Maracineni. For comparison of meteorological observations from Maracineni, with the values recorded at the meteorological station Pitesti located nearby, but placed under more representative conditions (plateau Cotmeana) than between Doamnei and Arges rivers, we made figures that follow. By processing the simple string of average (1982-2011) values from the 30 climatological stations managed by ANM Bucharest (Romania), compared with data from Maracineni and Pitesti, we can say the following: the annual means of maximum temperatures for Romania were 15.93°C, for Pitesti 16.48°C and for Maracineni 16.42°C; the annual means of minimum temperatures for Romania were 5.45°C, 5.86°C for Pitesti and 4.74°C for Maracineni and annual means of daily temperature amplitudes 10.48°C for Romania, 10.62°C for Pitesti and 11.68°C to Maracineni. The median varied very little from the mean values (eg. for Romania was 15.91°C for maximum temperature, 5.36°C for minimum and 10.54°C for mean daily amplitude). For Romania the daily amplitude temperature mode was placed on the right side of the average on the histogram (10.88°C) and for the other two indicators (maximum and minimum) on the left side of the average (14.76°C and 4.86°C respectively). The highest annual (1982-2011) mean value of

maximum temperatures was 17.55°C for Romania, in Pitesti 18.35°C and 17.94°C in Maracineni. The lowest annual mean value of minimum temperatures to Romania was 3.80°C for Romania, 4.10°C for Pitesti and 2.77°C for Maracineni and the highest values of annual mean of temperature amplitude was 11.48°C for Romania, 12.84°C in Pitesti, and 13.12°C to Maracineni. The most powerful statistical test to verify the normality of a distribution Shapiro-Wilk, accepted the normality for all strings of temperature values.

In figure 1, in the case of MINT, linear correlation is stronger ( $R^2 = 0.4144^{***}$ ), versus MAXT ( $R^2 = 0.243^{**}$ ), but highly significant in both cases. We can conclude that 41.4% of the MINT annual values oscillation during 1982-2011, was due to the time variable and for MAXT only 24.3%. This can be seen, also, in lower annual oscillations of MINT values versus MAXT, so a safer growing trend for minimum temperatures than maximum ones. Also the increasing rate of the two temperatures was almost equal: 0.48°C in a decade for MINT and 0.49°C for MAXT. In all three locations MDTA remained unchanged from 1982 to 2011. In the second figure we can see the same graph type but for Pitesti station. In this figure, in the case of MINT, linear correlation is stronger ( $R^2$  =  $0.5693^{***}$ ), versus MAXT ( $R^2 = 0.2116^{**}$ ), but in the same way as in Romania, highly significant in both cases. We can conclude that 56.9% of the MINT annual values oscillation during 1982-2011, was due to the time variable and for MAXT only 21.2%. This can be seen, also, in lower annual oscillations of MINT values versus MAXT, so (like in Romania). The increasing rate of the two temperatures was not equal in Pitesti: 0.64°C in a decade for MINT and only 0.51°C for MAXT. In the third figure we can see the same graph type as for Romania and Pitesti, but for Maracineni station. In the case of MINT, linear correlation is stronger ( $R^2 = 0.4184^{***}$ ), versus MAXT ( $R^2 = 0.1256^{*}$ ), but in the same way as in Romania and Pitesti, highly significant for MINT values and only significant in the case of MAXT. We can conclude that 41.8% of the MINT annual values oscillation during 1982-2011, was due to the time variable and for MAXT only 12.6%. This can be observed also, in lower annual oscillations of MINT values versus MAXT, so (like in Romania and Pitesti) a safer growing trend for minimum temperatures than maximum ones. The increasing rate of the two temperatures in Maracineni was higher for MAXT then for MINT (the same situation like in Pitesti): 0.49°C in a decade for MINT and only 0.33°C for MAXT.



Fig. 1. The dynamics of the annual means of air temperature during 1982 - 2011 for Romania

Fig. 2. The dynamics of the annual means of air temperature during 1982 - 2011 for Pitesti

The dynamics of the monthly means linear trends per decade for Romania versus Pitesti and Maracineni. The information presented in the tables 1, 2 and 3 is very

important because it gives us the time of year where MAXT, MINT and MDTA are significantly correlated with the time. We have described for each meteorological parameter, only the trends that had statistically insurance ( $P \le 0.05$ ). It can be noted that in general, the level of significance of maximum and minimum temperature linear trends per decade was low during winter, spring and autumn. But this level of significance was very high (between 0.01 and 0.001) for months of June, July and August. The degree of statistical assurance of MINT trends (figure 2) was higher (sig. less than 0.005) than of MAXT (sig. between 0.001 and 0.063). MDTA trends were not statistically assured for any month of the year (except for September in Pitesti and Maracineni weather stations). However, growth trends for maximum temperatures in 10 years (table 1) in June were lower in Pitesti (0.71°C) and Maracineni (0.55°C) compared with the average for Romania of 0.88°C. For July the increasing rate was higher for Pitesti (0.88°C) and lower for Maracineni (0.61°C) compared to Romania (0.82°C). For the month of August, although the values do not have statistical assurance for the two meteorological stations from Arges County, growth was higher only in Maracineni (0.84°C) compared to Romania (0.70°C). Very close are also the stations of Arges County from the November month national trends.





Fig. 3. The dynamics of the annual means of air temperature during 1982 - 2011 for Maracineni weather station

Fig. 4. 'Tuleu gras' plum cv. phenophase dynamics at Maracineni (1970-1976;1985-2007)

Regarding the minimum temperature trends shown in table 2, can be seen that in all three summer months, the growth rate is higher in the two analysed weather stations from Arges County, compared with the average for Romania. MINT growth was determined by time in percentages between 25.3% in July (Maracineni) and 53.6% in August (Maracineni).

In the same time, in February and November MAXT and MINT increased sharply by 0.9 up to  $1.2^{\circ}$ C per decade, but with large fluctuations from one year to another in all three locations, thus less safer than in the summer months (near, but under the limit of the statistically assurance). Due to accelerated growth of air temperature in February for the MAXT and MINT and in March only for MAXT ( $0.78^{\circ}$ C up to  $1.13^{\circ}$ C), the phenological dynamics of fruit trees had the tendency of starting earlier in the vegetation. For example (figure 4), 'Tuleu gras' plum tree cultivar used to have its onset of bud burst in the first days of April 44 years ago, but in 2007 it has its onset in vegetation in middle of March ( $R^2 = 0.3158^{***}$ , for swollen bud phenophase).

## Table 1.

	Romania (30 stations)			Pitesti Station			Maracineni Station		
Month	R ² (%)	Sig.	Mean trend in 10 years (°C)	R ² (%)	Sig.	Mean trend in 10 years (°C)	R ² (%)	Sig.	Mean trend in 10 years (°C)
January	0.1	0.869	0.09	0.2	0.841	0.11	0.1	0.900	-0.07
February	6.0	0.194	0.90	8.4	0.121	1.09	6.3	0.183	0.97
March	5.7	0.203	0.78	9.8	0.093	1.13	6.0	0.193	0.84
April	3.2	0.341	0.39	3.2	0.346	0.41	0.7	0.671	0.18
May	2.9	0.365	0.34	3.8	0.302	0.42	0.5	0.716	0.14
June	30.7	0.001	0.88	22.3	0.008	0.71	13.6	0.045	0.55
July	24.9	0.005	0.82	26.4	0.004	0.88	17.0	0.024	0.61
August	13.5	0.046	0.70	11.9	0.063	0.70	11.8	0.063	0.84
September	1.4	0.532	-0.28	3.2	0.348	-0.45	6.4	0.178	-0.61
October	0.1	0.891	-0.04	0.9	0.614	-0.18	2.4	0.414	-0.29
November	13.9	0.042	1.10	11.1	0.073	1.04	7.8	0.134	0.83
December	0.9	0.626	0.23	1.2	0.573	0.26	0.0	0.999	0.00
Average	8.6	0.343	0.49	8.5	0.282	0.51	6.03	0.377	0.33

Linear regression equations indicators, showing the dynamics of monthly means of maximum air temperatures during 1982-2011 (determination coefficient, significance level and average trend per decade)

#### Table 2.

Linear regression equations indicators, showing the dynamics of monthly means of minimum air temperatures during 1982-2011 (determination coefficient, significance level and average trend per decade)

	Romania (30 stations)			Pitesti Station			Maracineni Station		
Month	R ² (%)	Sig.	Mean trend in 10 years (°C)	R ² (%)	Sig.	Mean trend in 10 years (°C)	R ² (%)	Sig.	Mean trend in 10 years (°C)
January	0.6	0.680	0.22	2.0	0.455	0.37	0.0	0.922	0.05
February	8.2	0.125	0.94	15.7	0.030	1.21	8.6	0.117	0.90
March	3.9	0.298	0.41	8.1	0.127	0.63	1.8	0.479	0.27
April	3.1	0.353	0.25	12.1	0.060	0.53	2.1	0.444	0.20
May	2.3	0.421	0.18	9.8	0.093	0.43	5.9	0.195	0.30
June	35.4	0.001	0.61	39.9	0.000	0.79	38.7	0.000	0.76
July	26.7	0.003	0.67	26.6	0.004	0.72	25.3	0.005	0.67
August	44.5	0.000	0.75	44.4	0.000	0.77	53.6	0.000	0.88
September	4.7	0.250	0.30	7.6	0.141	0.39	11.6	0.066	0.54
October	4.4	0.267	0.30	6.5	0.173	0.37	5.7	0.206	0.39
November	13.0	0.051	0.94	20.2	0.013	1.11	10.8	0.076	0.75
December	1.0	0.602	0.25	5.0	0.233	0.46	0.7	0.658	0.19
Average	12.3	0.254	0.49	16.5	0.111	0.65	13.73	0.264	0.49

#### CONCLUSIONS

There were characterized the increasing trends of annual and monthly maximum, minimum and daily amplitudes of the air temperatures, of the last 30 years in Romania (30 climatological stations), versus Pitesti and Maracineni weather stations. In terms of annual means for Romania were found for MINT, that linear correlation with year of study variable was stronger versus MAXT, but significant in both cases. For MINT 41.4% of oscillation was due to the time and for MAXT only 24.3%. Also the increasing rate of the two temperatures was almost equal: 0.48°C in a decade for MINT and 0.49°C for MAXT. The same degree of statistical insurance for the increasing trend of MAXT and MINT was maintained for Pitesti and Maracineni too. It was noted, however the presence of a faster growth rate of both the MAXT and the MINT for Pitesti (0.51°C for MAXT and 0.61°C for MINT), versus Maracineni (0.33°C for MAXT and 0.49°C for MINT) and Romania.

For the three locations the monthly level of significance of MAXT and MINT linear trends per decade was low during winter, spring and autumn. But this level of significance was very high for June, July and August months. The increasing rate per decade of maximum temperatures for June was 0.88°C in Romania versus 0.71°C at Pitesti and 0.55°C at Maracineni. For MINT the same mean trend per decade was only 0.61°C for Romania versus 0.79°C at Pitesti and 0.76°C at Maracineni. For July the increasing trend per decade was similar to the previous month. For the month of August the increasing trend was lower for maximum temperatures (0.70°C per decade for Romania) and not statistically assured for Pitesti and Maracineni. For MINT the trend was the highest of the summer in August with 0.75°C per decade in Romania and 0.77°C at Pitesti and 0.88°C at Maracineni, and the best statistically assured.

In February and November MAXT and MINT increased sharply by 0.9 up to 1.2°C per decade, but with large fluctuations from one year to another in all three locations, thus less safer than in the summer months (near, but under the limit of the statistically assurance). Due to accelerated growth of air temperature in February for the MAXT and MINT and in March only for MAXT (0.78°C up to 1.13°C), the phenological dynamics of fruit trees had the tendency of starting earlier in the vegetation.

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# THE HIGHLY SENSITIVE C REACTIVE PROTEIN - MARKER OF THE CARDIOVASCULAR RISK IN OBESITY

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Key words: obesity, hs-CRP, cardiovascular risk

#### ABSTRACT

Obesity is a major public health issue of the twenty first century, through the increased risk for cardiovascular diseases 2-3 times and about 5 times the risk for type 2 diabetes mellitus (DM), serious conditions that reduce the quality and length of life for patients whose treatment is extremely costly to society. Inflammatory mediators are involved in the pathophysiological processes through which obesity, especially the abdominal one determines an increased cardiovascular risk, and the visceral adipose tissue being most likely located as the subclinical inflammatory centre.

Epidemiological studies have confirmed that patients with elevated basal plasma levels of CRP present an increased risk of coronary artery disease. Based on these data, we have studied the correlation between hs-CRP levels and the presence of the ischemic heart disease in a group of patients with different degrees of obesity. The results obtained confirm that the obese present a pro-inflammatory status and thus an increased risk of coronary ischemic disease (CID).

## **INTRODUCTION**

Obesity is a chronic disease defined as excessive adipose tissue accumulation in the body, with adverse effect on health, leading to the development of some very serious diseases and to reduced life expectancy. Obesity is a major health problem in the twenty first century, because it increases the risk for cardiovascular diseases 2-3 times and it increases the risk for diabetes mellitus type II approximately 5 times (Blaj Ş., 2003), serious diseases that significantly shorten the life expectancy and life's quality in patients who also receive expensive treatments. The prevalence of obesity grew significantly over the past decades, being noticed a real epidemic of obesity due to a sedentary life style with a high-caloric food intake, thus leading also to an increased prevalence of the metabolic syndrome (Bistriceanu Marian, 2000)

The inflammatory mediators are involved in the pathophysiological processes through which obesity; especially the abdominal one determines an increased cardiovascular risk. Adipocytokines stimulates the hepatic synthesis of acute phase proteins (C-reactive protein, fibrinogen), the visceral adipose tissue being most likely located as the subclinical inflammatory centre (Wolk R, 2004).

During the last years epidemiological studies have confirmed that patients with elevated basal plasma levels of CRP present an increased risk of coronary heart disease and myocardial infarction. Prospective studies conducted in European countries and the U.S.

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has confirmed the concordant results regarding the predictive value of CRP determinations on cardiovascular risk in both men and women. Thus CRP is a risk factor for coronary heart disease indirectly. hs-CRP is a strong predictor of future cardiovascular events (Glodeanu Adina, 2011). According to the 2003 AHA recommendations, including inflammatory markers currently used, highly sensitive C-reactive protein is only approved for clinical use. The decision intervals for cardiovascular risk assessment are set as recommended by CDC / AHA:

• <1 mg / 1 - low risk

• 1-3 mg / 1 - moderate risk;

 $\rightarrow$  3 mg/l - high risk.

#### MATERIALS AND METHODS

After studying the epidemiological reports presented above, we have done a prospective study on a group of 172 obese patients, admitted in the Department of Cardiology at Filantropia Clinical Hospital from Craiova during March 2008 – March 2011. The patient selection criterion was the excess weight associated with specific manifestations of cardiovascular damage. The cases were investigated through anamnesis, clinical examination according to a standardize examination form and paraclinical tests (blood glucose levels, full lipid profile, determination of hs-CRP - the Latex immunoturbidimetric method,), performance of 12-lead resting ECG, exercise test in selected cases. The degree of the obesity was established calculating BMI (represents the individual's body weight divided by the square of his or her height) and measuring waist circumference. According to WHO's and International Obesity Task Force's criteria the body weight is considered normal when the BMI values range between 18, 5 and 24, 9 kg/ m². A person with a BMI greater than 25 kg/m² is overweight and above 30 kg/m² is considered obese. Obese class I range from 30 kg/m² to 35 kg/m², obese class II from 35  $kg/m^2$  to 40 kg/m² and obese class III is defined as a BMI equal or over 40 kg/m². The waist circumference values obtained are related with the abdominal adipose tissue. According to IDF's recommendations values of 94 cm or higher for men and values of 80 cm or higher for women are associated with a greater cardiac risk (Klein I. Braunwald, 2008).

The CID probability was made based on the positive response to the WHO standard questionnaire for screening effort angina (Rose questionnaire) associated with the presence of changes in resting ECG (12 leads) suggestive for ischemia, according to the Minnesota code.

#### **RESULTS AND DISCUSSIONS**

The age distribution in our group of patients was as follows: the mean age was 60.65 years, with a minimum of 42 years old and a maximum of 81 years old, with a standard deviation of 9.98. Sex distribution: 118 women (68.60%) and 54 men (31.40%). All the women included in the study were at the age of menopause. Among the patients included in the study, 34.30% had first degree of obesity, 44.19% second degree of obesity and 21.51% third degree of obesity.

In the study we have highlighted 34.88% patients with painful ischemic heart disease and 23.84% patients with painless ischemic heart disease, diagnosed based on the clinical picture and resting ECGs performance. Following the exercise ECG test, another 4.65% of patients were diagnosed with CID.

The CID correlation hs-CRP level. In the case of the 60 patients with painful CID included in the study found the following (table 1, figure 1): 2 patients (3.33%) have

presented hs-CRP <1 mg / 1 (low risk); 17 patients (28.33%) had hs-CRP 1-3 mg / 1 (moderate risk); 41 patients (68.33%) had hs-CRP> 3 mg / 1 (high risk).

At a chi square of 33.760 0.00 it was highlighted p< 0.001, highly statistically significant.

The patients with painless CID have presented the following values of hs-CRP (table 2, figure 2): 4 patients (9.76%) with hs-CRP <1mg / 1 (low risk); 14 patients (34.15%) with hs-CRP 1-3 mg / 1 (moderate risk); m23 patients (56.10%) with hs-CRP> 3 mg / L (high risk).

Table 1

hs-CRP (mg/l)	<1	1-3	>3	Total
CIDd+	3.33%	28.33%	68.33%	100.00%
CIDd-	37.50%	34.82%	27.68%	100.00%
Total	25.58%	32.56%	41.86%	100.00%

The prevalence of painful CID based on the hs-CRP values



Figure 1. The correlation between the hs-CRP levels and the presence of painful CID.

# The Hs-CRP level correlation - degree of obesity

Patients with first degree obesity have presented the following values of hs-CRP (table 3, fig. 3): 30 patients (50.85%) with hs-CRP <1 mg / l; 19 patients (32.20%) with hs-CRP 1-3 mg / l; 10 patients (16.95%) with hs-CRP> 3 mg / l.

Table 2

The prevalence of elevated hs-CRP values at the patients with painless CID

hsCRP (mg/l)	<1	1-3	>3	Total
CIDd+	9.76%	34.15%	56.10%	100.00%
CIDBCId-	30.53%	32.06%	37.40%	100.00%
Total	25.58%	32.56%	41.86%	100.00%

In patients with second degree obesity, hs-CRP had the following values: 14 patients (18.42%) of hs-CRP <1 mg / l; 25 patients (32.89%) with hs-CRP 1-3 mg / l; 37 patients (48.68%) with CRP> 3 mg / l.





Table	3
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		hs-CRP (mg/l)				
Obesity	BMI	<1	1-3	>3	Total	
First degree	BMI 1	50.85%	32.20%	16.95%	100.00%	
Second degree	BMI 2	18.42%	32.89%	48.68%	100.00%	
Third degree	BMI 3	0.00%	32.43%	67.57%	100.00%	
Total		25.58%	32.56%	41.86%	100.00%	

The prevalence of elevated hs-CRP values depending on the degree of obesity

In patients with third degree obesity the hs-CRP dosage determination highlighted: No patients have had CRP hs <1 mg / 1; 12 patients (32.43%) had hs-CRP 1-3 mg / 1; 25 patients (67.57%) had hs-CRP> 3 mg / 1.

The prevalence of the moderate risk / increased for cardiovascular events significantly increase (p < 0.001) with the degree of obesity (Fig. 3).

At a chi square of 41.150 it was highlighted p<0.001 highly statistically significant.

The epidemiological studies conducted in the last years have confirmed that patients with elevated basal plasma levels of CRP present an increased risk of coronary heart disease and myocardial infarction.

Atherosclerosis and the atherosclerotic plaque evolution towards instability are clear implication inflammatory processes and the involvement of the C-reactive protein in the pathogenesis of atherosclerosis and coronary events can be explained through multiple mechanisms: CRP molecules are linked to LDL and VLDL plasma levels and may
contribute to their atherogenic accumulation; CRP serves as a ligand and binds to damaged plasma membranes; CRP was detected in the neointima and atherosclerotic plaque and it appears to activate the local complement system; CRP stimulates the production of tissue factors by macrophages in vitro; CRP activates the expression of endothelial cells and macrophage of the adhesion molecules, selectine, chemokine; CRP amplifies the proinflammatory effects of other mediators; CRP reduces the eNOS expression (endothelial nitric oxide synthase) in human endothelial cells; CRP has a procoagulant effect (Ridker PM., 2003).



Fig.3. The correlation between hs-CRP level and the degree of obesity

The prospective studies have shown that the C-reactive protein is an important predictor of the prognosis in cardiovascular events not only in healthy individuals, especially in patients with stable angina or acute coronary syndromes with or without ST-T steep segment elevation (Matsuzawa Y. 2005). The C protein level has been shown to be useful for the risk stratification of recurrent ischemia and death of patients with stable and unstable angina, of those who have made percutaneous angioplasty, and to assess the risk of myocardial infarction, stroke, peripheral arterial disease and sudden death.

The prospective studies conducted in the European countries and the U.S. has confirmed the consistent results regarding the predictive value of CRP determinations upon the cardiovascular risk in both men and women. Thus, CRP is an indirect risk factor for coronary heart disease and elevated levels may reflect some of the following: an inflammation of the coronary arteries in response to infectious agents, the severity of the inflammatory response in atherosclerotic vessels, extending inflammation associated with myocardial ischemia, expanding inflammation associated with myocardial necrosis, the amount and activity of circulating proinflammatory cytokines.

Although the role of the C-reactive protein as a marker of the subclinical inflammation is well established, there is still controversy regarding the role of risk factors

that the C-reactive protein appears to be due to the mechanisms that act on endothelial cells and smooth muscle cells from the vulnerable plaque (Rondinone CM, 2006).

Having regarded the current records concerning the involvement and the clinical value of using clinical testing of CRP in clinical practice, the American Heart Association (AHA) and Centres for Disease Control and Prevention (CDCP) published in 2003 the recommendations for clinical practice in the U.S. Thus, according to these recommendations, the inflammatory markers, high-sensitivity CRP (hs-CRP) is the only one approved for clinical use today.

## CONCLUSIONS

Hs-CRP is an inflammatory predictive marker of cardiovascular risk approved for clinical use today.

The risk of the cardiovascular events (evaluated by determination of hs-CRP) increases significantly (p < 0.001) with the degree of obesity.

Following the CRP dosage in the entire group of obese patients it has revealed a highly significant correlation from a statistical point of view (p < 0.001) between its elevated presence of coronary artery disease and ischemic pain and statistically significant in patients with painless coronary artery disease.

Consistent with literature data, positive correlation between elevated hs-CRP and the presence of ischemic heart disease confirms that obese patients have a proinflammatory status involving an increased risk of coronary heart disease.

The prophylactic long-term objectives for hypertension that develops in obese patients are: decreasing the body weight and diminishing the cardiovascular risk factors. To accomplish this we advise patients to lower the percentage of animal fat in their diet, to practice well dosed physical exercise and use the pharmacological therapy with the least adverse reaction.

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# RESEARCHES ABOUT THE AGRO-PATHO-SYSTEM CLAVICEPS PURPUREA (FR.:FR.)TUL.- AGROPYRON REPENS (L.)P.BEAUV.

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KEYWORDS: patho-system, Claviceps purpurea, Agropyron repens, sclerotia

### ABSTRACT

The control of dragged couch grass Agropyron repens, is difficult and expensive. Integrated weed management measures should be sought new ways to decrease the degree of infestation and control spending. Study Claviceps purpurea x Agropyron repens patho-system can bring some information on finding vulnerabilities in diseased weeds by fungus. From observations it was found that weed is quite sensitive to plant pathogen attack and thus be easier to control. In addition, couch grass crepping attacked by this fungus it produces a certain degree of sterility, very important in limiting its spread. The study shows that weed is infected by C.purpurea and its sclerotia is observed during the months of June-July. Attacked shoots are the flowers that appear in the second year of vegetation. Sclerotia analysis showed high variability. Thus, an ear of A.repens formed between 1-17 ergot-scerotia between 6-19 mm long and 0.5-1.5 mm thick. Correlation between C.purpurea fruit size shows positive trend ( $r=0.415^{***}$ ), the characteristic researched area.

#### **INTRODUCTION**

Bluegrass (couch grass) *Agropyron repens* (L.)P.Beauv. is one perennial monocots species in agriculture with a high cost of control (Anghel,1972). Chemical control included in all specific integrated plant management measures, could be easier given that different parasite species as pathogens including fungi commonly attacks it and *Claviceps purpurea* (Fr.:Fr.)Tul. are among them (Bobeş,1983).

In recent decades there have been brief references on the couch grass creeping fungus presence (Manoliu,1996). Spread and attack the fungus occurs more frequently in areas with cool, moist climate in spring, like that of the flowering period. Such conditions often occur in the podzolic soils of the south. The question is whether twich creeping plants are affected or not this fungus attack, and if so, at what level.

Studies of this kind are part of the new direction and perspective to find new vulnerabilities in the weeds to help control it more effectively (Comeş,1982). On the other hand, installed on weed disease may significantly reduce the possibilities of sexual propagation by seeds (Johnson,1996). As has been noted (Lazarus,1977), the fungus once

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installed in a flower, it affects both and on the other side. At that ear and the neighboring ears, sterility occurs, the important role in reducing the spread of weeds.

#### MATERIAL AND METHODS

In the last five years (between 2008 and 2012) have made studies on flowering stems of infected *A.repens* by *C.purpurea*. Points selected were characterized as three areas in cultivated cereals. At each site there was a twitch inventory with creeping stems with and without sclerotia and fungi for the separation was based on the number of sclerotia in the ear.

In a later stage there was a collection of sclerotia, they were measured in length and width (Fig. 1). Compared to other fungi monocots to twitch situation is different, specific.



Figure 1. *C. purpurea* life cycle and sclerotia variability

Such measurements can give some information on the morphology of sclerotia in this species of weed. Measurements revealed a high diversity offruit size and shape, and the correlation established between the two categories: long and thick, proved to be positive.

A special category of tests aimed attacked ears weight and mass sclerotia which they were formed. Between the two sizes you set a new correlation. Finally were weighted and weed stems, both attacked and the healthy (unchanlenged) and prepare a separate schedule.

In this study, the pathosystem values using laboratory balance and ruler, and the graphics expressed using Excel program.

#### **RESULTS AND DISCUSSIONS**

Pathosystem of fungus *C.purpurea* and weed *A.repens* has been less investigated. From what is known, the form sclerotia fungus infection grains instead.

<u>Number of sclerotia formed on *A.repens* strains.</u> The data obtained in the three analized points were found different distribution of sclerotia number of ears (table 1). Thus, the first point analized 14 strains were found to twitch with one sclerotic, two strains were approximately 5 sclerotia and one plant had 10-12 sclerotia. Other values were intermediate. In the second point 30 strains had one sclerotic, two strains were times 8 sclerotia and one strain each 11-15 sclerotia. In the third point of 43 strains were sclerotic in one ear, 12 strains were approximately 5 sclerotia formed 514 weed stems. Ears without attack were 60 % in the first case, 34 % in the second point and 9 % in last place.

#### Table 1

No.	Population 1			Pop	ulation 2		Population 3		
	Sclerotia	Stems	%	Sclerotia	Stems	%	Sclerotia	Stems	%
1	0	61	60	0	35	34	0	14	9
2	1	14	14	1	30	29	1	43	26
3	2	10	10	2	17	17	2	31	19
4	3	7	7	3	8	8	3	23	14
5	4	3	3	5	4	4	4	17	10
6	5	2	2	7	3	3	5	12	7
7	8	2	2	8	2	2	6	9	5
8	10	1	1	11	1	1	7	6	4
9	12	1	1	12	1	1	8	3	2
10	-	-	-	15	1	1	9	3	3
11	-	-	-	-	-	-	10	2	1
12	-	-	-	-	-	-	17	1	1
Total		101	100		102	100		164	100

The distribution of sclerotia number of Claviceps purpurea on the Agropyron repens stems

<u>C.purpurea</u> sclerotia size. Sclerotia or fruits of fungus were measured in length and in width. The data obtained show a relative variability. The number of fungus fruit generally ranged from1 to 10 on a stem and sizes ranged in length from 6 to 19 mm, and the width between 0.5 and 1.5 mm. The distribution of the two dimensions revealed different histograms (Fig. 2). Thus, the values most (maximum) were located 10 mm long and 1 mm wide. Compared to these oscillations were showing such biodiversity: length sclerotia had greater variability toward larger sizes and their tickness variability was below 1 mm.

Considering the specific form of *C.purpurea* sclerotia was established correlation between the two dimensions (Fig. 3). The correlation is positive and shows tendency to be larger due to the ground richer in food crops.

<u>Influence on weed fungus attack.</u> A separate determination was made by weighing fungus sclerotia and ears with fungus attack. The correlation between the two biomass was based on average values grouped on the weight categories (Fig. 4). From the figure it is found that the heavier ears formed the fungus sclerotia much higher (Prescott,1986).



Figure 2. Biodiversity of C.purpurea sclerotia formed on A.repens weed



Figure 3. Correlation between length and width of C.purpurea sclerotia formed on A.repens weed



Figure 4. Corelation between sclerotia weight of *C.purpurea* and ears weight of *A.repens* 516



Figure 5. The infection influence of *C.purpurea* on *A.repens* stem weight: 1-without fungus, 2-with fungus

Thus, on average 380 mg ears were formed sclerotia 100 mg and 190 mg in ears with 18 mg formed sclerotia. The explanation is that where plants have developed ears twitch larger and heavier fruit fungus produced.

By weighing weed strains without and with fungus attack, there was a difference in weight (Fig. 5). Average strain of weed free fungus weighted 0.693 g and one with fungi, 0.650 g weed attacked by this fungus showed such a degree of disease. Present sondition is favorable because it could lead to an easier control of weeds in an appropriate IWM.

### CONCLUSIONS

*A.repens* weed have attack by *C.purpurea* fungus, especially during cold and wet spring and warm and wet al flowering weeds period. Emerged from the study pathosystem degrees of infection by 40 % in the first location studied, 65 % in the second item and 19 % in the last point.

Fruit fungus- sclerotia, differ in number and in size on weed ears. In absolute values 1-17 sclerotia were formed in the ear. Dimensions were 6-19 mm in length and 0.5-1.5 mm width. The data obtained give this pathosystem original character and histograms obtained have demonstrated a certain degree of biodiversity.

The correlation between the weight of twitch ears and fungus fruit weight was positive. Ears heavier due to better growing conditions have made large quantities of sclerotia. Their response was due to the specific ecology of the area.

Weight of weed strains without attack and attack of the fungus demonstrated a certain degree of vulnerability of *A.repens*. These results might expect in a more consistent control of this species and calls for conducting research on natural enemies of weeds. The new recommendation calls for knowledge of weeds vulnerability, including specific diseases in order to reduce their degree of control.

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## PATHOLOGY OF ADAPTATION IN DAIRY COWS PURCHASED ON THE UE MARKET UNDER CLIMATE CHANGE

Ionita L.¹, Retea C.², Retea Genica², Ionita Carmen¹, Marinica, I.³

Keywords: climate change, homeostasis, metabolic profile

#### ABSTRACT

Dairy caws are very sensitive to heat stress conditions and its effects have a significant economic impact for animals and farmer by lowering productivity, changes in milk quality (increased somatic cell counts) and health's problems. Being aware that due to climate changes, especially during excessive heat times when new pathologies can develop in animals, we are dealing with a new adaptation pathology and we need to find other ways to manage these critical situations in order to ensure animal welfare in these conditions, including the ones due to very low environmental temperatures. Our study was over 4 years in a herd of 594 dairy cows purchased from the European Union market, from 5 countries. Due to adaptation stress, after 4 years, 335 animals were removed from the group, 241 cows remaining for reproduction. The severity of thermic stress is correlated to both environmental temperature and the humidity level and the temperature-humidity index is calculated by a formula (THI).

### **INTRODUCTION**

Dairy cows are very sensitive to thermic stress and its effects have a significant economical impact on both the animals and the breeder by decreasing productivity, changes in the quality of milk (increased somatic cell-counts) and health issues (De Renis, F., & coll., 2003). Being aware that due to climate changes, especially during excessive heat times when new pathologies can develop in animals, we are dealing with new adaptation pathology and we need to find other ways to manage these critical situations in order to ensure animal welfare in these conditions, including the ones due to very low environmental temperatures (Ionita, L., 2008).

Dairy cows are very sensitive to *thermic stress* (at temperatures over 25°C) and the level of stress is correlated to both the level of temperature and the level of humidity. The caloric stress affects the health and the productivity (Dikmen, S., and P.J. Hansen. 2009).

A key-element is that the effect of caloric stress for a certain temperature varies with the relative humidity. For example, there is no caloric stress effect at 28,3°C and 20% relative humidity, but the animal is stressed at 28,3°C and 90% relative humidity (Broucek, J., 2009;).

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Temperature Humidity Index (THI)									
C	20	30	Relati	50	60	70	80	90	100
22	20	50	40	50	00	70	70	30	70
22	00	00	0/	00	69	69	70	71	12
24	68	69	70	70	71	72	73	74	75
26	70	71	72	73	74	75	77	78	79
28	72	73	74	76	77	78	80	81	82
30	74	75	77	78	80	81	83	84	86
32	76	77	79	81	83	84	86	88	90
34	78	80	82	84	85	87	89	91	93
36	80	82	84	86	88	90	93	95	97
38	82	84	86	89	91	93	96	98	100
40	84	86	89	91	94	96	99	101	104
					a. 21				
	No he	at stre	SS						
	Mode	rate he	at stre	SS	1				
	Sever	e heat	stress						
	Dead	cows			1				
					8				

Figure 1. The level of stress in relation to THI

When THI>72, there is caloric stress (Zimbelman, R.B., & coll., 2009); when THI is 72-78 (79), there is a low stress; when THI is 79-89 (90), there is a high stress; when THI is 90-98 (99) there is a very high stress; when THI>98, death occurs (figure 2).





The mechanisms used by the cow to decrease the caloric stress are: increased respiratory rate (>70/min) is a primary change found in cows in order to reduce the caloric stress; decreased physical activity; increased water consumption – by 20 to more than 50%; the animals look for shading and cooler spots ( Gaughan, J.B., & coll., 2000).



Figure 3. The effect of raised environmental temperature on the health status and milk production



Figure 4. Animals suffering heat stress will often pant in an attempt to increase heat loss 521

### MATERIAL AND METHODS

The research was done on the animals from a commercial farm for *dairy kettle from Robănești city, Dolj county*. Our research was done over 4 years, on a group of 594 dairy cows bought from the EU market, from 5 European countries: Estonia, France, Czech Republic, Hungary, Germany during. The research was performed using clinical examinations, epizootological, laboratory, necropsy and paraclinical examinations (Broucek, J., & coll., 2009; Retea C., & coll., 2009).

For stress acclimatization determination was used the systematic study by *transverse methods*, that follow the phenomena, aspects and processes at a certain time and *longitudinal methods* that follow the processes and aspects over time.

During this time we monitored all the animals through clinical examinations to follow symptoms, adaptation and behavioural changes, including nutritional and metabolical monitoring by complex metabolic tests (Eicher, R., 2003, p28, Marinică I., 2006; West, J.W., 1999).

## **RESULTS AND DISCUSSION**

Our research was done over 4 years, on a group of 594 dairy cows bought from the EU market, from 5 countries. Due to adaptation stress, after 4 years, 335 animals were removed from the group, 241 cows remaining for reproduction.

Table 1

Country	Bought	Removed from	Stock after 4 years
		group	
Hungary	130	68	62
Estonia	139	79	60
France	31	19	12
Germany	263	163	100
Czech Republic	31	15	16
Total	594	353	241

The situation of bovines bought from EU countries and stock after 4 years

There were many aspects causing the removal from the group: tough acclimatization, poor housing and feeding conditions, the decrease of milk production, infertility, frequent pododermatitis and mastitis etc. The adaptation crisis was another determined factor for the economical loss, in relation to feeding, zoohygenic conditions for breeding.

One of the most important factors was the thermic stress.



Figure 5. Methods of heat stress mitigation

The environmental temperature is the main factor that impacts the metabolism and health of the animals; the direct ways are the ones acting immediate on the animals, impacting: increase in mortality, new pathologies development, reproduction, growth, the level of production, the quality of production.

Dairy cows are very sensitive to thermic stress and its effects have a high economical impact significant for both animals and farmer, by the decrease in productivity, changes in the quality of milk (increased somatic cell-counts) and health issues: ruminal and metabolic acidosis (fig. 3) and laminitis. Being aware that due to climate changes, especially during excessive heat times when new pathologies can develop in animals, we are dealing with new adaptation pathology and we need to find other ways to manage these critical situations in order to ensure animal welfare in these conditions, including the ones due to very low environmental temperatures.

The severity of thermic stress is correlated to both environmental temperature and the humidity level and the temperature-humidity index is calculated by a formula (THI). For dairy cows there is a discomfort when THI exceeds 72 units (in humans when THI is over 80 units).

### CONCLUSIONS

Nowadays, taking into consideration the intense climate changes, these affect the whole biodiversity

The transfer of animals between areas with different climate, both winter and summer induce in dairy cows the adaptation crisis, then nutritional-metabolic disorders linked with infertility, the decrease of productions and their removal from the group.

Dairy cows bought from the EU market bred in proper conditions (aired barns, appropriate feeding) overpass easier the adaptation and acclimatization.

Thermic stress influences the metabolism of the animals especially if the temperature-humidity index exceeds 72 units.

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## IMPACT OF CLIMATE CHANGE ON ANIMAL AND ECO-INNOVATIVE SOLUTIONS

Ionita Lucian¹, Câmpeanu Gheorghe¹

Keywords: eco-economy, climate change, eco-bio-economy, pollution

## ABSTRACT

Climate change is a reality of the contemporary world. They are the natural consequence of human intervention in the ecosystem, the effects of pollution on all levels: water, air, soil and plants. The current problems of humanity are focused on providing food for a growing population, but also on the issue of energy and food and environmental issues that degrade slowly but surely. Climate change is causing major changes in everyday life, has harmful effects on plants and animals and causes severe metabolic changes and adapts to new conditions. The issue of climate change calls seeking appropriate solutions and develop eco-innovative concepts such as bio-economy, and ecobioeconomy. Animals hardly bear climate change and try to adapt to these changes. Eco-innovative solutions aimed at obtaining varieties and breeds resistance and thermal comfort by building shelters to ensure scheduled microclimate conditions.

#### **INTRODUCTION**

Globalization is a problem of analysis environmental resources and the great challenges of the contemporary world.

Capital represented natural and semi- natural ecosystems form the" life support "providing resources underlying socio-economic development.

Biodiversity values form the" natural heritage" to be used by current generations without compromising future generations the opportunity to enjoy the same living.

In a multipolar world in which local or regional phenomena becomes global meanings, and medicine must make profound changes in the current medical ecosystems an actual medicine is primarily based on interdisciplinary, the indissoluble links with all branches of human and animal health, but and all that is related to other disciplines (life sciences), or those that intersect (economics, law, chemistry, physics, etc.).

Current medicine encompasses all methods with scientifically proven medical thinking and creates the emphasis is on the patient, not on illness (Bogdan, A.T., & coll., 2010).). More than ever, with profound changes in human life (scientific, technological proliferation, food crisis, climate change development of information system, etc.) new medical problems have arisen which need to be addressed diagnostic clarification and identification of anthropogenic ecosystem bioethical issues and traceability throughout the chain of biodiversity, the relationship: air-water-soil-plant-animal-food-humans (figure 1)

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Figure 1 Environmental challenges of the contemporary world

Global problems of mankind are social, economic, environmental and technical, each of them in composing other subdomains. For example the so-called environmental crisis refers to environmental problems depends on three factors interact among themselves overpopulation, resource depletion and pollution.

Global problems of mankind are social, economic, environmental and technical, each of them in composing other subdomains. For example the so-called environmental crisis refers to environmental problems depends on three factors interact among themselves overpopulation, resource depletion and pollution.

**Overpopulation.** Urban sprawl, industrial development and new infrastructure continue to spread at a rapid pace across Europe, often at the expense of natural areas. With them, the population will increase accordingly, the estimated world population growth (as a medium scenario) by the year 2100 and beyond by the year 2300 is presented in Figure 2.



Figure 2. The world population by the year 2300, in a medium scenario (correlation calculate using UN, 2007. World Population Prospects)

Figure 3 shows the dynamics of the world population in 2010, 2050 and 2100. In 2010 and now in the first 3 places by population ranks China, India and USA. In 2050 the order is India, China and USA and in 2100 the order will be India, China, Nigeria and USA (2012, How the feed the world in 2050).

#### **Resource depletion and pollution**

Since the beginning of its history, the man produced changes in ecosystem impact factor inducing the biocenosis. In the beginning people were too few and vegetation, abundant plants and animals, and today ratios were reversed, people are too much and exercise a variety of negative effects on the environment in which this population lived. Al these population consume energy, in same time will be held an important greenhouse gas that can amplify the greenhouse effect (Bogdan, A.T., & coll., 2010).



Figure 3. Population dynamics by 2100

As the human population increased, widened environmental stresses found in the disappearance of many species of plants and animals, some of them being sources of food or raw materials for the company.

Today man is the destroyer of his own ecosystem, anthropogenic fingerprint is determined by the level of civilization and industrialization which have the side effect of environmental destruction, to all components, including global warming phenomenon, with all the effects arising therefore (2012, Final Report Rio+20).

The population increases the demand for food increases, the planet had to feed both humans and animals.

In the rush to other energy sources and the food itself is suffering from pollution, leading to important changes in biodiversity will suffer at all levels.

Mankind tries to find renewable resources as the non-renewable (fossil) are practically exhausted. It puts emphasis on solar, wind, water, plants etc.

Reduction of greenhouse gas emissions is a central objective of European policy and more. Aware of this, overdeveloped countries, the emerging and international bodies should identify and help reduce greenhouse gas emissions (CO₂, methane, etc.) So gas emissions by 2050 to equal or much lower than emissions in 2009.

#### MATERIAL AND METHODS

Climate change has an immediate effect on the entire biodiversity.

There have been many scenarios, some optimistic, others pessimistic (providing a global warming by about 4°C (which would be a real disaster for life on the planet).

Impact of climate change on animals is several effects leading to disease previously unknown adaptation (Figure 4), decreased production, species extinction, etc.

In our study, the cattle, particularly watched ambient temperature influence on animals both in summer and in winter time, using general methods of clinical examination, observation of animal behavior modification, and statistical analysis laboratory determinations hygiene, nutrition, birth, infertility, livestock production.



Figure 4 Consequences of climate change

### **RESULTS AND DISCUSSION**

### Innovative concepts bio, eco- economy and eco-bio-economy.

The concept of "bio" invites thinking about global challenges of the future and how the biological sciences can contribute to solving these complex problems.

Much of tasks to respond to global challenges lay biological sciences from contributions through industrial biotechnology applications to environmental issues of climate change, improving health, and feeding the world with fruitful crops and delivery in good nutrients and vitamins in food.

Ecology and economy eco-economy is a phrase that launched the famous American economist Lester Brown Eco-bioeconomy is a phrase which was launched by Academician Prof. Dr. Alexandru T. Bogdan in School postdoctoral project manager for livestock biodiversity and food biotechnology and bio-economy based eco-economy necessary sanogenesis - ID 63258 (Bogdan, A.T., & coll., 2010).

These new ideas were prioritized global objectives, which include: environmental degradation of the planet through water, soil and air pollution (acid rain, global warming, destruction of the ozone rule etc.) ice cap leads to reflective surfaces and thus reduce temperature rise; death of coral reefs and ecosystems change, degradation (fires that have destroyed thousands of hectares of forest areas), etc.

Eco-economy has been addressed extensively by Lester Brown, who says in his book "Eco- Economy" (2001) that "ecology and economy are two disciplines that are based on the premise opposites ", which is not entirely so (Brown, L., 2006.).

Currently practicing "environmental economy " idea accepted by Lester Brown. Currently eco-economy approach phenomena, especially social sustainability is the main premise, eco-economy being directly related to ecosystems and biodiversity. Founder of the famous Worldwatch Institute, Lester Brown introduced the concept of the book " Eco -Economy: Building year for the Earth Economy", 2001 (Brown, L., 2010.).

#### Impact of climate change on animals

Basic factors act on the metabolism and animal health by:

- direct channels - are those that act directly on the animals reached: health, increased mortality, emergence of new diseases, reproduction, growth and body development, production levels, quality productions (Ionita, L., & coll., 2010)..



Figure 5 Schematic representation of the relationship between the thermal zones and average temperatures

*The influence of air humidity on animal health.* Usual accompanying low humidity and high temperatures only in rare cases low temperatures (winter outdoors).

In hot environments increase the incidence of airborne infections, skin disorders occur (solar dermatitis, pruritus) and Poda disorders, shortness of breath, increasing the amount of airborne dust.

In cold environments – the humidity is low and appear frostbites varying degrees of extremities (ears, tail, breast) growth of germs in the air from the animals expectorations.



Figure 6 Effect of heat stress in cows

Dairy cows are very sensitive to heat stress, to temperature from 25  $^{\circ}$  C and the severity of the thermal stress is related to both the temperature and the humidity. Heat stress affects the state of healthiness and the performance of production.

A key element is that the effect of heat stress at a given temperature varies with the relative humidity. For example, no thermal stress occurs at a temperature of 28.3 C and 20 % relative humidity, but the animal is stressed at 28.3 C and 90 % relative humidity.

The mechanisms by which cow are trying to decrease caloric stress:

- increased body temperature (38.6° C). A body temperature > 39,16° C indicates heat stress.

- increased respiratory rate ( >70/min ) is a primary change that cows can use to combat heat stress.

- reduction of physical activity,

- increasing the consumption of water - may be increased by 20 to > 50 %.

- looking shadow cool and of cool areas

Solutions. Ways of mitigating climate change:

-creation of animal populations (breeds, lines, hybrids) high temperature resistant; -livestock under shelter microclimate programmed with the optimum comfort for the body :

-increasing area under forage resistant at drought: sorghum, Sudan grass, sainfoin, artichokes, etc.

### CONCLUSIONS

Globalization is a matter of analysis of resources and great environmental challenges of the contemporary world.

Climate change affects the entire biodiversity.

Climate change is causing major changes in everyday life, have harmful effects on plants and animals and causes severe metabolic changes and adapts to new conditions.

Animals hardly bear climate change and try to adapt to these changes.

5. Eco-innovative solutions aimed at obtaining varieties and breeds resistance and thermal comfort in shelters that provide scheduled microclimate conditions.

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## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

## Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# STUDY OF CAPACITY OF *METCALFA PRUINOSA* (SAY) (HEMIPTERA: *AUCHENORRHYNCHA: FULGOROIDEA*) TO TRANSMIT GRAPEVINE LEAFROLL VIRUS IN GRAPEVINE

Lyubenova Tsonka*1, Genov Nikolay*2

Keywords: Metcalfa pruinosa, GLRaV, vectors, grapevine.

## ABSTRACT

Results of a study for establishing the potential capacity of planthopper Metcalfa pruinosa (Say) (Hemiptera: Auchenorrhyncha: Fulgoroidea) to transmit Grapevine leafroll virus (GLRaV) in grapevine are presented. The trial was carried out in the greenhouse of the IVE – Pleven with the varieties Sauvignon, Merlot, Shevka and Rubin. The results of the diagnostics by ELISA test did not show presence of GLRaV in the experimental plants, after artificial colonization with the planthopper. Serotypes 1 and 3 of GLRaV were found in the body of larvae and adults of the insect pest after a certain period of feeding on diseased plants, which defined M. pruinosa as a potential vector of the disease.

### **INTRODUCTION**

Grapevine leafroll is the commercially most important viral disease in grapevine, spread in all grapevine growing countries over the world. Diseased vines have very low productivity and low sugar content in grapes (Abracheva, 1991).

From the investigated viruses in Bulgaria, following the agent of Grapevine Fleck Virus (*GFkV*), relatively the most widely spread is *GLRaV-3*, while GLRaV-1 and GFLV have smaller share, and Arabis Mosaic Virus (*ArMV*) was not detected in any of the studied vines (Kamenova *et al.*, 2007; Genov *et al.*, 2009).

Except by grafting there is evidence for the transmission of some serotypes of *GLRaV* in grapevine by insects of the family *Pseudococcidae* and family *Coccidae* (Tanne *et al.*, 1989; Cabaleiro and Segura, 1997; Petersen and Charles, 1997; Golino *et al.*, 2002; Sforza *et al.*, 2003; Kuniyuki *et al.*, 2005; Belli *et al.*, 1994; Fortusini *et al.*, 1997; Sforza *et al.*, 2000). The common feature for these two insect families is that they belong to order *Hemiptera*. Representatives of this order have the largest percentage (34.74%) of alien insects in Bulgaria (Tomov *et al.*, 2007).

The planthopper *Metcalfa pruinosa* discovered in Bulgaria in 2004 also belongs to order *Hemiptera* (Trenchev *et al.*, 2007). That insect pest was found as well in Bulgaria's

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neighbouring countries: Greece – 2002 (Drosopoulos *et al.*, 2004); Turkey - 2003 (Karsavuran & Güçlü, 2004), Serbia - 2006 (Mihajlovic, 2007); Romania - 2009 (Preda & Skolka, 2009).

*Metcalfa pruinosa* belongs to family *Fulgoroidea*, suborder *Auchenorrhyncha*. The representatives of *Auchenorrhyncha*, order *Hemiptera* play a significant role in the epidemiology of the important plant diseases. Many of them are known as vectors of plant pathogens - viruses and phytoplasmas (Weintraub and Beanland 2006; Weintraub 2007).

Six years after the first registration of the planthopper in our country in Plovdiv region, it was found in the region of the town of Pleven in Merlot variety vines (Lyubenova, 2010). That necessitated various studied to be conducted both for monitoring and determining the range of hosts, as well as establishing its potential to transmit diseases. This paper presents the results of establishing the potential capacity of *M. pruinosa* to transmit *Grapevine leafroll virus* in grapevine.

### MATERIAL AND METHODS

The study was carried out in greenhouse conditions. Two-bud cuttings from healthy and diseased vines were rooted. In a screenhouse consisting of three sections 161 vine plants were grown in pots, including healthy ones and infected with Grapevine leafroll virus (fig. 1 and 2). The screenhouse was protected by insect net Biorete 55 of Arrigoni Company.



Figure 1. Screenhouse with three sections.



Figure 2. Test plants.

Colonies of averagely 50 first instar larvae of *M. pruinosa* were placed in entomological cage with vines, separately infected with one of *GLRaV-1* and *GLRaV-3* serotypes. In the course of 20 days the insects were feeding on the diseased vines and then pots with healthy vine plants were put into the cage.

The health condition of the trial plants was established by DAS-ELISA protocol three months after the infection of the healthy vines as diagnostic kits of Agritest – Italy were used. Leaf tissues from the trial plants, larvae and adults of *M. pruinosa* were utilized for the diagnostics. The samples were homogenized in 1.5 ml test tubes Eppendorf with extraction buffer comprising 0.2 Tris, 1% PVP, 0.8 % NaCl, 0.05 % TWEEN 20 (pH 8.0) in 1:5 (w:v) ratio. After the extraction the samples were centrifuged at 6000 rpm before they were used for diagnostics. All variants of the trial include four replicates (plants/insects) and each experiment was repeated at least twice.

### **RESULTS AND DISCUSSIONS**

The results of the study were summarized in Table 1. The results from the tests for the Grapevine leafroll virus demonstrated that in the carried out experiment it was not found *GLRaV-1* and *GLRaV-3* transmitted from the diseased plants to the healthy ones.

Table 1

	Results from ELISA test for nearth status of the experimental plants and insects									
Мо	Cultiver	Initial state	Variant	ELISA						
JND	Cultival	minial state	v arrant	GLRaV-1	GLRaV-3					
1	Sauvignon 5/16	healthy	M. pruinosa	-	-					
2	Sauvignon 12/70	healthy	M. pruinosa	-	-					
3	Sauvignon – p.	GLRaV-1	Donor of GLRaV-1	+	-					
4	Merlot 19/45	GLRaV-1	Donor of GLRaV-1	+	-					
5	Shevka	GLRaV-3	Donor of GLRaV-3	-	+					
6	Rubin	GLRaV-3	Donor of GLRaV-3	-	+					
7	M. pruinosa	healthy	Larva	+	+					
8	M. pruinosa	healthy	Imago	+	+					
9	Merlot 19/45 (PC)	GLRaV-1	Positive control GLRaV-1	+	-					
10	Rubin (PC)	GLRaV-3	Positive control GLRaV-3	-	+					
11	Sauvignon 5/16 (NC)	healthy	Negative control	-	-					
12	Sauvignon 12/70 (NC)	healthy	Negative control	-	-					

Results from ELISA test for health status of the experimental plants and insects

The diseased vines showed positive sample only for GLRaV, for which they were donors. The vines of Sauvignon variety had positive sample for GLRaV - 1, and the vines of the other varieties (Merlot, Shevka and Rubin) - for GLRaV-3.

In the healthy vines of Sauvignon variety, colonized with *M. pruinosa* larvae it was not found any of the leafroll serotypes.

Fifth instar larvae and adults of *M. pruinosa* were captured at the time of reporting (fig. 3 and 4).

The samples of larvae, participated in the disease transmitting, were positive for *GLRaV-1* and *GLRaV-3*. Both *Grapevine leafroll virus* serotypes were also found in the adult insects.

The results showed that already in the larval stage *M. pruinosa* was ingesting the Grapevine leafroll virus in its body through feeding.



Figure 3. Larva of Metcalfa pruinosa.



Figure 4. Metcalfa pruinosa - adults.

The obtained results confirmed the data of Materazzi et al (1998), who were investigating the potential of *M. pruinosa* to transmit three viral diseases - *Grapevine leafroll-associated virus 3*, *Grapevine fanleaf virus* (*GFLV*) and *Grapevine fleck virus* (*GFkV*) in vine. By ELISA-test they found out that the insect ingested *GFLV* and *GLRaV-3* while feeding on infected vines as in the nymph stage it assimilated more efficiently the viruses compared to the adult planthopper.

Although in our study it was not accounted for transmitting *GLRaV-1* and *GLRaV-3* from the diseased plants to the healthy, *M. pruinosa* ingested the virus and it is assumed as one of the dangerous pests of vine.

## CONCLUSIONS

The data indicating the presence of *Grapevine leafroll virus* in the planthopper body determined *M. pruinosa* as potential vector of Grapevine leafroll disease.

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## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

## Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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## STUDIES REGARDING THE EFFECT OF FOLIAR FERTILIZERS ON PRODUCTION AND NUTRIENT SOLUTION CONSUMPTION OF THE CORNICHON CUCUMBER PROTECTED CULTURE

Mărăcineanu Ecaterina, Mărăcineanu Liviu¹

Keywords: cornichon, greenhouses, foliar fertilizers

#### ABSTRACT

This paper presents the main results obtained in the Işalnita greenhouses regarding the influence of foliar fertilizers on the production and consumption of nutrient solution for the culture of cucumbers, Cornichon type. There were used more hybrids characterized by early maturing, high strength of the plant, the absence of male flowers, fruits without bitter taste,  $L/\phi = 3.0/1.0$ , a good consistency of the fruit and high productivity. There was highlighted the different nutrient solution consumption depending on the stage of harvesting the fruits and the foliar fertilizers influence on the consumption or on the recorded production. The obtained data indicate the positive effect of the Cropmax product, ensuring a superior capitalization of the available water and nutrient resources, compared with the control plant (the witness) and with the variants based on Bionat and Folibor.

### INTRODUCTION

The cucumber is grown for its fruits that at the technological maturity are very appreciated in alimentation, for the attributes and diverse content of nutrients and for their therapeutic effect. In the vegetable area of Oltenia, perhaps the most important vegetable area in the country, the production of cucumbers has grown spectacularly since 1990 (Ciună S., 2001). The author considers that this growth is due to the increased consumption of fresh and canned cucumbers. In recent years, in some greenhouses the crops profitability can be ensured only by the cold cultivation, the culture that enabled the use of greenhouses in the two crop cycles was the cucumbers, Cornichon type. This was possible because the cucumber, Cornichon type crop has a shorter growing period, provides high productions for a consumption spread over a long period of time, which confers the culture a high economic value. The water consumption of different vegetable species is not correlated with the soil moisture so that the cucumber is placed in the group of vegetables with high consumption and reduced absorption (after Trummer 1952 quoted by Butnariu H. et al., 1993). As a result, these species require a stringent management of fertilizers and irrigation, being the first which need irrigation, especially since the soil solution is the direct source of nutrients supply for the plant.

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## MATERIAL AND METHODS

The experiments regarding the influence of foliar fertilizers on the consumption of nutrient solution for the cucumbers, Cornichon type protected culture, carried out in three years, were placed in the Isalnita greenhouses, located at a distance of about 18 km from Craiova, in the north - western part of the city, on the both sides of the national road between Craiova and Timisoara. The climate is temperate continental with some specific characteristics due to the proximity of the Jiu River, as following: high atmospheric humidity, almost daily presence of fog in autumn, temperatures lower than 2-3°C in comparison to the temperatures recorded in Craiova and the amount of yearly precipitations of 625.5 litres/m².

In order to carry out the experiment, two hybrids of cucumber, Cornichon type were used: Bianca and Renato, grown in the 1st production cycle. The crop density was of 22,000 plants/ha achieved by planting three rows on the span, with a distance of 40 cm between the plants in the row, on a flatten, shaped field, the density being imposed by the distance between the dropping system. The fructification of the used hybrids was made by removing all the shoots that appear on the main stem, so all the production will grow on the stem. The fertilizer administration was done through irrigation by the dropping system, in the same time with the poured water. The foliar fertilizers were administered according to the manufacturer indications.

### **RESULTS AND DISCUSSIONS**

The consumption of nutrient solution, as the average of the years of study, under the conditions of equal irrigation rate for the two hybrids, for all the eight stages of harvesting, led to differences between variants. Thus, for the Renato hybrid, the lowest consumption of nutrient solution is during the treatment with Cropmax on the entire harvesting period. The consumption has as a limit 162.3 - 67.3 ml/g fresh substance and it decreases when the harvest starts (stage I) until it ends (stage VIII). Also it can be seen that the irrigation rate varies in reverse, so that a low nutrient solution consumption/gsp is correlated with a high standard of irrigation rate and vice versa (table 1).

The following two variants (V₄ and V₂) record close nutrient solution consumption of 182.3 - 74.0 ml/gsp, and of 173.9 - 67.3 ml/gsp. At the end of the production cycle, the highest consumption of nutrient solution is recorded by the witness (control plant) with a value of 111.1 ml/gsp and the lowest values are recorded when applying Cropmax (88.6 ml/gsp).

Table no: 1

The	$V_1$	$V_2$	<b>V</b> ₃	V ₄	Irrigation	V ₁	V2	<b>V</b> ₃	$V_4$	Irrigation
harvesting	Mt	Bionat	Cropmax	Folibor	rate	Mt	Bionat	Cropmax	Folibor	rate
stage			Renat	0		Bianca				
Ι	215,8	182,3	215,8	182,3	215,8	182,3	215,8	182,3	215,8	182,3
II	147,3	133,1	147,3	133,1	147,3	133,1	147,3	133,1	147,3	133,1
III	113,6	103,9	113,6	103,9	113,6	103,9	113,6	103,9	113,6	103,9
IV	104,7	95,6	104,7	95,6	104,7	95,6	104,7	95,6	104,7	95,6
V	83,2	77,9	83,2	77,9	83,2	77,9	83,2	77,9	83,2	77,9
VI	67,9	60,4	67,9	60,4	67,9	60,4	67,9	60,4	67,9	60,4
VII	75,3	70,3	75,3	70,3	75,3	70,3	75,3	70,3	75,3	70,3
VIII	81,3	74,0	81,3	74,0	81,3	74,0	81,3	74,0	81,3	74,0
Average	111,1	99,7	111,1	99,7	111,1	99,7	111,1	99,7	111,1	99,7

The effect of foliar fertilizers on the nutrient solution consumption  $(m)(\alpha$  fresh substance)

For the Bianca hybrid the variation of the nutrient solution consumption in relation to the administered treatment is very similar to the Renato hybrid. We notice that the most important consumption of nutrient solution is recorded by the control plant (the witness), with an average of 111.4 ml/gsp. At the opposite site it is the variant 3 with a consumption that ranges between 192.7 ml/gsp in stage I and 63.1 ml/gsp in stage VIII, with an average of 87.5 ml/gsp.

The dynamics of the production and of the quantity of nutrient solution indicates that, as the production increases, the consumption of nutrient solution decreases, stage by stage. The stage VI can be considered for the both hybrids, a stage when the production is supported by the most reduced consumption of nutrient solution. It corresponds to the second decade of June (table no: 2). The more accentuated effect of Cropmax is manifested from the first stage of harvest and remains the same along the whole other stages. Therefore, the production cumulated on stages demonstrates once again the superiority of this product in the existing microclimate conditions.

Table no:2

	$V_1$	$V_2$	<b>V</b> ₃	$V_4$	$V_1$	$V_2$	<b>V</b> ₃	$V_4$			
Stage	Mt	Bionat	Cropmax	Folibor	Mt	Bionat	Cropmax	Folibor			
		Rei	nato			Bianca					
Ι	0,145	0,179	0,200	0,187	0,127	0,136	0,161	0,143			
I-II	0,221	0,247	0,295	0,266	0,207	0,248	0,296	0,259			
I-III	0,295	0,322	0,365	0,340	0,307	0,342	0,400	0,354			
I-IV	0,381	0,418	0,479	0,444	0,471	0,530	0,604	0,541			
I-V	0,537	0,573	0,629	0,597	0,529	0,599	0,688	0,612			
I-VI	0,656	0,740	0,816	0,727	0,665	0,728	0,814	0,741			
I-VII	0,605	0,652	0,696	0,657	0,625	0,699	0,749	0,709			
I-VIII	0,549	0,599	0,658	0,626	0,583	0,628	0,702	0,633			
Total	3,389	3,728	4,137	3,843	3,516	3,909	4,415	3,991			

The fruit production  $(kg/m^2)$  on variants and harvesting stages

Regarding the number of fruits/m², there is a favourable situation for all the considered variants. It can be also observed a more accentuated effect of Cropmax (table no: 3), followed by Folibor and Bionat. Analyzing the stages of harvesting, it appears that the permanent treated variant with Cropmax and the one treated with Folibor had a superior behaviour for the Renato hybrid. For example, they have provided production increases between 125.81 and 117.06% in 2002. Although the Bianca hybrid recorded lower productions during the harvesting stages, it ensured higher production growth of 137.18-121.86% compared to the similar variants of the Renato hybrid. It can be also noticed in this case the positive influence of Cropmax, followed by Folibor and Bionat during all the years of experimentation.

The highest production growth was recorded in 2002, and ranged between 127.72% and 136.58% (Cropmax), 118.36% - 121.28% (Folibor) and 113.63 - 117.96% (Bionat).

We notice that the most significant consumption of nutrient solution was recorded by the control plant, being of 195.5 ml/gsp in stage I. In contrast version 3 recorded a consumption which fluctuates between 138.2 ml/gsp in stage I and 64.4 ml/gsp in stage VIII, with an average of 72.0 ml/gsp. The dynamics of the production and of the quantity of nutrient solution indicates that, as the production increases, the consumption of nutrient solution decreases, stage by stage. The stage VI can be considered for both hybrids the stage when the achieved productions are supported by the most reduced consumption of nutrient solution. This means the period between 17 and 23 June.

e no	o:3
	e no

	<b>V</b> ₁	$V_2$	V ₃	$V_4$	$V_1$	$V_2$	V ₃	$V_4$		
Stage	Mt	Bionat	Cropmax	Folibor	Mt	Bionat	Cropmax	Folibor		
		Rei	nato			Bianca				
Ι	21,67	26,92	30,58	27,17	18,67	20,75	24,67	21,67		
I-II	33,92	37,92	45,75	40,25	30,83	33,83	41,58	39,00		
I-III	46,17	50,25	57,00	52,33	46,00	51,92	61,25	53,50		
I-IV	60,00	65,67	75,42	69,17	70,42	79,58	92,67	82,42		
I-V	82,83	87,83	97,33	91,92	77,67	89,83	103,58	92,83		
I-VI	101,58	111,25	122,92	110,00	100,42	108,42	124,25	110,08		
I-VII	92,42	99,17	109,25	98,50	93,75	103,00	113,67	105,33		
I-VIII	83,75	91,17	101,08	94,17	85,58	95,08	106,92	95,92		
Total	522,33	570,17	639,33	583,50	523,33	582,42	668,58	600,75		

The fruit production (fruits/m²) on variants and harvesting stages

It can be observed in the I-st cycle of 2003, that variant 3 provided for both hybrids the lowest consumption of nutrient solution, being of 74.1 ml/gsp for Renato and 61.2 ml/gsp for Bianca. The similar values are recorded by the variants 4 and 2, for the both hybrids, the highest consumption of nutrient solution being recorded by the control plant. Also it continues the trend of gradually decreasing the consumption of nutrient solution as the harvest reaches stage VIII, during which time the irrigation rate gradually increases stage by stage. The dynamics of the production and of the consumption of nutrient solution varies in inverse directions, so that a high consume of nutrient solution is correlated with a reduced production. This is the situation of the harvesting stage I. On the other hand, in the stage VI (16 - 22 VI) it is recorded the lowest consumption of nutrient solution and the highest production.

The data from the I-st cycle of production of 2004 reflects a dynamic of the nutrient solution consumption similar to the previous years. Thus, the data are favourable to variant 3 which recorded the lowest average values of 101.4 ml/gsp for Bianca and of 104.0 ml /gsp for Renato. It is followed by variant 4, where the average consumption ranged between 110.4 ml/gsp and 110.1 ml/gsp, then the variant 2 with a nutrient solution consumption of 114.5 ml/gsp and 112.4 ml/gsp, according to the hybrid. The highest consumption of nutrient solution was between 125.8 and 124.5 ml/gsp and it was recorded by the control plant. Also it continues the inverse correlation recorded during all the 8 stages of harvest, between the production and the consumption of nutrient solution. Also, the stage VI (14-20 VI) records the highest productions and the lowest consumption of nutrient solution.

In order to highlight the links between the various recorded features, it can be very helpful the graphical representation of the established relationship (regression line), the regression function and the correlation coefficient. Thus, the statistical analysis revealed the intensity of the links between the variables and their meaning. The correlations between the irrigation rate and the production lead to the conclusion that when the irrigation rate increases, the volume of production also increases. This issue is due to the role of water in the plant life and due to the mineral salts administered, according to the phenophase. Therefore, the term irrigation rate is not the most appropriate in this situation. Regardless of the used terminology, the correlations indicate a close and direct connection between the two investigated elements, the correlation coefficients recording high values in the most cases, between 0.83 and 0.95, depending on the year and variant (figure no: 1).



production, V1

The correlations between the irrigation rate and the number of fruits, as in the case of the correlations between the irrigation rate and production, show a direct link between the two variables, presented statistically through the correlation coefficients. Furthermore, the graphs are very similar to those recorded for the irrigation rate and fruit production (figure no: 2). However, there are cases when the correlation coefficients have low values, resulting a close relationship between the two studied elements. These are characteristic only in the second cycle of production, regardless of the year or the studied hybrid. Although in these cases it can be recognized a slight influence of the irrigation rate on the number of fruits, the most important influence belongs to some other factors.



Figure: 2 The correlation between the irrigation rate and the number of fruits, V1

#### CONCLUSIONS

In the 1st cycle, during 2002-2004, it can be observed that Bianca hybrid records higher production.

In comparison to Folibor and Bionat, the Cropmax provides the highest production growth while reducing the consumption of fertilizing solution.

The correlations between the irrigation rate and the number of fruits, and those between the irrigation rate and the production indicate that between those two factors there is a direct connection, presented statistically also through the correlation coefficients.

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## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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# EXCEPTIONAL CLIMATIC VARIABILITY IN OLTENIA IN THE SPRING OF 2013

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Keywords: precipitation excess, drought, scorching heat

#### ABSTRACT

In the spring of 2013 in Oltenia the weather evolution was characterized by an exceptional variability: quick changes from warm to extremely cold (excessively so in some time intervals) and from exceptionally rainy to droughty weather. These extreme transitions are related to the increasing climate variability connected to global warming. The floods of 3-4.04.2013 were followed by an excessively draughty interval (10.04-21.05.2013) which affected the crops. The high air temperature had a serious impact on the plant growth: the barley crops in the South and South-East of the region started ripening. The analysis of the climatic conditions of the spring 2013 in the South-West of Romania is a continuation of the extended studies of the regional climate variability (Bogdan Octavia et al. 2010, Marinică I. et al., 2012a, Marinică I. et al., 2012b) and is of great use to specialists, doctoral and master students as well as all those interested in the climate evolution.

#### **INTRODUCTION**

In the spring of 2013, in Oltenia, weather evolution has been marked by an exceptional variability with fast transitions from a warm weather to an extremely cold and even excessively cold in some intervals and from an excessively rainy weather to a droughty weather. These fast transitions from one extreme to another of weather aspect are due to the increase of climatic variability, an aspect which is directly connected to climatic global warming. We will further analyse this climatic variability and its causes.

## MATERIAL AND METHODS

For our research on this analyse interval we analysed the database archive of Oltenia MRC, NAM Bucharest (National Administration of Meteorology), the maps drafted by Agrometeorology Laboratory (ANM), synoptic maps, data provided by the archive of satellite and radar images, as well as facilities provided by Office.

## **RESULTS AND DISCUSSIONS**

Climatic conditions in March 2013 - Pluviometric regime in March Monthly quantities of precipitations registered in March were comprised between 47.9  $l/m^2$  in Bechet and 132.0 47.9  $l/m^2$  in Apa Neagră, and their percentage deviations from the multiannual means were comprised between 32.0% in Bechet and 126.9% in Tg. Jiu, which

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lead to the classification of pluviometric time type in March from very rainy (VR) to la excessively rainy (ER). *The monthly mean of precipitation* for the entire region was 79.0  $47.9 \text{ l/m}^2$ , and its deviation from the normal mean was 87%, which confirms the classification of excessively rainy month for the entire region. 25 days with precipitations were registered, of which 20 days with general light precipitations, and the rainiest period was in the interval March 11-14 and on March 21. Only in the interval March 25-28 precipitations under the form of snowfall were registered, and also in this interval a snow layer was registered whose thickness was comprised between 4 cm in Rm. Vâlcea and 21 cm in Târmigani in Mehedinți County, being the biggest in the country.

**Thermal regime in March** Monthly temperature means were comprised between 2.2°C in Voineasa and 5.4°C in Dr. Tr. Severin, and their deviations from the multiannual means calculated for the interval 1901-1990 were comprised between -1.4°C in Bâcleş and 0.1°C in Polovragi, and in the mountainous area 0.5°C in Parâng. According to Hellmann criterion, March 2013 was thermally normal at all meteorological stations excepting a small area in Mehedinți Hills in Bâcleş where it was cool.

*The lowest daily temperature means* were registered during *the late cold wave of spring* registered in the interval March 25-28, 2013.

This late intense weather cooling was due to a synoptic situation of late blizzard of spring, caused by the blending between a Mediterranean Cyclone and Greenlander Anticyclone with Scandinavian ridge (baric group "news items", VIth baric type, according to the classification of synoptic situations type – blizzard provided by O. Bălescu and N. Beşleagă, 1954). This cold wave was the most dangerous climatic risk of the spring of 2013 and caused the significant delay of spring arrival.

*Monthly minimum air temperatures* were comprised between -6.9°C in Voineasa (registered on March 5) and -2.4°C in Dr. Tr. Severin (registered on 3 and March 17), and the monthly mean of minimum temperatures was -5.2°C.

*Monthly maximum air temperatures* were comprised between 15.7°C in Polovragi (registered on March 8) and 21.7°C in Bechet (registered on March 10), and the mean of monthly maximum temperatures for the entire region was 17.7°C.

Monthly minimum temperatures at the soil surface were comprised between - 6.6°C in Caracal (registered on March 18) and -3.8°C in Calafat (registered on March 18).

Monthly maximum temperatures at the soil surface were comprised between 20.0°C in Apa Neagră (registered on 1 and March 8) and 31.6°C in Băilești (registered on March 11). The highest soil surface temperatures were registered on 8 and March 10, and the highest mean at the soil surface for the entire agricultural area from Oltenia was 25.0°C registered on March 10.

Maximum thermal values mean at the soil surface was  $26.8^{\circ}$ C. 18 days with frost and thaw at the soil surface were registered (transitions of soil surface temperature through the value of 0°C), 10 days in which the soil maintained thawed and only 3 days with continuous frost (in the last five days of March 26-28).

The long period of warm days and high temperatures at the soil surface led to the continuation of the vegetation phases of plants from agricultural crops and especially those which were highly reactive to temperature increase (rape, apricot tree, wheat, etc.). As a consequence of these continuations in phases, the intense weather cooling in the interval March 25-28 had some damaging effects on the concerned crops and also on some species of migratory birds which had come from tropical countries and had been unable to find sources of food. The chart of air temperature variation in Oltenia highlights the periods of weather warming and cooling in March 2013.

A weather warming registered in the intervals March 7-14, March 19-22 and March 30-31, namely for 14 days, and the amplitude of air temperature for the entire region was 28.6°C. *The linear tendency of air temperature variation* was highly decreasing, which constitutes an *anomaly for March* because this is the last month of cold season.

*The climatic phenomena* registered in March were: hoarfrost, fog, drizzles, rains and sometimes heavy rainfalls, lightning, thunderstorms, glazed frost, sleet, sleety, snowfall, blizzard, weather warming in the first part of the month, intense cooling in the last decade, frost and thaw at the soil surface.

Climatic conditions in April 2013 - Pluviometric regime in April. Monthly quantities of precipitations registered in April were comprised between  $37.3 \text{ l/m}^2$  in Caracal and  $94.8 \text{ l/m}^2$  in Polovragi, and their percentage deviations from the multiannual means calculated for the interval 1901-1990 were comprised between -17.3% in Caracal and 56.7% in Tg. Logresti.

The classifications of pluviometric time types according to Hellmann criterion were comprised between little droughty (LD) in Romanați Plain in Caracal, in Olt Couloir in Rm. Vâlcea and in the mountainous area in Parâng and excessively rainy, in the south of Gorj Hills in Tg. Logrești.

*The quantities of precipitation mean* calculated for the entire region was  $63.3 \text{ l/m}^2$ , and its percentage deviation from the multiannual mean was 12.0% which means a little rainy month (LR) on average for the entire region.

*The rainiest interval* was April 2-3, when the quantities of precipitations registered in the two days were comprised between 8.2  $1/m^2$  in Caracal in the east of the region and 64.6  $1/m^2$  in Polovragi and Tg. Logrești, and in the mountainous area 73.1  $1/m^2$  in Ob. Lotrului. The quantities of precipitation mean calculated for the entire region was 39.7  $1/m^2$ , and in the hilly area the quantities of precipitations have often exceeded 50.0  $1/m^2$  and caused flood, humidity excess in soil and water bogging on agricultural lands and grasslands.

**Thermal regime in April.** Monthly air temperature means were comprised between 9.5°C in Voineasa and 13.9°C in Calafat, and their deviations from the multiannual mean calculated for the interval 1901-1990 were comprised between 1.5°C in Polovragi and 2.8°C in Drăgășani and Rm. Vâlcea, which lead to the classification of pluviometric time type in Oltenia according to Hellmann criterion comprised between warmish (WS) and warm (W).

The monthly mean for the entire region was  $12.5^{\circ}$ C, and its deviation from the multiannual mean was  $2.1^{\circ}$ C which confirms the classification of warm month (W) for the entire region.

Monthly minimum temperature values were comprised between -0.8°C registered in Voineasa on April 17 and 3.7°C in Rm. Vâlcea on April 9, and the monthly minimum mean for the entire region was 1.5°C.

Most of the monthly minimum values were registered in the first five days of the month.

*Monthly maximum temperature values* were registered in the last day of the month and were comprised between 28.8°C in Polovragi and 32.4°C in Bechet in the extreme south of the region, and the maximum values mean for the entire region was 30.0°C.

At the soil surface the minimum temperatures were comprised between -2.1°C in Polovragi in the sub-Carpathian area registered on April 11 and 2.8°C in Craiova registered on 9 April.

Negative values were registered in Subcarpathians in the second decade, on April 16 (-0.5°C in Apa Neagră). *Monthly maximum thermal values at the soil surface* were

registered in the last day of the month and were comprised between 39.8°C in Caracal and 59.4°C in Dr. Tr. Severin and Calafat. The chart of air temperature variation in April highlights weather cooling and warming periods. *Cooling* intervals occurred on: April 3-5, April 7-9, April 18-19, and warming intervals on: April 1-2, April 6, April 10-17, April 20-30. In the interval April 10-30 weather warming was almost continuous, excepting some short intervals of cooling. We notice *the fast decreasing slope* of maximum and average temperature means.

*Continuous warming*, associated with the lack of precipitations caused fast the phenomenon of atmospheric and pedological drought, *accentuating the thermal and hydric stress in crops*, and leading to gradual enforcements and negative effects.

*The climatic phenomenon* registered in April were: fog, drizzle, rainfalls and sometimes heavy rainfalls, lightning, thunderstorms, hoarfrost in Subcarpathians, soil passing frost in Subcarpathians, weather cooling and warming, excessive precipitations in the first decade which caused floods and bogging of streams in crops, fields and grasslands, drought with different degrees of intensity in the interval April 11-30, wind gusts.

*Climatic conditions in May 2013 - Pluviometric regime in May. Monthly quantities of precipitations* registered in May were comprised between 41.4 l/m² in Craiova and 127.9 l/m² in Voineasa and their percentage deviations from the multiannual means calculated for the interval 1901-1990 were comprised between -49.8% in Drăgășani and 33.9% in Voineasa, which determines classifications of pluviometric time type comprised between very droughty (VD) on extended areas in the east and central Oltenia (Caracal, Slatina, Craiova, Drăgășani, Rm. Vâlcea and Apa Neagră) and very rainy (VR) on restricted areas in Băilești Plain and Voineasa intra-mountainous depression.

The monthly mean for the entire region was  $73.3 \text{ l/m}^2$ , and its percentage deviation from the multiannual mean was -8.4% which determines a classification of normal month on average from a pluviometric point of view. The temporal-spatial distribution of precipitations shows that the significant values from an agricultural point of view were registered beginning with May 22. In the interval May 1-21 there were no precipitations on extended areas, and when it rained the precipitations were isolated and lights. In the same period air temperature had high values with daily maximum thermal values comprised between 25 and 33°C, which caused a high thermal and hydric stress on crops.

The drought in the spring of 2013 was registered on a period of 41 days in the interval 11 April-21 May and was associated with high temperatures in the air and at the soil surface which caused significant gradual enforcements at wheat, barley and rape crops. On May 18, 2013 in the south-east of the region in Corabia area (with sandy soil) in barley crop the maturation phase was beginning (a characteristic aspect only in the south of Balkan Peninsula on that date) and beans were shrivelling.

This intense episodic drought caused the damage of wheat crops which were in the phase of beans formation, namely at the maximum need of water. Stagnations of plants occurred also in corn crop. *On May 20, 2013*, in autumn wheat crop (soil layer of 0-100 cm) the moderate drought (MD) was extended in most part of the region, and the strong drought (SD) in most part of Romanați Plain, and in corn crop (soil layer of 0-50 cm) the moderate drought (MD) was present in the entire Oltenia, and the strong drought (SD) in most part of Oltenia Plain.

There has been a similar situation in most part of the month although rainfalls started to be significant on May 22, because most of them were on restricted areas, and warm weather and intense water consumptions as well as intense evapo-transpiration did not enable a proper and fast rehabilitation of water reserve. This period of intense drought marked the third significant climatic variation of this spring.
**Thermal regime in May 2013.** Monthly air temperature means were comprised between 13.6°C in Voineasa and 19.8°C in Calafat in the extreme south-west of the region, and their percentage deviations from the multiannual means calculated for the interval 1901-1990 were comprised between 1.5°C in Voineasa and 3.0°C in Drăgășani which determines classifications of thermal time type comprised between warmish (WS) on restricted areas in the east of the region in Caracal, at the southern hilly limit in Tg. Logrești and in Voineasa intra-mountainous depression and warm (W) in most part of the region.

The monthly air temperature mean calculated for the entire region was  $17.8^{\circ}$ C, and its deviation from the multiannual mean was  $2.4^{\circ}$ C which confirms the classification of warm month (W) for the entire region.

*Monthly minimum air temperature values* were comprised between 2.9°C in Voineasa registered on May 16 and 8.4°C in Dr. Tr. Severin registered on May 28 in the period of weather cooling in the end of the month.

Monthly minimum air temperature mean calculated for the entire region was 6.0°C.

Monthly maximum air temperature values were comprised between 28.4°C in Polovragi in the Subcarpathian area registered on May 1 (in the second day of weather warming in the end of April) and 33.1°C in Calafat in the south-west of the region registered on May 19 (two days before the beginning of May rainfalls), and the maximum temperature means for the entire region was 30.2°C which confirms the prevalence of warm weather.

At the soil surface the monthly minimum temperatures were comprised between 3.2°C in Polovragi registered in the middle of the month on May 16 and 8.4°C in Calafat registered on May 28, and the minimum temperatures mean was 5.8°C. Monthly maximum temperatures at the soil surface were comprised between 30.4°C in Caracal registered on May 1 (which confirms the low regime of soil surface temperature in the central part of Romanați Plain in this spring) and 62.0°C registered on May 21 in Calafat in the southwest of the region. The maximum thermal values mean for the entire region at the soil surface was 55.5°C, which approach the characteristic values of the first summer month, June.

The chart of air temperature variation in May highlights the periods of weather warming and cooling, therefore periods of weather cooling registered in the intervals: May 13-17, May 22-28 and May 31 (amounting 12 days); periods of weather warming in the intervals May 1-12, May 18-22 and May 29-30 (amounting 19 days). We notice a decreasing linear tendency of air temperature, which constitutes *a climatic anomaly of May*, because of the intense weather cooling in the second half of the month.

These cooling occur systematically in most of the years which causes *the frequency of this anomaly*. The cooling from the second part of May have also a beneficent aspect because "tempers" the gradual development of plants, often causing stagnations which are in the beginning of June close to the normal stage of the specific calendar period. The significant thermal variations which occur during summer determine and complete their development.

*The climatic phenomena* registered in May were: rainfalls and sometimes heavy rainfalls, lightning, thunderstorms, hail, torrential rains, drought, weather warming and cooling, wind gusts and sometimes storm.

#### CONCLUSIONS

In the spring of 2013, the spring arrival was little early (LE), although warm weather predominated in an interval of 84 days during winter and during the first 14 days in March. The intense weather cooling and late spring blizzard in the interval March 25-28 led to the registration of some indexes of spring arrival lower than it would have registered if this cooling had missed. The first climatic alternation was the warm weather from the first 24 days of March. Weather cooling and later spring blizzard from the interval March 25-28 marked the second climatic alternation. The excessively rainy weather from the first decade of April was the second climatic alternations, and the drought in the interval April 11- May 21 was the first and intense warming in the last 20 days of April continued until 20 May was the fifth alternation. The cooling in the last decade of May marked the sixth alternation, and the return of rainfalls in the interval May 21-31 was the seventh alternation.

These fast transitions from a weather type to another, the rainy periods associated with cold weather, the drought periods associated with warmish weather show *an exceptional climatic variability* in this spring and *led to significant effects of thermal and hydric stress of crop plants which caused them significant damages*.

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## RESEARCH ON THE DYNAMICS OF TORTRIX VIRIDANA AND LYMANTRIA DISPAR FROM FORESTS OF OAK FORESTS FROM ARGES

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Keywords: insects, defoliators, butterflies

#### ABSTRACT

The impact products defoliators of Tortrix viridana L. and Lymantria dispar L. on physiological processes to the trees of cvercinee as well as to make available to staff from forestry and horticulture development populations taken in the study in order to take measures to reduce the negative impact caused by insects defoliation on forest ecosystems and horticulture. These effects include reduction in tree growth, crown dieback and tree mortality. Tree mortality is usually associated with other insects (wood borers) and pathogenic fungi that attack stressed trees. In extreme situations, nearly 100% tree mortality may occur over large areas. The most important impacts occur in urban/suburban settings. Defoliation and tree mortality may be very serious if impacted trees are valuable shade or street trees in urban settings.

#### INTRODUCTION

Research purposes presented in this work is, in the first place, to present the impact of defoliation caused of *Tortrix viridana* L. and *Lymantria dispar* L. on physiological processes to the trees of cvercinee as well as to make available to staff from forestry and horticulture dynamic populations taken in the study in order to take measures to reduce negative impact caused by defoliating insects on forest ecosystems and horticulture. Total area of forestry in Arges county is 276 832 ha representing 41,1 % of the total area of the county. The largest share have deciduous forests occupying 193 978. hectares (70.1%) and the remaining 82 854. ha being distributed coniferous forests (29.9%) (Inventory Forest Fund - ICAS 1984).

## MATHERIAL AND METHODS

Layout relief in increments corresponding natural setting of the main types of soils in the county, determined by temperature variation with altitude, an obvious vertical zonality of vegetation.Of the 193 978 hectares of deciduous forests, cvercineele occupies a total area of 47,809.4 ha of which require - 3,474 ha (1.9%), flasks - 7.890 ha (4.06%), oak - 34375.6 ha (17.7%), english oak - 1836.8 ha (0.9%), gray oak - 22 ha (0.01%), red oak - 211 ha (0.10%).

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*Tortrix viridana* L. (green oak moth) is a pest that attacks stands defoliator of oaks, especially those of english oak. Detection and weather *Tortrix viridana* can be achieved in all stages of development. During the month of May or even at the end of April is observed crown defoliation trees or even this larvae on the leaves. The adult stage is catching butterflies using pheromone races plastic 40/30 cm, glued on one side, which is set amidst pheromone lure (synthetic sex pheromone Atravir) (Dissescu, G. and colab., 1981). Use of the check points, each with as many 2-3 trees, as well as closer, which on the side of the sunny exhibition at a height of 1.2 - 1.5 m shall be fixed panels. Checkpoints are placed at the edge of the forest, the massive and inside stand near a meadow or a portion of the stand is reduced consistency. Location pheromone traps is pest before flight. The average density of population *Lymantria dispar* obtained by multiplying the average number of deposits on the shaft with the average number of eggs from one deposit. Once determined the average density of the insect population can proceed to calculate the coefficient of infestation is determined by the average population density corrected for natural mortality and corresponding critical number.

## **RESULTS AND DISCUSSIONS**

In sequential analysis conducted for pest *Tortrix viridana* three trees in the forest reservation Mihăesti administration on the degree of defoliation by infestation with eggs was found that the percentage of defoliation probably has values between 1 and 12 (Table 1), values which correspond to very low levels of infestation and poor.

Dynamic mestation of <i>Torrix virtaana</i> eggs during 2010 – 2013 nom Forestry Minaesti						
	Ecrest cal: sessile cal:	Number o	f branches	The degree of infestation		
	Forest oak – sessile oak	Harvested	Observed	Previous	Current	
	Stage	I octomber 2010	- february 2011			
1	Mihaesti Park	18	8	5	2	
2	Băjești	18	8	7	9	
3	Cireșului Valley	18	8	3	0	
	Stage II octomber 2011 - february 2012					
4	Mihaesti Park	18	12	2	10	
5	Băjești	18	12	9	3	
6	Cireșului Valley	18	12	0	3	
	Stage III octomber 2012 - february 2013					
	Mihaesti Park	18	12	10	12	
8	Băjești	18	8	3	14	
9	Cireşului Valley	18	8	3	2	

Dynamic infestation of *Tortrix viridana* eggs during 2010 – 2013 from Forestry Mihaesti

For forests under management of forest reservation Costesti (Table 2) values of the degree of infestation are between 8 and 20, results that establish a low to medium degree of infestation, a situation which still requires careful monitoring of the evolution of livestock populations carnation leaf-rollers. A special case is the forest Smei - Cerbu defrosted where test results have ranged from 17 to 20 with a medium degree of defoliation likely.

Table 2

#### Dynamic infestation of *Tortrix viridana* eggs during 2010 - 2013 from Forestry Costesti

	from Forestry Costesti				
Nr.	Forest oak –	Number of branches		The degree of	infestation
crt	Italian oak	Harvested	Observed	Previous	Current
	St	age I October 20	010 - February 2	011	
1	Smei - Cerbu	18	16	18	20
2	Podeanca	18	12	10	8
3	Negoveanca	18	10	10	10
	Sta	ige II October 2	011 - February 2	2012	
4	Smei - Cerbu	18	16	20	17
5	Podeanca	18	14	8	10
6	Negoveanca	18	14	10	12
Stage III October 2012 - February 2013					
7	Smei - Cerbu	18	16	17	19
8	Podeanca	18	16	10	12
9	Negoveanca	18	16	12	12

In forests under management of forest reservation Mihăești test results *Lymantria dispar* L. after egg deposition showed that insect populations are at a minimum scale entry into neextind danger (table 3).

After analyzing the deposition of eggs in three stands of oaks in the forest reservation management Costești and summarized in table 4 revealed that the pest is in phase II and III with an average degree of infestation. A significant increase in the pest population was found in Gavan Trees where average fertility and the average weight of an egg deposits reached 932, or 0,740 g / tip, a situation corresponding to the maximum average degree of infestation. 2013 Forest Gavana will be included in the control area.

Pest population dynamics *Tortrix viridana* L. and *Lymantria dispar* L. in forests monitored showed no remarkable findings, however, that in some herds stands pests are in the early stages and numerical growth showed that these forests continue to be closely monitored.

A typical situation where a reprezită *Lymantria dispar* in the last half century intervals called time cvercineelor infested large areas of habitat . *Lymantria dispar* gauge regular successive intervals of 7 -12 years, but sometimes more frequently (3-5 years), but in most cases the gauge has been stopped during the increase in number and rash due to chemical treatments and biological frequently applied, for a long period of time. In other situations, scales were not developed due to the influence elmentelor unfavorable climate, especially temperature and precipitation.

# Table 3

	Forest oak	Average laying	Average weight	Average weight	Eggs (%)			Eggs	
	/sessile oak	of eggs /tree	grams/ laying of eggs	Fecundity	Fecundity	Parasitized	Sterile	Viable	examined
	Stage I October - December 2010								
1	Parc	0,012	0.147	154	24	16	60	500	
2	Titesti	0.36	0.260	201	18	18	64	500	
3	Stalpeni	0.24	0.187	187	22	18	60	500	
	Stage II October- December 2011								
4	Parc	0,013	0.136	164	27	16	57	500	
5	Titesti	0.32	0.218	209	28	18	54	500	
6	Stalpeni	0.21	0.184	188	19	18	63	500	
	Stage III October- December 2012								
7	Parc	0,024	0.160	155	22	16	62	500	
8	Titesti	0.31	0.270	192	26	18	56	500	
9	Stalpeni	0.27	0.203	201	28	16	56	500	

# Ponte analytical results of Lymantria dispar in oak forests Mihăești forest reservation area for the period 2010 - 2012

## Table 4

<u> </u>				T				1
	Forest	Average	Average weight grams/ Fecundity			Eggs (%)		Eggs
	Italian oak	eggs /tree	laying of eggs	Feedmenty	Parasit.	Sterile	Viable	examined
		5	Stage I Octo	ber - Decemb	per 2010			
1	Gavana	0,150	0,318	260	12	6	82	500
2	Negoveneasca	0,160	0,290	280	14	3	83	500
3	Brosteni	0,200	0,310	270	16	9	75	500
	Stage II October- December 2011							
4	Gavana	0,090	0,101	210	15	6	79	500
5	Negove neasca	0,100	0,095	270	20	4	76	500
	Stage III October - December 2012							
6	Brosteni	0,085	0,110	240	25	6	69	500
7	Gavana	0,300	0,740	932	3	1	96	500
8	Negove neasca	0,300	0,585	744	2	0	98	500
9	Brosteni	0,200	0,627	796	4	1	95	500

Ponte analytical results of Lymantria dispar in oak forests Costesti forest reservation area for the period 2010 - 2012

Scales large areas developed and *Tortrix viridana* L. , but not as regular as those typical of *Lymantria dispar* L.

## CONCLUSIONS

The most important insect defoliators of cvercineelor, *Tortrix viridana* and *Lymantria dispar* were found in all investigated stands, the likelihood of their manifestations is high risk of occurrence of damage over the economic damage threshold.

Presence in the ecosystems studied many species of lepidopteran defoliators (*Euproctis chrysorrhoea L., Malacosoma neustria L. Thaumaetopoea procession L.*) and *geometride* attacks that can produce adverse consequences associated with forests of oaks, as well as other valuable forest species in terms economically.

The infestation of trees is strongly influenced by a complex of factors: biotic – entomofauna useful and insectivorous birds, abiotic factors (temperature and precipitation), anthropogenic - grazing abusive and illegal felling of trees.

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## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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# RESEARCH IN VITRO ACTION OF FUNGICIDES OF DYNAMIC NEPERFECT PATHOGENS OF *TUBERCULARIA VULGARIS* TODE AND *ALTERNARIA SOLANI* SORAURER

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Keywords: pathogens, vitro, fungicides

## ABSTRACT

The influence of different pesticides used in cultivation on the growth of the fungus Tubercularia vulgaris and Alternaria solani, a natural pathogens of crops, was evaluated under the laboratory conditions. Our investigations approached a series of bioecological aspects of this pathogens: isolation, purification and obtaining of the pathogen. The fungicides studies were clorotalonil, thyophanat methyl, mancozeb, copper sulphate, procloraz and tebuconazole.

#### **INTRODUCTION**

Pesticides have taken a long time among the most important means of combating pests. Their use often irational abuse, phenomena led to many side effects, among which are the most important class.

Order to establish the lowest effective dose is to reduce human exposure and environmental elements biotic undue quantities of pesticides and increase the economic efficiency of treatment (Richiteanu, 2004).

## MATERIAL AND METHODS

The strains of *Tubercularia vulgaris and Alternaria solani* used in this study was isolated from leaves and stems of plants from the forest and plantation near Pitesti. The isolates were cultivated on media PDA. After 4 days the fungus covered surface was evaluated.

The pesticides studied were selected from wide spectrum of the pesticides commonly used in plants cultivation (Codexul produselor de uz fitosanitar omologate pentru a fi utilizate în România, 2004). Pesticides were added to sterilised medium after cooling to approximately  $40^{\circ}$  C. Plates were incubated at  $24^{\circ}$  C. Colony diameter was measured every three days for 21 days.

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#### **RESULTS AND DISCUSSIONS**

Coral spot is a weak pathogen of broadleaf trees. This gregarious ascomycete is hardly ever seen on conifers. Beech is the host most often infected, but this parasite is also fairly common on sycamore, horse chestnut and hornbeam. Particularly susceptible are trees that have already been weakened by other stressing factors such as drought, another fungal infestation or physical damage (Alexopoulos and coll. 1996)

The effect of Coral spot infection is that (usually small) twigs and branches die back, and then dense clusters of soft, pinhead-sized pink fungal blobs (the sexual stage in the complex lifecycle of this fungus) break through the thin bark. Later the blobs harden and turn dark reddish-brown (this is the conidial stage in the lifecycle), and by this time the infected timber is so weak that it tends to snap off during windy weather (figure 1).



Figure 1. Nectria cinnabarina

The pathogen grown in culture medium PDA (potato - glucose - agar) formed pink colonies with a radial development from the point of inoculation (figure 2). In this culture medium, the fungus is very rapid increase in 4 days it reached a diameter of 4/4 cm, after 10 days the surface is covered petri plate. Mycelium hyphae formed on conidiome differentiated is directly hyaline neseptați conidiophores. On conidiophores formed conidia ovoid or spherical, unicellular, isolated, hyaline, 2-3 micrometers. The malt-agar culture medium, the growth of the pathogen was similar to that of the PDA, after four days it reached a diameter of 3/3, 2 cm, and after 10 days 7/7 cm.

For this pathogen experimental variants of the product Tebuconazole were 700 ppm, 500 ppm and 100 ppm. The first variant, efficacy was remarkable pathogen growth rate is zero. Shortly after applying active substance penetrates the aerial parts of the plant and is distributed within acropetal tissues. This mode of action of the product allows for prevention, clean and in some cases even eradicative, resulting in high efficiency against pathogen at concentrations of 700 ppm and 500 ppm. The other two variants growth rate was significant evidence that tebuconazole only applied in the recommended work correctly on pathogen growth (figure 3, 4).



Figure 2. Isolates of Tubercularia vulgaris on PDA

Thiophanate-methyl is a systemic fungicide which has been used in our experiments at concentrations of 1000 ppm, 500 ppm and 300 ppm, fungistatic action is little effect on the rate of growth of the pathogen (figure 5).

Mancozeb was used in our experiments at the concentrations 2000 ppm, 1000 ppm and 500 ppm. The radial growth of mycelia was not inhibited by Mancozeb (figure 6).



Figure 3. Effect of Tebuconazole 700 ppm on Tubercularia vulgaris

Alternaria solani infects stems, leaves and fruits of tomato (Solanum lycopersicum L.), potato (S. tuberosum), eggplant (S. melongena L.), bell pepper and hot pepper (Capsicum spp.), and other members of the Solanum family. On tomato, foliar symptoms of A. solani generally occur on the oldest leaves and start as small lesions that are brown to black in color. These leaf spots resemble concentric rings - a distinguishing characteristic of the pathogen - and measure up to 1.3 cm in diameter. Both the area around the leaf spot and the entire leaf may become yellow or chlorotic. As the disease progresses, symptoms may migrate to the plant stem and fruit. Stem lesions are dark, slightly sunken and concentric in shape. In fruit, A. solani invades at the point of attachment to the stem as well as through growth cracks and wounds made by insects, infecting large areas of the fruit Fruit spots are similar in appearance to those on leaves – brown with dark concentric circles.



Figure 4. Effect of Tebuconazole 100 ppm on Tubercularia vulgaris



Figure 5. Effect of Thyophanate-methyl 500 ppm on Tubercularia vulgaris



Figure 6. Effect of Mancozeb 1000 ppm on Tubercularia vulgaris

The pathogen *Alternaria solani* grown in culture medium PDA (potato glucose agar) formed white colonies with a radial development from the point of inoculation. In the middle of the mycelia hyphae dark brown color within a range of 1.5-1.7 cm. Mycelia hyphae are brown, septate, richly branched, often interwoven. In this culture medium, the fungus is very rapid increase in 4 days it reached a diameter of 4/3,6 cm Petri dish after 10 days surface of the plate is covered in its entirety. In our *in vitro* observations I noticed a turn colony color from dark brown to white to olive concentric zonal uniformly throughout the mass by about 2 weeks.



Figure 7. Isolates of Alternaria solani on PDA



Figure 8. Effect of Copper sulphate 5000 ppm on Alternaria solani



Figure 9. Effect of Thiophanate methyl 1000 ppm on Alternaria solani

Copper sulphate neutralized dose of 5000 ppm had a strong fungistatic action, the growth rate of the fungus is close to that of the control at doses of 1000 ppm and 500 ppm (figure 8). Thiophanate methyl is a systemic fungicide which has been used in doses of 500 ppm, 1000 ppm, 2000 ppm and 4000 ppm. Very weak fungistatic action against the pathogen, its growth rate at a concentration of 1000 ppm is close to that of the control (figure 9).



Figure 10. Effect of Prochloraz 1000 ppm on Alternaria solani

Prochloraz is a fungicide endowed with properties of preventive and curative translaminar acting on a large number of pathogens and has been a strong fungicidal activity and in the concentration of 200 ppm the effect is the same, and the concentrations of 1000 ppm and 4000 ppm (figure 10).

## CONCLUSIONS

Coral spot (*Tubercularia vulgaris*) is a weak pathogen of broadleaf trees. The pathogen grown in culture medium PDA formed pink colonies with a radial development from the point of inoculation. Mycelium hyphae formed on conidiome differentiated is directly hyaline neseptati conidiophores. On conidiophores formed conidia ovoid or spherical, unicellular, isolated, hyaline, 2-3 micrometers. The growth of pathogen was not inhibited by Mancozeb, but the Tebuconazole fungicide 700 ppm and 500 ppm inhibited fungal growth the most.

*Alternaria solani* infects stems, leaves and fruits of tomato, potato, eggplant, bell pepper and and other members of the *Solanum* family. On tomato, foliar and fruit, symptoms of *A. solani* generally occur on the oldest leaves and start as small lesions that are brown to black in color. Copper sulphate neutralized dose of 5000 ppm had a strong fungistatic action on mycelia of *Alternaria*.

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# STUDIES ON THE SANDY SOILS ENTOMOFAUNA BETWEEN OLT AND JIU RIVERS

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Keywords: entomofauna, sandy soils

#### ABSTRACT

Research on sandy soils entomofauna focused mainly on the spread and frequency of insect populations from sandy soils area comprised between the rivers Olt and Jiu, from agricultural and horticultural crops, forest plantations and young forest plantations, fallow lands as well from the crops in the greenhouses of individual producers.

On this occasion were inventoried 208 species of insects belonging to 10 orders, 62 families and 155 genera, differentiated by the attack mode in 3 categories: harmful insects, beneficial insects and indifferent insects.

In this paper present a new species for the sandy soils between Olt and Jiu river, as well for Oltenia (Tuta absoluta Povolny - tomato leaf-mining) an quarantine pest.

#### INTRODUCTION

Sands and sandy soils occupy most of the land area of about 209400 ha. Located on the terraces of the Danube, about 97100 ha of these, occupies all terraces of the Danube, between Olt and Jiu river, forming a triangle with one side on the Danube, another part on the Jiu river and the third part very sinuous, crossing Plain Leu-Rotunda, after the cities Craiova and Corabia.

Sandy soils entomofauna in this area has undergone profound structural changes, as a result of their use category change, resulting after applying the modeling and leveling process, offering possibility for cultivation of various new species of plants, which adds to the traditional ones, as well the emergence of new species harmful to our country.

## MATERIAL AND METHODS

Research on the taxonomy and ecology of sandy soils entomofauna between Olt and Jiu rivers were conducted in 2011-2013,

Entomological material collection was performed every two weeks from April to October, with an entomological net, with an equal number of 50 collecting for each stage correlated with crop vegetation to detect feeding periods of the pest.

For collection of microlepidoptere species were used traps with specific pheromone and for nocturnal lepidoptera there were used light traps.

Entomfaunistic material was collected from both agricultural and horticultural crops, forest plantations and young forest plantations, fallow land as well from the crops in the greenhouses of individual producers.

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Important contributions regarding the entomofauna of Oltenia were made by various authors including: Bobîrnac & Matei 1985, Costescu & Mitrea 1989, Mitrea et al. 2000, Mitrea et al. 2002, etc.

Determination of entomological material was performed using various specialized determinators, after insects were collected than separated on systematic groups (orders, families, genera and species). To determine the entomofaunistical material there have used studies of various authors: Balachowschy et al. 1966, Bobîrnac et al. 1994, Panin S. 1951, Panin S. 1955, Stănoiu & Năstase 1994, Stănoiu et al.1995, etc.

## **RESULTS AND DISCUSSIONS**

From the data obtained on the structure of sandy soils entomofauna between the Olt and Jiu rivers we reported the presence of 10 orders of insects with 63 families included 154 genera and 208 species.

Most numerous species belong to the order Coleoptera, with 17 families, 70 genera and 98 species, followed by those belonging to Lepidoptera Order with 36 species, Heteroptera Order with 18 species, Hymenoptera Order with 16 species and Homoptera Order with 14 species.

The lowest number of species was recorded in the order Neuroptera order, one single species, followed by the Order Odonata with 2 species. (fig. 1).

After species frequency most numerous families are: Scarabaeidae (28 species), Chrysomelidae (16 species), Pentomidae (11 species), Coccinelidae (10 species), Carabide (9 species), Curculionidae (8 species), Tortricidae and Pieridae (with 6 species each).

Analyzing the collected entomofauna by economic importance, I found that insects collected are divided into 3 categories: harmful insects, beneficial insects and indifferent insects.

Table 1.

Family	Species	
Ord. <b>ODONATA</b> Fabr.		
Fam. LIBELLULIDAE	Libellula depresa L.	
	Lestes dryas Kirby.	
Ord. (	ORTHOPTERA Latr.	
Fam. TETTIGONIIDAE	Tettigonia viridissima L.	
Fam. DECTICIDAE	Decticus verrucivorusL.	
Fam. GRYLLIDAE	Gryllus desertus Pall.	
	Gryllus campestris L.	
Fam. GRYLLOTALPIDAE	Gryllotalpa gryllotalpa Latr.	
Fam. ACRIDIDAE	Acrida mediterranea Dirsch.	
	Acrida hungarica Herbst	
	Dociostaurus maroccanus Thumb.	
	Calliptamus italicus L.	
Ord. D	ERMAPTERA Leach.	
Fam. FORFICULIDAE	Forficula auricularia L.	
Ord. THYSANOPTERA Haliday		
Fam. THRYPIDAE	Limothrips denticornis Haliday.	
	Thrips tabaci Lindemann.	
Fam. PHLOEOTHRYPIDAE	Frankinella occidentalis Pergrande	

Structure of the sandy soils entomofauna between Olt and Jiu

	Haplothrips tritici Kurdj.		
Ord. HETEROPTERA Latr.			
Fam. PENTATOMIDAE	Eurygaster austriaca Schrank		
	Eurygaster integriceps Put.		
	Eurygaster maurus L.		
	Groapbosoma lineatum L.		
	Aelia acuminurata L.		
	Aelia rostrata Bohem.		
	Eurydema ornată L.		
	Eurydema oleracea L.		
	Palomena prassina L.		
	Carpocoris fuscipinus Bohem.		
	Carpocoris (Dolycoris) baccarum L.		
Fam. COREIDAE	Coryzus hyosciami L.		
Fam. LYGAEIDAE	Lygaeus equestris L.		
Fam. PYRRHOCORIDAE	Pyrrbocoris apterus L.		
	Pyrrbocoris marginatus L.		
Fam. CAPSIDAE	Lygus pratensis L.		
	Lygus pubescens L.		
Tam TINGIDAE	Stephanitis (Tingis) pyri Fabr.		
Ord.	HOMOPTERA Latr.		
Fam. APHIDIDAE	Aphis pomi De Geer.		
	Aphis fabae Scop.		
	Dysaphis devecta Wwlker		
	Myzodes persicae Sulz.		
	Myzus cerasi Fabr.		
	Hyalopterus pruni Geoffr.		
	Toxoptera graminum L.		
	Ropalosiphum maidis Fitch		
Fam. LECANIDAE	Eulecanium (Lecanium) corni L. Bouche		
	Lecanium hesperidum L.		
	Aspidiotus hederae Vall.		
Fam. DIASPIDIDAE	Quadraspidiotus perniciosus Comst		
	Aulacaspis rose Bché		
Fam. SCHIZONEURIDAE	Eroisoma lanigerum Hausm.		
Ord. H	YMENOPTERA Linné		
Fam. CEPHIDAE	Cephus pygmaeus L.		
Fam. TENTHREDINIDAE	Hoplocampa minuta Christ.		
	Hoplocampa testudineea Klung		
	Hoplovampa brevis Klung.		
Fam. ERYTOMIDAE	Eurytoma schrainerii Schr		
Fam. SCOLIIDAE	Scolia flavifrons Fabr.		
	Scolia quadripunctata Fabr.		
Fam. FORMICIDAE	Formica fusca L.		
Fam. VESPIDAE	Vespa germanica L.		
	Vespa vulgaris L.		

	Vespa crabro L.
Fam. APIDAE	Apis melifera L.
	Xylocopa violacea L.
	Bombus terrestris L.
Fam. TRICHOGRAMATIDE	Trichograma embriofagum Htg.
	Trichograma dendrolimi Mats
Ord.	COLEOPTERA L.
Fam. CICINDELIDAE	Cicindela hybrida hibrida L.
	Cicindela hybrida ab. in-humeralis Beuth
Fam. CARABIDAE	Zabrus tenebrioides Goeze
	Harpalus distinguendus Dufn.
	Harpalus calceatus Duftschmid
	Amara similata Gyll.
	Carabus cancelatus Illiger
	Carabus ulrichi Germar
	Carabus violaceus L.
	Calosoma sycophanta L.
	Calosoma inquisitor L.
Fam. SILPHIDAE	Aclvpea undata Mull.
Fam. STAPHILINIDAE	Paederus fuscipes Curt.
Fam. HISTERIDAE	Hister quadrinotatus Scriba.
Fam. MELOIDAE	Mylabris variabilis Pall.
Fam. CANTHARIDAE	Cantharis obscura L.
	Malachius aeneus L.
	Malachius bipustulatus L.
Fam. ELATERIDAE	Agriotes sputator L.
	Agriotes lineatus L.
	Agriotes ustulatus Schall.
Fam. BUPRESTIDAE	Copnodis tenebrioides Pall
Fam. DERMESTIDAE	Antbrenus scropbulariae L.
	Dermestes lardarius L.
Fam. COCCINELLIDAE	Subcoccinella 24-punctata L.
	Adonia variegata Goeze
	Coccinella 7-punctata L.
	Coccinella (Coccinella) 14-pustulata
	Coccinella 12-punctata L.
	Coccinella tessulata Scop.
	Coccinella (Propylea)14-punctata tetragonata
	Coccinella (Adalia) bipunctata L.
	Chilocorus bipunctatus L.
	Exochomus quadripustulatus L.
Fam. TENEBRIONIDAE	Gnaptor spinimanus Pali.
	Blaps mortisaga L.
	Blaps letifera Marsh.
	Opatrum sabulosum L.
	Pedimus femoralis L.

Fam. SCARABAEIDAE	Scarabaeus affinis Brulle
	Copris lunaris L.
	Geotrupes (s. str.) mutator Marsch.
	Geotrupes (s. str.) spiniger Marsh.
	Oryctes nasicornis L.
	Valgus hemipterus L.
	Epicometis hirta Poda.
	Oxythyrea funesta Poda
	Cetonia aurata L.
	Potosia (Cetonischema) aeruginosa Drury
	Potosia (s. str.) affinis Andersch.
	Polyphylla fullo L.
	Melolontha melolontha L
	Anoxia (Protanoxia) orientalii Kryn.
	Anoxia (s. str.) pilosa Fabr
	Amphimallon (s. str.) solstitialis L
	Phyllopertha horticola L
	Rizotrous aechinoctialis I
	Anomala solida Fr
	Anomala dubia Scop
	Anomala dubia ab marginata Schilsky
	Anomala dubia ab maculata Schilsky
	Anomala vitis Fabr
	Anomala vitis ab curreonitens Baudi
	Anomala errans ab desertorum Motsch
	Anisoplia (Chaetopteroplia) segetum Herbst
	Anisoplia (Autanisoplia) austriaca Herbst
	Anisoplia agricola Poda
Fam CERAMBYCIDAE	Vadonia livida Fabr
	Chlorophorus varius Fabr
	Purpuricentis hudensis Götz
	Dorcadion (Pedestredorcadion) pedestre Poda
	Phytopecia cylindrica I
Fam CHRYSOMFLIDAE	Lema melanona I
	Diabrotica virgifera virgifera Le Conte
	Labidostomis longimana I
	Cvaniris cvanea Fabr
	L'entinotarsa decembriata Say
	Entomoscelis adonidis Pall
	Chrysomela menthastri I
	Chrysomela aenea I
	Chrysometa populi I
	Phytodacta fornicata Bruggm
	Calaruaa pomonaa Soop
	Haltica olaracea I
	Dhyllotrota atra I
	r nyuoireia aira L.

	Phyllotreta nemorum L.
	Cassida nebulosa L.
	Cassida vibex L.
Fam. LARIIDAE	Bruchus pisorum L
	Acanthoscelides obtectus Say
Fam. CURCULIONIDAE	Sitona lineatus L.
	Hypera variabilis Hrbst.
	Bothynoderes punctiventris Germ.
	Tanymecus dilaticolis Gill.
	Tanymecus paliatus F.
	Otiorrhynchus ligustici L.
	Apion pomonae Fabr.
	Apion apricans Herbst.
Orc	1. NEUROPTERA
Fam. CRYSOPIDAE	Chrysopa perlla L.
Ord. LE	PIDOPTERA L. et auct.
Fam. LEUCOPTERIDAE	Leucoptera malifoliella (Costa)
Fam. LITOCOLETIDAE	Phyllonorycter (Lithocolletis) blancardella F.
Fam GELECHIIDAE	Anarsia lineatella Z.
	Tuta absoluta Povolny*
Fam. TORTRICIDAE	Cydia pomonella L.
	Laspeyresia funebrana Tr.
	Cydia (Laspeyresia) piricola L.
	Grapholitha (Cydia) molesta Busch.
	Lobesia botrana Schiff.
	Clysia amiguella Hb.
Fam. PYRALIDIDAE	Salebria semirubella ab. intermedia Kohl.
	Pyrausta nubilalis Hübner.
Fam. GEOMETRIDAE	Operophtera brumata L
	Erannis defoliaria Cl.
Fam. NOCTUIDAE	Agrotis exclamationis L.
	Autographa (Phytometra ) gamma L
	Mamestra (Barathra) brassicae F.
	Chloridea (Helicoverpa) armigera Hbn.
Fam. LIMANTRIIDAE	Lymantria dispar L.
	Stilnoptia (Leucoma) salicis L.
Fam. ARCTIIDAE	Hyphantria cuneea Drury
Fam. LASIOCAMPIDAE	Malacosoma neustria L.
Fam. PAPILIONIDAE	Iphiclides podalirius L.
Fam. PIERIDAE	Pieris brassicae L.
	Pieris rapae L.
	Pieris napi L.
	Pontia daplidice L.
	Colias hyale L.
	Colias croceus Fourc.
Fam. LYCAENIDAE	Chrysophanus dispar Hw.

	Lycaena icarus Rott.
Fam. NYMPHALIDAE	Pyrameis atalanta L.
	Pyrameis cardui L.
	Melithea phoebe Knoch
	Epinephele jurtina L.
Fam. ARCTIIDAE	Hyphantria cuneea Drury
Ord. I	DIPTERA L. et auct.
Fam. SYRPHIDAE	Sphaerophoria scripta L.
FAM MUSCIDAE	Musca domestica L.
FAM ANTHOMYIDAE	Delia (Hylemia) antigua Meig.
	Delia (Chortophila) brassicae Bechéé

*a new species for the Oltenia



Fig.1 Structure of the sandy soils entomofauna between Olt and Jiu rivers

#### CONCLUSIONS

Sandy soils entomofauna in southern Oltenia eight consist of 10 insect orders, 63 families, 154 genera and 208 species.

After the economic importance the insects collected were classified into three groups: pests insect, beneficial insects and indifferent insects.

The most common and numerous insect order was Coleoptera, with 17 families, 70 genera and 98 species, followed by Lepidoptera Order with 15 families, 31 genera and 36 species, and the orders Heteroptera 18 species, Hymenoptera 16 species and Homoptera 14 species.

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# LEGAL FRAMEWORK FOR THE REGULATION OF GM PLANTS IN PAKISTAN

Muhammad Sajjad Ali¹

Keywords: GMPs; regulatory instruments; monitoring mechanism

## ABSTRACT

A host of international accords and laws govern the cultivation, evaluation and commercialization of GM plants. Following is the identification of particular policy and regulatory characteristics and options for Pakistan in connection with the development and commercialization of GMPs. The regulatory scheme about GM crops in Pakistan is outlined and the guidelines are elaborated which must enable policy-makers to think through the various opportunities raised by agricultural biotechnology. The analysis of the monitoring procedures reveals a need for several improvements.

#### **INTRODUCTION**

Regulation is necessary for the use of modern biotechnology to be safe for humans and the environment. It is so because the integration, expression and stability of the DNA insert is not absolutely predictable in the receptor and the introduction is strictly regulated due to fears of release of transgenes into the genome of another organism. Consequently, many countries have developed their own regulatory systems for a rigorous evaluation of GMPs and derived products before their release into the environment or food chain. A national biosafety framework consists of an administrative system with public participation in decision making. Auxiliary legal, technical and administrative units make sure that the GM products are utilized in a safe and sustainable basis. This paper aims to identify the features of management and monitoring structure for genetically modified plants in Pakistan.

A model regulatory system is accompanied with the following features (figure 1):

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Figure 1:Features of an ideal regulatory system (source:modified from Jaffe,2004)

## MATERIAL AND METHOD

A review of contemporary agricultural policy documents regarding the research, import/export and field evaluation of genetically modified plants as well as the handling of trials' data by the monitoring committees in Pakistan is carried out. Visits to the concerned Government departments, individual meetings with relevant scientists and consultation of a vast literature about the regulatory framework for recombinant DNA technology make up various sources of data and information.

#### **RESULTS AND DISCUSSION**

## Regulation of agricultural biotechnology in Pakistan

Pakistan lacks a proficient capacity to regulate agri-biotechnology domestically due to a weak compliance to regulatory principles although a robust regulatory mechanism exists. Pakistan has ratified international agreements and is a member of major regulatory accords. Yet the progress in making national biosafety rules and regulations has been slow.

There are regulatory faults in the current monitoring mechanism as well (Foresster, 2008). The national biosafety committee set up under its provisions has the following composition.



Figure 2: Association of National Biosafety Committee with other stakeholders of agricultural biotechnology in Pakistan

Regulations, while still being the result of political expediency in finding a middle ground for social, industrial, trade and consumer interests, are formulated on the basis of different criteria from those used in the industrialised world.

#### **REGULATORY INSTRUMENTS**

In order to strengthen research and development in biotechnology, the government of Pakistan took several steps e.g. the establishment of the National Commission on Biosafety; the formation of the Intellectual Property Organization, Pakistan (IPOP); amendments to the Seed Act 1976; and approval of the Plant Breeders Rights Act. These are discussed below.

## Amendments in Seed Act 1976

The seed industry of Pakistan is dominated by the private sector and in recent years, the participation of private local and multinational companies has increased considerably. Currently the private sector is playing an important role in seed production and marketing. The seed industry is regulated by the Seed Act, 1976. This Act, however, does not cater to the needs of the private sector. Several amendments to the Seed Act, 1976 were made by the Ministry of Food and Agriculture to facilitate the exchange of recombinant germplasm with the private sector.

## Plant Breeders' Rights Act

Under the sui generis system for GM plant material, the Plant Breeders' Rights Act was formulated in 2007 which is regulated by the Federal Seed Certification and Registration Department (FSC&RD). The legislation encourages the private sector, especially the multinationals, to initiate large-scale research in genetic engineering and seed production in the country. Under this act, a Plant Breeder's Rights Registry is established under MINFA.

#### **Biosafety Rules**

A very intractable issue is the cost of regulation (environmental safety assessment procedure) of GM events while complying with safety principles. The Cartagena Protocol should in principle lead to a significant reduction of that burden, because it allows importing countries to accept new GM products without additional requirements. In order to promote GM plants' evaluation domestically, Pakistan biosafety rules are set under Pakistan environmental protection Act,1997.They cover the import, storage and manufacturing of GMPs and their products as well as all genetic manipulations of plants, animals and microorganisms for research and commercial purposes.

## **Intellectual Property Rights**

Being a member of the World Trade Organization (WTO), Pakistan has the obligation to develop a strong intellectual property regime within the parameters set by the Trade Related Aspects of Intellectual Property Rights (TRIPS) agreement. A few Pakistani institutes have applied for patents of novel Bt genes because big multinational companies in the developed countries own patents of many Bt-gene(s), their sequences, expression, vectors etc. so any violation of the agreement and commitment with lenders or owners may attract patent problems. GM cotton is one example of crop where IP protection affects investment and research opportunities. A brief summary of regulations relevant with GM crops are given in the table 1.

Table 1.

Laws and Regulations adopted in the country				
Regulation/ Act	Function			
Cotton Control Act 1949	The Cotton Control Act 1949 regulates the cultivation of recommended varieties according to the agro-climatic conditions of the region. However, in practice, farmers grow multiple varieties on one farm. This gives non-uniform plant population and differences in fiber quality (Ahmad and Ali, 1994). It is obligatory for the Government to supply 100 percent of the seed requirements of each registered variety. At present, 55 percent of the seed requirement is realized by the public sector. Rest of the requirement is furnished either by the private sector or by farmer-to-farmer exchange.			
Seeds Act of 1976	Approved varieties of a crop need to be registered and their sale, exchange and barter is subject to this act.			
Amendments to Seed Act 1976	The amendments allow the R&D national centers to transfer genetic material to private companies.			
Pakistan Environmental protection Act 1997	The Pakistan Environmental protection Act was enacted on 6th December 1997, to provide for the protection, conservation, rehabilitation and improvement of environment with a prospective release of GMOs. It maintains the implementation of National Biosafety Rules, 2005.			
National Biosafety Guidelines & Pakistan Biosafety Rules 2005	These rules are applicable not only to the manufacture, import, storage, export, sale and purchase of Living Modified Organism (LMOs), but also to the different stages of research in biotechnology. It provides legal cover for the National Biosafety Guidelines and their implementation within the country. The national biosafety guidelines establish the proper procedures for carrying out research and the commercial release of GM crops.			

The national biosafety committee set up under National Biosafety Rules, 2005 involves an active interaction among different institutions. Its functions include:

- 1. Risk assessment and labelling procedures of GMPs;
- 2. To regulate the release of GMPs and their products in the country;
- 3. To align the existing monitoring procedure regarding the research and release of GMPs in concurrence with international standards and procedures;
- 4. To ensure inspection and regulation of GMPs at the provincial level;
- 5. To prohibit any potential hazardous experiments or trials with GMPs;
- 6. To ensure measures for development of Biotechnology research in domestic public institutions;
- 7. To ensure safety related aspects of research upon GMPs

## MONITORING SYSTEM FOR GMPs IN PAKISTAN

In order to monitor the introduction and after-approval status of genetically modified plants, various agencies are involved (figure 4).



Figure 4:The Coordination among various agencies for the monitoring and approval of GM events in Pakistan

A regulatory system must set up the standards of biosecurity for consumer and environmental protection. The safety system must clealry define the factors to be followed before granting approval to an application. In case of transgenic plants to be used as food, there must be high/strict regulation for minimum tolerance to deviation from the set standards of biosafety limits. The consumers do not have trust in a regulatory system which is dysfunctional for biosafety because there is a perception that the benefits of the products are out weighed by their risks. The standard for transgenic plants can be set by a thorough compositional analysis of the transgenic food product. A series of laboratory analysis must be applied before commercial approval. Pakistan imports large quantities of GM food products from other countries. They include wheat, palm oil, sugar, maize etc, but hardly any biosafety tests are applied for the safety of these imports for consumers'health. Only the true-to-kind regulation is applied at the port of import to confirm the identity of the import. There needs to be a proper laboratory system to execute the contents' analysis. It must show whether the produce meet international legal limits of toxins in the products.

## **Transparency & Public Involvement in Decision Making Process**

Transparency is an important component of a regulatory system. A transparent system assures:

- Information about regulatory process including all those involved in regulatory, its responsibilities and the mode inwhich these responsibilities will be carried out;
- The access to contents of notifications and all authoritative documents which attest to the fact that notifications is conforming to legal obligationsgale;
- A vivid presentation of document of decision which describes motives behind the adopted decision;
- Informations about the submission of notification for approval and the ways in which public can access these notifications

Pakistani agricultural regulatory authories, although, provide the information about GM events developed and the ones in the process of development. However, there is no provision of information i.e. complete data about the submitted file for approval of GM event. There is a spread of common knowledge through newspapers and TV about the arrival of a GM event in the field but no formal system of communication exists which is helpful to give biotechnological and biosafety details , even after a crop is released. In this regard, the official websites need to be updated for such data especially Bt cotton, transgenic maize and more recently Bt rice which are poised for an imminent release on a commercial cultivation scale. It is important because the solicitor for approval and public will have a better understanding of the regulatory process and the specific products which must pass through the system.

In order to gain the trust of consumers, public participation is an integral part of regulatory process. It gives an opportunity to obtain information on public attitudes, comments and opinion about a regulation, procedure for application and the notifications/ applications submitted for approval. Before taking a regulatory decision, the regulatory authority should consult all stakeholders for their reservations/suggestions. It is necessary to make sure that all aspects of commercializing the GM event are studied/evaluated. So in the concluding remarks of the regulatory decision, helpful suggestions might be mentioned to enhance the trust level among public.

In Pakistan, during the process of deliberate release of a GM event either for commercial or experimental purpose, there does not exist any system of public consultation. The developers present their case with all data and informations about the transgenic event directly to NCB and the process of evaluation begins. There is not any kind of public participation in the assessment procedure.

## **Consultation with the Subject Specialists**

In most of the regulatory systems, scientific experts are consulted by making scientific committies. It is important for the regulatory process that the consultation with the experts must continue at all stages; from evaluation of filed data contents to field evaluation reports. But their opinion must be consultative only and should not be implied in making a decision. It is so because the decision making involves other social, religious and ethical factors as well as public perception about the technology. It is beneficial that the decision comes from an organized central authority becuase it leads to more trust and confidence among public. In all cases, the involved regulatory stakeholders must avoid the clash of interests. Ideally, the authority for making the final decision should come from the department of public health safety and environmental protection to get the maximum trust of consumers.In Pakistan, because only GM cotton is approved so far for commercial cultivation and it is a cash crop; so the decision was done by PARC and it was communicated to farmers through proper channel i.e agricultural extension services.

## CONCLUSIONS

GMPs may not only pose a risk for human health or the environment but they must be treated from various other angles such as ethical, social and economic implications. But because the principal scope of regulatory/ monitoring authority is the evaluation of effects of GMPs on the environment and consumers' health, other aspects must not be treated/ included in the evaluation scheme and approval mechanism. The analysis of Pakistani regulatory regulations reveals the need for implementation of following activities in Pakistan:

- 1. Constitution of the National Biosafety Framework to implement international agreements and regulations effectively and to align existing laws and regulations accordingly;
- 2. Assuring the legislative framework upon import/export of GMPs, their labeling and traceability in food and feed,
- 3. Constitution of a National Catalogue (also available online) of GMPs accepted in Pakistan for commercialization or trials evaluation;
- 4. Capacity building and infrastructure development of the laboratories specialized in detecting the GMPs;
- 5. Formulation and implementation of the legislation for Post commercialization monitoring of GMPs;

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## CASE STUDY ON THE USE OF PESTICIDES IN ROMANIA

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Keywords: pesticides groups, class, active substances

#### ABSTRACT

European Parliament and the Economic and Social Council in its communication "Towards a Thematic Strategy on the Sustainable Use of Pesticides', recognized the need for detailed, harmonized and updated on pesticide sales and use in the Community. EC Member States should use national action plans designed to establish quantitative objectives, measures, timetables and indicators to reduce risks and impacts of pesticide use on human health and the environment to encourage the development and introduction of integrated pest management. The main objective of this study was to test the methodology for case assessment of pesticides (plant protection products) placed on the market, to obtain information on plant protection products placed on the market and sold for use in plant protection treatments to crops, the quantity of products sold, product categories and statistical investigation active. From statistical surveys revealed a large number of plant protection products sold in Romania because each firm producing products that have specific names for the same active substance. Regarding the amount of active substances regions there is a similarity with the type of crops as vegetables.

## INTRODUCTION

EC Member States should use national action plans designed to establish quantitative objectives, measures, timetables and indicators to reduce risks and impacts of pesticide use on human health and the environment to encourage the development and introduction of integrated pest management, and alternative approaches or techniques to reduce dependence on pesticides. Should monitor the use of plant protection products containing active substances of particular concern and establish timetables and targets for reducing their use, especially if they are an appropriate means for achieving risk reduction. In a broad sense, a pesticide can be defined as a product of inorganic or organic nature, used to combat agricultural pests and stored, and to combat biological vectors of human and

animal diseases.

Biologically active ingredients are generally toxic potential of environmental degradation. Many pesticides are toxic to humans. For these reasons the production, packaging, storage, transportation and use of pesticides are regulated by law.

European Parliament and the Economic and Social Council in its communication "Toward Strategy on the Sustainable Use of Pesticides", recognized the need for detailed, harmonize and updated on pesticide sales and use in the Community. Indicators on the risks for health and the environment related to pesticide use.

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The main objective of this study was to test the methodology for case assessment of pesticides (plant protection products) placed on the market. Through this research to obtain information on plant protection products placed on the market and sold for use in plant protection treatments to crops, the quantity of products sold, product categories and statistical investigation active.

Through pesticides substances were collected data on the quantity of plant protection products placed on the market by counties, regions, macro and national level.

## MATERIAL AND METHODS

The study was conducted by authorized operators with distribution of pesticides (plant protection products) on the market in Romania (1746 operators in 2010). This created a questionnaire in collaboration with the National Institute of Statistics, the questionnaire Placed Plant Protection Products Market (PPPPP). Reference period was 2009 questionnaire interviews sand were conducted through *face to face* interview. The statistical surveying pesticide shave collected data on the amount of plant protection products placed on the market by district and national level, detailed:

•manufacturers, importers and distributors of plant protection products;

•pesticide categories : fungicides (including bactericide), insecticides, herbicides and molluscocides, growth regulators and other plant protection products (vegetable oils, sterilizants of soil, rodenticides);

•major chemical groups for each category of pesticides;

•chemical classes, each class of pesticides and major groups (approximately 100 chemical classes);

•active substance (cca.500 substances).

To collect information on the amount of active substances from 2009 and for the purposes of statistical research in the field have used the following documents:

-Questionnaire statistical research-plant protection products placed on the market (PPPPP)

-Methodological guide field operator;

- The list of pesticides (plant protection products);

- Nomenclature of units authorized (www.madr.ro).

The results were summarized and interpreted statistically .

### **RESULTS AND DISCUSSIONS**

List of pesticides (plant protection products) sold in Romania includes 1159 products grouped in 11categories of classification.

The data on the total amount of pesticides placed on the market (Table 1) shows that in 2009 has been placed on the market 8,854,0277,173,647 kilograms and liters of plant protection products. Quantitatively, the distribution is very uneven, there are counties with very small quantities of products sold both in the solid and liquid forms(Mehedinti 1,723 kg, Sibiu 2,816 kg, Gorj 3,514 kg, Harghita 4,789 l, Maramures 8,093 l, Mehedinti 8,483 l and Baia-Mare 8,556 l).

Highest quantities of plant protection products used are found in Buzau 1,099,857 kg, Botosani 954,756 kg, Galati 871,059 kg, Ilfov 1,908,934 l and Timis 1,603,729 l. In general, higher consumption of products plant protection may also be associated with the fact that these counties are grown mainly cereals. On the other hand, in these areas there are large farms that plant treatment is performed more easily and regularly.

Aggregate quantity categories (Fig. 1), is observed in the total share their different plant protection products placed on the market in 2009. For solid products, 39% were in the

category of other pesticides, while only 5% were herbicides. The situation is reversed in the form of conditioning liquid, which, herbicides accounted for more than half (53%), while other pesticides and insecticides category had a weight of 14% and 11% respectively.

Table 1.

No	Counties	Total quantity of pesticides		No	Counties	Total quantity of pesticides	
1.01	countres	kg	liter	1101	countro	kg	liter
1	ALBA	95,281	66,234	22	HARGHITA	7,446	4,789
2	ARAD	43,895	26,890	23	HUNEDOARA	6,004	13,214
3	ARGES	45,681	121,944	24	IALOMITA	5,251	136,193
4	BACAU	242,453	127,626	25	IASI	18,667	17,053
5	BIHOR	52,034	245,820	26	ILFOV	711,937	1,908,934
6	BISTRITA-	8,086	8,556	27	MARAMURES	8,111	8,093
	NASAUD						
7	BOTOSANI	954,756	54,141	28	MEHEDINTI	1,723	8,483
8	BRAILA	314,544	223,466	29	MURES	66,315	93,785
9	BRASOV	396,830	649,317	30	NEAMT	28,196	99,160
10	BUCURESTI	279,027	574,801	31	OLT	21,943	28,868
11	BUZAU	1,099,857	67,043	32	PRAHOVA	12,478	8,567
12	CALARASI	12,477	411,952	33	SALAJ	8,460	11,951
13	CARAS-	59,144	24,778	34	SATU MARE	140,084	492,146
	SEVERIN						
14	CLUJ	68,786	32,178	35	SIBIU	2,816	10,133
15	CONSTANTA	177,116	499,830	36	SUCEAVA	25,609	33,600
16	COVASNA	27,851	22,614	37	TELEORMAN	37,253	149,402
17	DIMBOVITA	90,936	120,828	38	TIMIS	429,250	1,603,729
18	DOLJ	159,586	293,870	39	TULCEA	26,124	29,905
19	GALATI	871,059	45,956	40	VASLUI	31,639	376,623
20	GIURGIU	5,649	12,579	41	VILCEA	328,928	102,430
21	GORJ	3,514	10,551	42	VRANCEA	246,851	75,995
					TOTAL	7,173,647	8,854,027

Total quantity	of pesticides	placed in 2	2009 on	Romanian	markets

The analysis of the units placed on the market of plant protection products, as well as data on manufacturers, distributors and importers can see from the data that most companies acquire plant protection products from distributors.

The investigation revealed that the same company may purchase products from one, two or all three sources. Also, manufacturers, importers and distributors may sell plant protection products.

The investigation has been inventoried 121 manufacturers that sold plant protection products. Of these, 78 are from Romania and 43 are foreign. Expressing percentage, 64% are Romanian manufacturers and 36% of those foreigners (Fig. 2, Table 2).

According to information provided by the survey conducted, they sold 4,114 tons of active substances in the form of various plant protection products. Of these, 1,729 tons were fungicides, herbicides 1,733 tons, 576 tons and 76 tons of other insecticides of plant protection products



Fig. 1.Percents of plant protection products (a-kg, b-l)



Number of producers/importers/distributors of plant protec	ction products that are purchased
plant protection products in Ron	mania

Number of producers	121
Number of importers	102
Number of distributors	282
The number of companies who	
purchase from producers	232
The number of companies who	
purchase from importers	298
The number of companies who	
purchase from distributors	711



Fig. 2.Manufacturers that are purchased plant protection products

Distribution of the active substance groups of plant protection products (Fig. 3) is comparable to the situation in the European Community. In both cases the highest sales were fungicides, herbicides followed by insecticides. The group of insecticides, the active ingredient sold in Romania in 2009 was 3.5 times higher than that sold in the EU

(average/country) in 2003.Note that in the European Union, EUROSTAT report cited shows a clear trend of decreasing consumption of plant protection products.

About one third of the active substance for **fungicide group** came from products derived from sulfur (625,272.32 kg). And at EU level is the same substance found in the first two positions in the top 10 active ingredients in fungicides group, only sulfur percentage was higher, 54.8% (EUROSTAT report on the use of plant protection products in the EU in During 1992-2003, edition 2007).



Fig. 3.The active ingredients of 2009 by group of plant protection products in Romania and the European Union

A similar analysis of **herbicide active substances** shows that the group sold the active herbicides acetochlor was in 2009, the share was 32.9% of the total group. At EU level, glyphosate stood first, followed by isoproturon (EUROSTAT report on the use of plant protection products in the EU in the period 1992-2003, edition 2007).

Regarding the **group of insecticides** can see that the first two active ingredients, imidacloprid and thiamethoxam, representing more than 70% of the active substance of insecticides. At European Union ranked first chlorpyriphos, its share was only 15.6% of the total active substance group (EUROSTAT report on use of plant protection products in the EU in the period 1992-2003, Edition 2007)

Evaluation of the results of the first **class of fungicide** active substances is observed that organic sulfur products represent 35.6% of total fungicides marketed compounds, phtalamid is then 17.5%. Plant protection products containing dithiocarbamate in their composition, third with a share of 14.8% and copper-based fungicides 4th place with a total of 164 tons sold and a percentage of 9.3%.

If herbicide active substance classes ranked highest quantities sold were the type cloracetanilida with 626 tons, based herbicides aminophosphats and dinitroanilin with amounts 267 tons and 145 tons respectively.

## CONCLUSIONS

It is necessary to regularly update the nomenclature of products approved for marketing in Romania because it active substance change by abolishing or start-up sand changes to the contact; From statistical surveys revealed a large number of plant protection products sold in Romania because each firm producing products that have specific names for the same active substance;

In areas where companies, large farms are operated by agronomists, there is also interest in proper against pests and diseases;

On plant protection products groups and classes, the top 10 were found there the same products in the European Community which means that pest and disease infestation is similar to our country.

# ACKNOWLEDGMENT

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Harmonization of Romanian agricultural and environmental statistics with European norms and standards 2007/19343.07.01

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## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

# Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# PHYSIOLOGICAL RESEARCHES IN *CITRULLUS LANATUS* (THUNB.) MATSUM. & NAKAI GRAFTED PLANTS CULTIVATED ON SANDY SOILS FROM THE AREA OF OLTENIA

Nicolae I.1*, Lascu N.1, Ploae Marieta²

*Keywords*: chlorophyll, photosynthesis, transpiration, watermelon plants

## ABSTRACT

This paper presents the physiological researches results carried out in watermelon plants (Citrullus lanatus Thunb. Matsum. & Nakai) grafted on Lagenaria siceraria (Molina) Standley rootstock cultivated on sandy soils from the area Oltenia (CCDCPN, Dăbuleni, Dolj). It was noticed that photosynthesis and transpiration intensity varies depending on climatic conditions, presenting low values in the morning, high values at after noon and low values towards the evening. The linear regressions performed between the physiological processes intensity and the photosynthetic active radiation of watermelon plants show a positive correlation with specific variations in the grafted plants, in compared to not grafted plants. The chlorophyll content in mature leaves of grafted plants is low, between the content of chlorophyll and the photosynthesis being a positive correlation.

# **INTRODUCTION**

The physiological researches have been carried out within the national research program (PN II, No. 52147 / 2008) entitled: "Research on the foundation and development of technology for cultivation of watermelon grafted plants, in order to obtain biological production in sandy soils areas". On the sandy soils due to the low natural fertility and climatic conditions characterized by thermal and hydric stress, watermelon cultivation requires the application of biological green technologies.

Grafted plants improved plant growth and yield without any harmful effects on fruit quality. The positive effects of grafting can change according to the rootstock being used (Ozlem et al. 2007). The observations carried out watermelon showed that grafting seems to be an effective tool for disease resistance as well as having a positive effect on yield and quality.

Except controlling soil-borne diseases, grafted plants which have stronger root systems can be used to increase low temperature tolerance and yield and reduce fertilizer and agrochemical application (Yetişir et al. 2003).

The grafting influences growth and yield at watermelon plants. These increases can be explained advantages of grafting plants; tolerance of low temperature, tolerance of salinity, enhanced water and inorganic nutritient uptake (Rivero et al. 2003).

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The fruit size of watermelons grafted to rootstock having vigorous root systems is significantly increased compared to fruit from intact plants, and many growers are practicing grafting mainly for this reason (Lee 1994).

The biological grafted response is the culmination of a series of physical, biochemical, and physiological events, beginning with vascular connection in the rootstockscion interface and ending with effects on plant yield. Sufficient connection of vascular bundles between rootstock and scion increases water and nutrient flow, allows an enhancement of the photosynthesis; and as a consequence, the availability of carbohydrates as energy resources for the active uptake ions that lead to growth plants (Martínez-Ballesta et al. 2010).Photosynthesis is a core function and its functional status has been considered an ideal physiological activity to monitor when the health and vitality of plants is under scrutiny (Calatayud et al. 2003).

### MATERIAL AND METHODS

The physiological researches were carried out on watermelon plants (*Citrullus lanatus* Thunb. Matsum. & Nakai) grown on sandy soil at the CCDCPN, Dăbuleni, Dolj.

The research consisted of analyzing physiological at watermelon *Montana* and *Oltenia* varieties grafted on the *Macis* F1 hybrid of *Lagenaria siceraria* (Molina) Standley rootstock. The *Montana* and *Oltenia* varieties are from the group of early hybrids, but they are harvesting after 4-5 days, than the early hybrids.

The photosynthesis and transpiration were made by using the photosynthesis analyzer LCpro+. The chlorophyll content was estimated by Minolta SPAD 502.

## **RESULTS AND DISCUSSIONS**

The researches were carried out on July 7th 2009 and consisted of analyzing some physiological processes in grafted plants, in comparison to not grafted plants. The morphological aspect of watermelon plants of the *Montana* variety is presented in Figure 1 and 2 and of the *Oltenia* variety is presented in Figure 3 and 4.



Figure 1. Grafted watermelon plants - *Montana* variety (Original).



Figure 2. Not grafted watermelon plants - *Montana* variety (Original).



Figure 3. Grafted watermelon plants - *Oltenia* variety (Original).



Figure 4. Not grafted watermelon plants - *Oltenia* variety (Original).

The physiological researches of watermelon plants has been carried out in accordance with environmental factors. The photosynthesis intensity during the day increases from early morning due to the increase of light intensity, temperature and the stomata opening level until after noon, and then gradually decreases due to the reduction of light intensity and temperature, as well as to the closing of the stomata (Figure 5 and 6).



Figure 5. The photosynthesis intensity at the leaves of *Citrullus lanatus - Montana* variety. leaves of *Citrullus lanatus - Oltenia* variety.

The transpiration intensity during the day in watermelon plants increases starting in the morning due to the opening of stomata, increase of light intensity and temperature, presents high values after noon, when light intensity and temperature are high and air relative humidity is lower, and then decreases in intensity towards evening (Figure 7 and 8).





The photosynthesis and transpiration intensity is correlated with the photosynthetic active radiation, but it presents different values in grafted watermelon plants, in comparison to not grafted watermelon plants.

In watermelon plants - *Montana* variety can be seen as that photosynthetic active radiation increases starting in the morning (9 a.m.) when the values are of 1482  $\mu$ mol/m²/s for grafted plants and of 1302  $\mu$ mol/m²/s for not grafted plants, it grows until after noon (1 p.m.) when the values are of 1589  $\mu$ mol/m²/s for grafted plants and of 1609  $\mu$ mol/m²/s for not grafted plants and of 1609  $\mu$ mol/m²/s for not grafted plants and of 1586  $\mu$ mol/m²/s for grafted plants and to 1704  $\mu$ mol/m²/s for not grafted plants.

In watermelon plants - *Oltenia* variety can be seen as that photosynthetic active radiation increases starting in the morning (9 a.m.) when the values are of 1287  $\mu$ mol/m²/s for grafted plants and of 1415  $\mu$ mol/m²/s for not grafted plants, it grows until after noon (1 p.m.) when the values are of 1678  $\mu$ mol/m²/s for grafted plants and of 1761  $\mu$ mol/m²/s for not grafted plants and towards the evening (5 p.m.) the values decrease gradually to 1523  $\mu$ mol/m²/s for grafted plants.

The linear regressions performed between photosynthesis intensity and the photosynthetic active radiation at watermelon plants *Montana* variety show a positive correlation - the coefficient of determination ( $\mathbb{R}^2$ ) being of 0.83 for grafted plants and 0.69 for not grafted plants and linear regression made between the photosynthesis intensity and photosynthetic active radiations at watermelon plants *Oltenia* variety shows a positive correlation - the coefficient of determination ( $\mathbb{R}^2$ ) being of 0.64 for grafted plants and 0.63 for not grafted plants (Figure 9 and 10).

The linear regressions performed between transpiration intensity and the photosynthetic active radiation at the watermelon plants - *Montana* variety show a good positive correlation, the coefficient of determination ( $\mathbb{R}^2$ ) being of 0.87 for grafted plants and 0.99 for not grafted plants and linear regression made between the transpiration intensity and photosynthetic active radiations at the watermelon plants - *Oltenia* variety shows a positive correlation between these, the coefficient of determination ( $\mathbb{R}^2$ ) being of 0.76 for grafted plants and 0.85 for not grafted plants (Figure 11 and 12).



Figure 9. The correlation between the photosynthesis intensity and the photosynthetic active radiation at *Citrullus lanatus - Montana* variety. Figure 10. The correlation between the photosynthesis intensity and the photosynthetic active radiation at *Citrullus lanatus - Oltenia* variety.



Figure 11. The correlation between the transpiration intensity and the photosynthetic active radiation at *Citrullus lanatus - Montana* variety. Figure 12. The correlation between the transpiration intensity and the photosynthetic active radiation at *Citrullus lanatus - Oltenia* variety.

The mature leaves of grafted watermelon plants show a slight decrease in chlorophyll content of 1.66 % for *Montana* variety and of 5.78 % for *Oltenia* variety, in comparison to not grafted watermelon plants (Figure 13).



Figure 13. The chlorophyll content at the leaves of Citrullus lanatus.

### CONCLUSIONS

In watermelon plants it was observed that photosynthesis and transpiration intensity presents a minimum in the morning, a maximum after noon and a minimum toward the evening, but the values recorded in grafted plants show variations compared to not grafted watermelon plants.

The linear regressions performed between photosynthesis intensity and the photosynthetic active radiation incident on the surface of the leaf show a positive correlation at the watermelon plants - *Montana* variety (coefficient of determination  $R^2 = 0.83$  for grafted plants and 0.69 for not grafted plants) and at *Oltenia* variety ( $R^2 = 0.64$  for grafted plants and 0.63 for not grafted plants). The linear regressions performed between transpiration intensity and the photosynthetic active radiation show a good positive correlation at the *Montana* variety (coefficient of determination  $R^2 = 0.87$  for grafted plants and 0.99 for not grafted plants) and at the *Oltenia* variety ( $R^2 = 0.76$  for grafted plants and 0.85 for not grafted plants).

The mature leaves of grafted watermelon plants show a slight decrease in chlorophyll content, in comparison to not grafted watermelon plants.

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## RESEARCH REGARDING THE BEHAVIOR OF SOME VINE VARIETIES AT THE ATTACK OF THE MAIN HARMFUL AGENTS

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Key words: vine, pathogenic, acarian, variety, behavior²

### ABSTRACT

During our research on the behavior of eight varieties of vines cultivated in Craiova Banu Mărăcine Didactic Station at the attack of the main harmful agents it come out that they showed some variability during the three years of study in the light of changing climatic conditions.

To analyze the behavior of 8 vine varieties at the attack of the main pathogenic fungi for each variety has been calculated the degree of attack (GA %), depending on this value has been established the character expression and the behavior of these varieties, as well has been established

the attack frequency for the main pests.

# INTRODUCTION

Like other cultivated plants, vine, during the growing period is subject incidence of harmful agents attack, but only few species are more frequent and truly damaging (Tomoioagă and colab., 2006).

Grapevine downy mildew and powdery mildew can cause damage higher than other 15-20 diseases taken together, as well *Lobesia botrana* and acarians are more dangerous than other pest that occur only rarely on certain environmental conditions (Comes 1995).

Specialists estimate that damage produced by the main hramful agents, even when applying a rational agriculture, may amount to about 25-35 % of the crop, including the storage period until consumption (Pārvu 1998).

Moreover, for viticulture, practitioners believe that crop losses caused by pests and phytopathogenic agents, although not exceeding 10%, are normal (Irimia 2011). Varieties have a role in limiting the damage caused by harmful agents; a role that is often overlooked in plant protection, the main attention is given to other control measures (Mitrea 2000).

Concerns about the behavior of vine varieties at the main harmful agents attack had over the years a number of researchers (Blaich & Stein 1986, Heiny et al. 1985, Mitrea 2007, Stan 2007, Mitrea & Tudose 2011, Balaban & Mitrea 2012).

## MATERIAL AND METHODS

Biological material studied was represented by 8 varieties of vines from wich: 4 varieties for white wines (Fetească Regală, Italian Riesling, Fetească Albă, Sauvignon), 1

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variety for aromatic wines (Muscat Ottonel) and 3 varieties for red wines (Cabernet Sauvignon, Fetească Neagră and Merlot).

Behavior of vine varieties to attack of the major harmful agents was assessed in the field. Estimation of the attack produced by *Plasmopara viticola*, *Uncinula necator* and *Botryotinia fuckeliana* has been made according to the methodology used in the network of Stations Forecasts and Warnings. For each pathogen and variety has been established the frequency (F %), intensity (I%) and the degree of attack (GA %), the collected data has been processed by the usual formula. The attack was noted for each specific pathogen depending where the damage occurs, the number of control is in line with the methodology applyed in the diagnosis, prognosis and warning. The attack of the pathogens under study was evaluated on five levels set by Vonica 1998.

Acarians attack has been evaluated according to their frequency on the leaves, using the methodology control according to the Forecasting and Warning Network.

## **RESULTS AND DISCUSSIONS**

Centralization of data about the behavior of the 8 varieties of vines at the main harmful agents attack in climatic conditions of the period 2006-2008 (Table 1) shows that although varietal resistance is a stable character that has its basis in the fund genetic variety, it can be changed, each climatic factor having an influence clearly defined on the harmful agents that attacks vines. Regarding the main pathogen attack during the period under study as seen from the data listed in Table 2, Muscat Ottonel, Sauvignon and Riesling Italian varieties, showed medium resistance to *Plasmopara viticola* fungus attack and from the varieties with good resistance we noted Merlot, a variety with an attack degree value below 5%. To the *Uncinula necator* fungus attack good behavior has been recorded at Cabernet Sauvignon with an average value of the attack degree of 2.16%, while Muscat Ottonel and Riesling Italian presented an average value of the attack degree higher than 10%.

To the attack of *Botryotinia fuckeliana* good behaviour has been recorded at Cabernet Sauvignon, Merlot and Fetească Neagra while Italian Riesling, Muscat Ottonel, Sauvignon, Fetească Regala, manifested medium resistance, the average attack degree ranging between 19.21% and 24.93%. Regarding accarians attack vine varieties behaved differently as shown in Table 3. The highest attack frequency of *Eriophies vitis* (38.9 %) was recorded at Muscat Ottonel, while the highest attack frequency of red spider mite (*Tetranychus urticae*) has been recorded at Fetească regala.

Two other species of acarians whose attack is present in the vineyard ecosystem of Banu Mărăcine are *Panonychus ulmi* and *Calepitrimerus vitis*. In the case of Panonychus ulmi highest frequency of attacks 6.7% was recorded at Muscat Ottonel while in the case of *Calepitrimerus vitis* highest frequency of attack 2.8% was recorded at Cabernet Sauvignon.

On the other hand in terms of weight species of acarians during the growing season in the vineyard ecosystem the average for *Eriophies vitis* was 53.01%, 36.92% for *Tetranychus urticae* 6.70%, 3.34% for *Panonychus ulmi* and the Calepitrimerus vitis. Thus it can be said that during the investigation the most common species of acarians of Banu Maracine vineyard was Eriophies vitis control measures applied to control this species ensure the protection against the other species of acarians.

### CONCLUSIONS

In the three years of research, due to the interaction of climatic factors during critical periods of vines to the "key" pathogens attack has been recorded mainly averages low degrees of attack, which ranged between 4.54% - 16, 24% in the case of the

*Plasmopara viticola* fungus, between 2.16% - 14.88% in the case of *Uncinula necator* and between 3.86% - 24.93% in the case of *Botryotinia fuckeliana* fungus.

This has led to the conclusion that some vine varieties recorded in the literature as sensitive to the pathogen attacks in this research has been recorded with a medium strength or better, which allowed us to obtain yields close to "clean" in terms phytopathological.

At the vine varieties studied, in 2006-2008, there has been recorded the attack of the acarians species *Eriophies vitis*, *Tetranychus urticae*, *Panonychus ulmi* and *Calepitrimerus vitis* whose weight fluctuated depending on the variety and the viticultural year. The most common species remained *Eriophies vitis* and the Muscat Ottonel variety has been recorded as the most sensitive to the attack of acarians, with a number of attacked leaves of 584 out of 1500 analyzed, during the three years of research.

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Month		April			May			June			July		A	August		Sep	otemb	er	C	October	
	T°C	P mm	U	T°C	Р	U	T°C	Р	U	T°C	Р	U	Т°С	Р	U	Т°С	Р	U	Т°С	Р	U
Year			%		mm	%		mm	%		mm	%		mm	%		mm	%		mm	%
2006	12,1	58,9	70	16,7	48,8	65	19,7	125,6	72	22,8	29,7	61	21,0	132,8	70	17,4	12,5	69	12,8	22,2	75
2007	12,9	0,0	46	18,7	93,6	60	23,0	57,5	57	26,5	5,6	36	22,9	148,6	66	15,8	65,6	71	11,3	138, 8	77
2008	12,4	59,6	69	17,1	31,0	65	21,7	28,4	62	22,8	88,2	55	24,3	0,6	50	16,3	55,6	66	12,5	66,8	76
Average 1999- 2008	11,5	57,5	67	17,5	53,8	65	21,3	69,5	66	23,2	66,1	63	22,2	57,5	67	16,7	65,7	75	11,2	65,8	76

Climatic elements during the growing vine that influence the main harmful agents attack in 2006, 2007 and 2008

Table 1.

No	Variety				Pathogen							
		Plasmopara viticola				Uncinula neca	tor	Botryotinia fuckeliana				
		Average GA%	Character	Resistance	Average	Character	Resistance	Average	Character	Resistance		
			expression		GA%	expression		GA%	expression			
1	Fetească Regală	8,05	7	High	7,62	7	High	19,54	5	Middle		
2	Italian Riesling	12,45	6	Middle	10,91	6	Middle	24,93	5	Middle		
3	White Fetească	9,77	7	High	7,45	7	High	19,21	5	Middle		
4	Sauvignon	13,64	6	Middle	8,65	7	High	20,51	5	Middle		
5	Muscat Ottonel	16,24	5	Middle	14,88	6	Middle	24,21	5	Middle		
6	Cabernet	6,94	7	High	2,16	8	High	3,86	8	High		
7	Fetească Neagră	6,96	7	High	4,37	8	High	7,78	7	High		
8	Merlot	4,54	8	High	3,52	8	High	5,24	7	High		
9	Average	9,76	7	High	7,44	7	High	15,66	5	Good ⁺		

Behavior of vine varieties from Banu Mărăcine Didactic Station at the attack of the main pathogens in 2006-2008

Table 2

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Variety	Eriophies vitis		Tetranychus urticae			Pa	anonychus i	ılmi	Calepitrimerus vitis			
	Number	of leaves	%	Number	of leaves	%	Number	of leaves	%	Number	Number of leaves	
	Total	Attacked		Total	Attacked		Total	Attacke		Total	Attacked	
								d				
Fetească	1500	405	27,0	1500	495	33,0	1500	46	3,1	1500	20	1,3
Regală												
Italian	1500	522	34,8	1500	193	12,9	1500	36	2,4	1500	13	0,9
Riesling												
Fetească	1500	394	26,3	1500	271	18,1	1500	63	4,2	1500	28	1,9
Albă												
Sauvignon	1500	470	31,3	1500	420	28,0	1500	64	4,3	1500	30	2,0
Muscat	1500	584	38,9	1500	302	20,1	1500	101	6,7	1500	26	1,7
Ottonel												
Cabernet	1500	467	31,1	1500	345	23,0	1500	56	3,7	1500	42	2,8
Sauvignon												
Fetească	1500	458	30,5	1500	311	20,7	1500	55	3,7	1500	36	2,4
Neagră												
Merlot	1500	463	30,8	1500	294	19,6	1500	53	3,5	1500	41	2,7
Total	12000	3763	100	12000	2599	100	12000	474	100	12000	236	100
2006-2008												

Behavior of vine varieties from Banu Mărăcine Didactic Station at the attack of *Eriophies vitis, Tetranychus urticae, Panonychus ulmi* and *Calepitrimerus vitis* in 2006-2008

Table 3.

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## THE OCCURENCE AND DISTRIBUTION OF ADRENOMEDULLIN IMMUNOREACTIVITY IN THE AMPHIBIAN INTESTINE

Luminița Mariana Olaru¹

Key words: adrenomedullin, immunoreactivity, newts, frogs.

### ABSTRACT

The research objectives aimed at demonstrating the presence, nature and spreading area of the immunoreactive structures for the adrenomedullin in the gastroenteric tract of several species of amphibians (Triturus vulgaris, Rana esculenta, Hyla arborea, Xenopus laevis). Within this research the attention was focused on the quantitative distribution of the endocrine and neural imunomarked elements in various regions of the stomach and intestine, in order to shape some functional models of the gastroenteric tract specific to the newts and frogs studied. Constant AM identification only in the neural structures which innervates the enteric wall layers has been interpreted as a proof of the neuropeptide's participation in the modulating of blood influx from the stomach and intestine and of these organs' motility.

### **INTRODUCTION**

Adrenomedullin (AM), peptide  $\alpha$  - amide composed of 52 amino acids residues, was isolated by Kitamura et al. (1993a) from human pheochromocytomas. Peptide in rats, unlike the human, contains only 50 amino acids (Sakata et al., 1993).

The quantity and consistence of scientific information which demonstrates the distribution of the tissue neuropeptide in poikiloterme vertebrates, although superior to those of invertebrates, prove, however, insufficient compared with the data from the study of mammals. I mention the immunohistochemical investigations of AM in the hypothalamic- hypophysis tract (Gonzalez et al., 1998), hypophysis (Gonzalez et al., 1998; Collantes et al. 2003), pancreas (Del Corral and Rubio, 1998; Lopez et al., 1999) and intestine (Trandaburu et al., 2000), several representatives of fish, amphibians and reptiles. Information provided by these investigations seems to be rather sporadic, if relate to organotipical and taxonomy diversity of poikiloterme vertebrates, much greater than that of homeoterme vertebrates.

This exposed situation determined me to make actual immunohistochemistry research in order to identify and establish AM distribution patterns in the organs of the gastro-entero-pancreatic system (GEP) of some amphibians.

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### MATERIALS AND METHODS

## Animals

The research has been carried on a total number of four species of amphibians, most of them common in our country. The adult specimens of amphibians of both sexes were captured in spring time or purchased from commercial sources (Xenopus laevis). The amphibians had been kept in fresh-water aquaria and in terraria (Hyla arborea) for 2-4 days before being sacrificed. The animals were not fad while being captive. The employed species and the number of specimens are listed below:

Urodela newts: Anura frogs: Triturus vulgaris (5 specimens) Rana esculenta (4 specimens) Hyla arborea (2 specimens) Xenopus laevis (4 specimens)

### **Tissue preparation**

The animals were sacrificed under chloroform anesthesia and the target organs (gastrointestinal tract) were excised and divided into several regions, such as: stomach (upper, middle and lower), small intestine (duodenum, middle and distal) and large intestine. Fragments of different length for certain species have been taken and histologically processed from these regions. This has been done in order to compare the results for various species. The divided fragments of both organs were immersed in Bouin's fluid for 24-36 h, dehydrated through a graded ethanol series, cleared with toluene and embedded in paraffin. Serial sections of 5-6  $\mu$ m-thickness, prepared on a sledge microtome, were mounted on poly-l-lysine (Sigma, USA)-coated slides.

## Primary antibody

The rabbit polyclonal antibody used in this study was raised against rat adrenomedullin. It has been purchased from R.E. Lang, Marburg, Germany.

# Immunohistochemical protocol

For optical microscopy research, I applied the peroxidase anti-peroxidase complex (PAP) method having the changes listed below. To the original protocol PAP (Sternberger, 1974), the changes made in order to visualize AM in optical microscopy, included:

- the replacement of secondary and tertiary incubation steps with only one incubation in donkey anti-rabbit IgG coupled with peroxidase-antibody complex, a product of Amersham Pharmacia Biotech Company, England;

- the inserting, before developing the reaction 3,3 '- diaminobenzidine (DAB), of one incubation in double ammonium sulphate and Ni (from Riedel Haën, Germany). This step has been introduced in order to enhance specific immunocolor.

For optical microscopy investigations I have used the dilution of 1:500. The control of the specificity of this antibody was achieved by preadsorption with appropriate antigen (AM human 1-52 - Peninsula Lab., GmbH, Germany) or with antigens with related molecular structures (rat CGRP - Chemicon, Temecula, USA; rabbit IAPP - Biotrend, Germany).

The antigen-antibody binding sites were visualised by a 5 minutes – immersion of the section in a solution of 0,7 mM 3,3' – diaminobenzidine tetrahydrachloride (Serva Feinbiochem, GmbH., Germany) preparated in 0,05 M Tris-HCl buffer (pH 7,6), to which 0,02% hydrogen peroxide was added just before use. 0,01 M phosphate buffer saline (PBS) (pH 7,4) was used as diluent for every step of the procedure and as rinsing solution between each step.

The sections were finally dehydrated in ethanol, cleared with xylene, mounted in

Entellan (E. Merck, Germany) and examined in a Zeiss (Oberkochen, Germany) Photomicroscope II.

## **Specificity controls**

The control of the specificity of immunohistochemical methods and the primary antibody has been done according to the recommendations in the literature (Grube et al., 1986; Cetin et al., 1989, 1993; Trandaburu et Nürnberger, 1995).

Method - dependent specificities were tested by incubations of tissue sections with normal rabbit serum instead of primary antibody, with primary antibody supplemented with poly-L-lysine (2 mg/ml dilued antibody) and also by omission of single steps in the immunohistochemical procedure.

Controls for the antibody specificity were performed by using the primary antiserum preadsorbed (24h at 40C) with varying quantities (6-100  $\mu$ g/ml working dilutions of antiserum) of corresponding synthetic bovine antigen (Sigma, USA) or with structurally related antigens (xenopsin – Biotrend, FRG; neuromedin N – ICN Pharmaceuticals, Inc., USA). Preadsorption of the antiserum with the coresponding antigen at concentrations as low as 6 $\mu$ g/ml completely abolished immunoreaction, while its preadsorption with related antigens at concentrations up to 100  $\mu$ g/ml had no effect on immunostaining.

### **RESULTS AND DISCUSSION**

AM immunoreaction marked strongly stomach and intestine innervation in all species of amphibians investigated, given that immunopozitive enteroendocrine cells couldn't be identified. Reaction appliance to anurans, showed not only nerve fibers, but also terminals distributed in the superficial myentheric plexus (Auerbach) and the deep myentheric plexus (Meissner) (Fig.1 - 4). Semiquantitative evaluation of immunoreactive nerve fibers located in different layers of the intestinal wall, showed a tendency to increase their density, starting from the proximal segment of the organ to the distal one.

Finally, one last remark concerns the great incidence in the Hyla and Xenopus intestine of the nerve fibers and terminals distributed in the mucosa chorion, even at the very foundation of the villous epithelium (Fig. 5).

Immunohistochemical investigations to identify the AM presence and distribution in the gastro-entero-pancreatic system (GEP) are relatively few and often contradictory, even in mammals (Mulder et al., 1996; Sakata et al., 1998; Martinez et al., 1998). Generally, they show a heterogeneous expression of the neuropeptide in the endocrine and / or neural components of the system, sometimes negated by the biochemical test results and RIA (radioimmunological) (Martinez et al., 2000; Kiyomizu et al., 2001; Letizia et al., 2001).

Research dedicated to AM immunohistochemical identification and distribution in stomach and intestine, although they prove to be relatively numerous in mammals, however, address a limited range of species (human, rat and pig)(Mulder et al., 1996; Sakata et al., 1993; Ahrén et al., 1996; Kitani et al., 1998). All of them support the localization of immunoreactive material exclusively in endocrine elements distributed uniformly along the entire gastrointestinal tract. According to the opinion of several authors (Ahren et al., 1996; Kitani et al., 1998; Mulder et al., 1996), AM cells would be in human intestine and in rat one a subpopulation of 5-HT enterochromafine cells (serotonin-producing) and in the pyloric region of stomach, gastrin-producing cells. In comparison to those described above, the previous investigations (Trandaburu et al., 2000), as a matter of fact the only ones in the literature that approached the amphibians' stomach and intestine, evoke an immunohistochemical picture of the distribution AM that is quite different. It reveals the neuropeptide distribution in neural structures (nerve fibers and terminals) and

its absence of enteroendocrine cells. Such a model seems to be close to the one found in the echinoderms stomach (Martinez et al., 1996a), but also in the respiratory tract and in the central nervous system of mammals (Martinez et al., 1998; Satoh et al., 1995).

The AM exclusive nervous distribution in the amphibians' gastrointestinal tract is even more exciting, as the application of the same primary antibody, under identical methodological conditions, revealed in the pancreas, both of some neural structures and of endocrine immunostained elements. The discrepancy of immunohistochemical results could be attributed at least partly, to the different proteolytic processing in the amphibians' pancreas and intestine of the natural bioactive precursor of AM (PAMP and prepro-AM).



Figure 1. Ganglion cells (arrowheads) and the profiles of some nerve fibers (arrows) immunostained for AM in myentheric superficial plexus and circular muscle (mc) of the Hyla arborea' stomach. ml = longitudinal muscle. PAP method; AM dilution  $1:500. \times 430$ .



Figure 2. AM-immunostained nerve fibers (arrows) in the longitudinal (ml) and circular (mc) muscle layers of the Xenopus laevis' stomach. PAP method; AM dilution 1:500.  $\times$  430.



Figure 3. Cross section through newt's large intestine (Triturus vulgaris) with a network of nerve fibers is strongly immunostained in submucosa (sm) (arrows) and in the longitudinal musculature (ml) (arrowheads) of the organ. Fibers that innervate capillaries (C) in the submucosa also appear immunostained. mc = circular musculature. PAP method; AM dilution 1:500. × 276.



Figure 4. Fragment of Rana esculenta large intestine wall. In the myentheric deep plexus we note neurons (1-3) and the profiles of some nerve fibers (arrowheads) strongly immunostained. Longitudinal muscle innervation (ml) of this intestine segment (arrowheads) also appears strongly immunoreactive. PAP method; AM dilution 1:500. × 21

Regarding the functional role of AM quartered in the amphibians' gastrointestinal tract, unlike other authors (Kitani et al., 1998; Mulder et al., 1996; Sakata et al., 1998), who confer it, at mammals, the status of hormone with insulotropic function and regulation of local and systemic circulation, my observations rather plead for qualities of neurotransmitter involved in modulating motility, endocrine secretion, and local visceral circulation.



Figure 5. Intestinal villus from the Hyla arborea's distal region of the small intestine. The profiles of some immunoreactive nerve fibers from the mucosa appear parallelly directed to the long axis of villus. PAP method; AM dilution 1:500. × 276.

## CONCLUSIONS

The research covered in this paper represents the first comparative and comprehensive tackling of the AM appearance and expression in the organs of the amphibians' GEP system. As for the information revealed, it appears all original.

Gastrointestinal tract research of the same species of amphibians revealed:

a.the absence of the enteroendocrine immunoreactive for AM;

b.immunostained nerve fibers and terminals in all the layers of wall intestine;

c.the exclusive presence of nerve fibers in urodeles and the presence of terminals and nerve fibers in anurans, in the superficial myentheric plexus (Auerbach) and the deep myentheric plexus (Meissner),;

d.gradual increase of the incidence of neural immunoreactive structures in the proximal enteric region to the distal one.

The AM topographic distribution patterns suggest, in the stomach and intestine, the participation in modulating the motility of both organs and of local visceral circulation.

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## STUDY REGARDING INTRAUTERINE CONTRACEPTIVE DEVICES

Olimid Diana¹

Keywords: contraception, intrauterine device, side effects

### ABSTRACT

The intrauterine device is a long-acting and reversible method of contraception. The aim of this study was to determinate the prevalence of the IUDs usage among women at reproductive age and its possible side effects.

The majority of the IUD users were parous (96,42%) and over 30 years old (92,85%).

They are satisfied with this method and choose to use it until the menopause. Only 5,35% from IUD users reported discomfort, side effects or other problems.

### INTRODUCTION

The intrauterine device (IUD) is one of the most commonly used, reversible, sage and long-active contraceptive method. The first IUD appeared in the early 1960s. Currently there are available the following types (ACOG, 2010):

-biologically inert IUD, made of polyethylene;

-Cooper bearing IUD: TCu-380A, TCu-200C, Multiload (MLCu 250, 375) and the NovaT;

-IUD with hormons: with progesterone (Mirena) and with levonorgestrel (LevoNova);

-IUD with cooper and gold (Goldlily).

The most commonly used IUD is the TCu-380A.

The mechanism of action of the IUD depends on the type of the IUD (inert, cooper or hormonal). The biologically inert IUD determinate an inflammatory response of the endometrium (the uterine mucosa) that inhibit the nidation (the implantation).

Cooper-IUD releases a cantity of Cooper into the uterus. Cooper has spermicidal effect and reduce the motility, viability and the fertilization potency of sperm. Also, Co determinate the damage of the ovum and biochemical changes in the cervical mucus (Stanford and Mickolajczyk, 2002).

If the fertilization occurs, the IUD is acting by the following mechanisms:

-slowing or speeding the transport of the eggs through the fallopian tubes;

-the damage of the early embrio (blastocyst);

-the prevention of the implantation due to the endometrial changes.

The endometrial changes depend on the type of IUD. Cooper IUD do increase the number of leukocytes and determinate a chronic inflammation.

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Hormonal IUDs cause endometrial suppression with decreased thickness, size of the glands and amount of secretions. They, also, inhibit the implantation of the blastocysts by the alteration of the cellular metabolism. (Stanford & Mickolajczyk, 2002).

It is desirable to insert the IUD during the menstrual period when the cervical canal is most patent or in the postpartum period, after the six weeks postpartum medical examination (AGOC, 2007).

## MATERIAL AND METHODS

The study was made on 56 women, with ages between 24 and 45 years old, who were current IUD users and who came for a medical examination to the gynecological clinic. It was performed an anamnestic study which followed the next questions referring to the IUD users:

-the age;

1).

-the parity (nulliparous or parous);

-a monogamous relationship in the present or not;

-the number of the sexual partners during lifetime;

-microbiological and cytological cervical screening before the insertion; -side effects or others problems;

-follow up after the insertion (clinical and ultrasound examination);

-the desire to change or not this contraceptive method with another one.

### **RESULTS AND DISCUSSIONS**

Conform to their age the study participants were divided into five groups (Table

Table 1

The age	<25	25-29	30-34	>35
No. of cases	1	3	23	29
Percentage %	1,78%	5,35%	41,07%	51,78%

The repartition of the patients conform to their age

The repartition showed that the majority of the IUD users were over 30 year old (92,85%). The youngest IUD user was 24 year old.

The repartition of the IUD users conform to the parity showed that only two patients were nulliparous and majority (96,42%) were parous (Table 2, Figure 1).

Table 2

The repartition of the patients conform to their parity

Parity	Nulliparous	Parous
No. of cases	2	54
Percentage %	3,57%	96,42%

47 parous women (87,03%) reported that they don't want another pregnancy in the future and they finished their reproductive activity.



Figure 1. The repartition of the patients conform to their parity

Nulliparity is not and absolute contraindication for IUD insertion, but the risk of expulsion is high.

The insertion to a nulliparous woman is best to be avoided (in my opinion) because of the increased risk of reproductive tract infections and the consequences on the future fertility.

A study made in the USA surveyed 252 women, aged 14-27 years, about sexual and birth control history and their opinions about the IUD. The study showed that 55% had not heard of the IUD. The parous women were 4,4 time more interested to use an IUD than nulliparous. The majority of the participants were nulliparous (84%) and currently in a monogamous relationship (85%). 80% had more than one lifetime sexual partner (Fleming et al., 2010).

A rapport by the Center for Disease Control showed the birth rate among teenagers in the United States rose between 2005 and 2007 and the teenage pregnancy rate (83,6‰) is almost twice that of Great Britain (46,7‰) or Canada (45,7‰) (Hamilton et al., 2009). In european countries, like France or Sweden is a lower rate (22,2‰ and 25,0‰) (Darroch et al., 2001).

IUDs are used by fewer than 3% of reproductive-aged women in the United States. Women who have had children are more motivated to prevent pregnancies and are more likely to consider this contraceptive method. The attitude regarding the IUD usage among teenagers and young women in the USA is to encourage them to now about this contraceptive method. The reproductive tract infections risk in teenagers and young women should not be an absolute contraindication to use this highly effective and safe method of birth control (ACOG, 2007).

The World Health Organization (WHO) supports the use of IUD contraception in women from menarche to age 20 years, stating that the benefits outweight the risks (WHO, 2004).

51 IUD users (91,07%) reported that they have a monogamous relationship in the present. In only 7 cases (12,5%) the study participants reported that they had only one sexual partner until the present day and the others reported two or more partners during lifetime.

Multiple sexual partners increase the risk of the reproductive tract infections (ACOG, 2007).

Before the IUD insertion there is need to perform a microbiological and cytological cervical screening. The majority of the patients were investigated. Only in 4

cases (7,14%) the IUD was inserted without performing the screening of the vaginal discharge.

After 6 weeks of insertion there is necessary to perform a vaginal and a ultrasound examination to ensure that the IUD is still in its place (Figure 2).



Figure 2. Normal appearance of an IUD (transvaginal longitudinal ultrasound image of the uterus)

This examination should be repeated every year (WHO, 2004).

The IUD users should check her IUD strings after every menstrual period.

The procedure has the following steps:

-sit in squatting position;

-insert second or middle finger into the vagina to find the cervix;

-feel for the strings.

It is possible that the transcervical thread disappear.

When the threads is not visible, it may be that it was cut too short and can be found within the cervix. In this case it is advisable to remove the device and reinsert a new one, leaving the thread projecting at least two centimeters from the external cervical ostium (www.fsrh.org).

Regarding the follow up after the insertion, only 23 IUD users (41,07%) reported that they are followed the medical indications and repeated every year the cervical screening and ultrasound examination. The attitude of the majority is to forget about the necessary medical examination.

The possible side effects of the IUD usage are:

-menstrual pain;

-excessive bleeding between periods, or spotting;

-pain during sexual activity;

-the expulsion of the IUD out of the uterus;

-the perforation of the wall during insertion (1‰);

-inflamation of the uterus and fallopian tubes (pelvic inflammatory disease);

-increased risk of an ectopic pregnancy (WHO, 2004).

In this study I found no cases of expulsion or perforation. In one case I observed the migrations of the IUD into the cervix. The patient had no symptoms, she reported that she noticed the IUD into the cervix from the beginning, right after the insertion, so it could be a defectuous insertion. I found no intrauterine or ectopic pregnancies. In 2 cases the sexual partners of the IUD users reported discomfort during sexual intercourses because of the strings. The IUD users asked to the doctor to cut the strings.

52 IUD users (92,85%) were satisfied about this method and would'nt change it with another one. In 3 cases the patients had longer and heavier menstrual periods and in one case it has been installed the menopause.

Migration of IUD from its normal position in the uterine fundus is a frequently complication. Expulsion or intrauterine displacement of the IUD leads to decreased contraceptive efficacy. There are possible: the embedment of the IUD into the myometrium, the complete uterine perforation and migration into the peritoneal cavity. Expulsion of the IUD is occurring in up to 10% of the patients. It is favorised by early insertion in the menstrual cycle, nulliparity, immediate postpartum insertion. (Boortz et al., 2012).

Younger age and previous IUD expulsion are increasing the risk of failure (Thonneau et al., 2006).

One study examined all reported cases of elective surgical removal of peritoneally migrated IUDs. Regarding the symptoms at presentation almost half (48,1%) of included patients were asymptomatic and had discovered incidentally. 28,7% of patients were diagnosed as a result of pregnancy, 17,8% presented with abdominal pain and 4,7% presented with vaginal bleeding (Mosley et al., 2012).

A study which included 7034 women from six countries (USA, UK, China, Italy, Egypt and Netherlands) showed that IUDs are a highly effective method of emergency contraception, as 99,86% of users did not become pregnant after unprotected intercourse when an IUD was inserted post-coitally.

The maximum time from intercourse to insertion of the IUD ranged from 2 days to 10 or more. The majority of insertions (74%) occurred within 5 days of intercourse. The pregnancy rate was 0,09% (Cleland et al., 2012).

The ACOG recommended that the IUD may be used for emergency contraception within 5 days of unprotected intercourse (ACOG, 2010).

The use of IUD is absolutely contraindicated in women with:

-cervical or endometrial cancer;

-endocarditis or rheumatic heart disease;

-in the presence of pregnancy;

-acute pelvic inflammatory disease;

-acute cervicitis with cervical discharge;

-disfunctional uterine bleeding;

-allergy to any component of the IUD or Wilson disease;

-anomalies of the uterine cavity, polyps, submucous or intramural fibroids;

-3 months after a septic abortion or pelvic infection.

Insertion is more likely to produce bleedings, pain and sincope in nullipoarous women (WHO, 2004).

### CONCLUSIONS

The intrauterine device is a safe and reversible contraceptive method. The majority of patients who choose to use it are parous and don't want anymore children in the future.

The IUD is not the first option for the nulliparous women because of the risk of the reproductive tract infections.

The majority of the users were satisfied with this method and they reported only a few side effects or problems.

After the IUD insertion the patients should be instructed on how to check the presence of the transcervical threads. There is need to performed a follow up after the insertion by cervical screening, clinical and ultrasound examinations.

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## WINTER MOTH AND MOTTLED UMBER, THE MAIN DEFOLIATING SPECIES ENCOUNTERED IN FORESTRY PLANTATIONS

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Keywords: winter moth and mottled umber, sticky rings, number density

## ABSTARCT

Deciduous trees have suffered a strong impact through graduations of defoliating species making enormous efforts to avoid the trees that produced defoliated repeatedly, would have led to their drying.

Winter moth and mottled umber are a group of important pests for forestry and fruit plantations in the temperate zone, producing significant damage unless it is acting at the right time to fight or maintenance under the economic threshold for lowering.

Catching adult insects using sticky rings placed on the stem of trees leads to the maintenance of population under control and reduce damaging the reserve of biological pest control for next year, and at the same time as representing organic and effective control.

In forestry plantations of oak in Arges County, in the study on biological activity of adult stage occurred in early November and during the second decade of the month December, being influenced by changes in microclimate conditions.

## **INTRODUCTION**

Development of fruit plantations and productive forest stands of valuable species it is possible if it is assured a good state of health. This objective can be achieved only if animal pests affecting the vegetation are known, setting out the areas on which they shall be multiplied, and the degree of intensity of the attack. According to the above mentioned factors are appropriate protection measures in order to avoid extensive damage.

Due to the fact that forests and orchards were faced with particularly difficult issues, changes in this area have occurred at intervals of time not too big.

An important group of particularly dangerous pests is the winter moth and mottled umber (*Operophtera brumata* 1.- winter moth and *Erannis defoliaria* CI- mottled umber), the importance of the knowledge resulting from their ability to be multiplied in the table on large areas, producing defolieted with undesirable effects. Account should be taken of the fact that these insects are frequently associated with other defoliating such as *Tortrix viridana*, *Lymantria dispar* and disappear, thus creating a very strong pressure on the infested areas. Winter moth and mottled umber can be encountered in the oak groves and formations of the hills of the southern sub-Muntenia (Gaines, m., Litescu, m., 2008, Sabic, Williams Gh, et al., 2003).At the national level, after Simionescu A., 2012, in the period

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1959-2010 winter moth and mottled umber have infested an area of 8854, 4 thousand hectares, of which about 35 percent is in the South of the country.

Defoliating insects were a constant concern for many scholars, the study of the biology, ecology and control, both nationally (Ene, 1979, Stanoiu et al. 1979, Ciochia & Stanca, 2000, Trifan, 2009, Rosca et al. 2011) and international (Abgrall & Soutrenon, 1991) due to the damage it causes to the fruit crops and forestry.

## MATERIALS AND METHODS

Research has been carried out on the Argeş County, the two stationary: Colibaşi and Armeneasa, in the period 2011-2012, where they identified several types of stands in stating the presence of harmful organisms mentioned.

Evolution of populations of winter moth and mottled umber has been studied in the oak forests and plantations of fruit trees through the installation of stationary networks, in which trees and fruit trees were ringed with glue of caterpillars in the form of strip with a width of 5-10 cm (Figure 1).



Figure 1. Sticky rings on the tree (orig.)

In each location were installed five rings. In the days preceding the notification of the presence of insects, along with the first, copies selected brume was the glue of caterpillars. 2-3 days the rings have been checked, and females and males identify butterflies have been recorded in the logbooks. The sample trees were analysed carefully so as not to find females cracks of ritidom or at the base of the samples.

Determination of the degree of infestation to establish control and supervision areas was done according to some technologies for detection and forecasting.

It should be noted that there have been some improved working methods in the detection, forecast, prevention and control of pests, some of them being studied and the analysis of other groups of defoliators including on the *Lymantria dispar* and *Tortrix viridana*.

The material seized in the land with the help of the rings was brought into the laboratory and subjected to analysis and operation of identification and separation by species and sexes.

### **RESULTS AND DISCUSSIONS**

According to data recorded during the period 2011-2012, in Argeş County, it was found that the first adult insects are reported, you can place both male butterflies in flight and caught on the rings of glue (figure 2). According to data recorded has been found that the first male butterflies are insects reported, you can place both in flight and caught on glue rings. A few days can be detected and the females. As shown in the records made in the logbooks and winter moth and mottled umber made graphs have entered the business with the cooling time.

At the time of adult female wingless, climb on the tree trunk looking for partners or for the eggs, they are caught on the rings. Unfertilized females caught in the glue release sex pheromones that attract male butterflies. Abundance of males varies according to the number of females caught.

On the occasion of forecasting which took place during the period 2011-2012, on the rings of glue placed on the sample trees were captured and many butterflies the males.

Making an analysis of the emergence of adults in the two stationary taken in the study we can notice that Armeneasa station, in 2011 the first adults emerged on the 6th November, and females on the 8th of November (Table 1).

Table 1

2011	Armen	easa	Colibași s	station	2012	Armen	easa	Colibași station	
2011	male	female	male	female	2012	male	female	male	female
4XI	0	0	0	0	4XI	0	0	0	0
6XI	1	0	0	0	6XI	0	0	0	0
8XI	2	1	0	0	8XI	0	0	0	0
10XI	2	0	2	0	10XI	0	0	0	0
12XI	4	2	5	0	13XI	0	0	0	0
14XI	11	5	12	2	15XI	3	0	0	0
17XI	10	5	18	3	17XI	10	0	1	0
20XI	26	7	32	5	20XI	10	1	14	2
23XI	31	10	44	6	24XI	12	2	15	1
26XI	14	2	28	4	26XI	18	3	26	2
28XI	7	1	25	3	28XI	31	5	41	3
1XII	15	3	11	3	1XII	14	3	34	1
3XII	4	1	8	2	3XII	15	2	17	1
5XII	2	1	4	0	5XII	9	0	12	0
8XII	0	0	4	1	8XII	5	0	7	0
10XII	1	0	2	0	10XII	3	0	2	0
12XII	0	0	2	0	14XII	2	0	1	0
15XII	1	0	1	0	18XII	2	0	0	0
17XII	0	0	0	0	22XII	0	0	0	0
19XII	0	0	0	0	-	-	-	-	-

In Colibași station the first males were record in 2011, on 10 November, compared with females that were reported 10 days later (November 12).

Analyzing data recorded on the occurrence of adults in 2012, we found that in the Armeneasa station the first males were released on November 15 and the first female on the 20th of November. In the Colibaşi station the emergence of the first males has been reported on 17 November, compared with females that were recorded on November 20.

Evolution of the population of winter moth and mottled umber was recorded in the wake of the catches made by using sticky rings placed on the stem of trees.

On the basis of the catches recorded in the period of activity of adults we drew up the curve. The analysis of the data recorded in the year 2011, in Armeneasa station, we have found that male butterflies have evolved during the period from November 8-December 10, the maximum value of the catches being registered on November 20 (figure 2). Biological activity of females ranged between 10 November and 5 December, with a maximum of 10 females recorded catches of 23 November.



Figure 2. Evolution of catches of *winter* moth and mottled umber in 2011, in Armeneasa area

In Colibaşi station, in 2011, the evolution of males occurred in the period from 10 November-December 15, with a maximum of 44 individuals on 23 November (figure 3). In this stationary, females had developed between 11 November and 8 December, the peak being in the numerical date of 23 November (6 females captured).



Figure 3. Evolution of catches of winter moth and mottled umber in 2011, in Colibași area

The data recorded in the year 2012, in Armeneasa area biological activity of male butterflies were recorded during the period November 15-December 18, reaching a maximum of 31 individuals on 28 November (figure 4).



Figure 4. Evolution of catches of winter moth and mottled umber in 2012, in Armeneasa area

In Colibaşi station, in 2012, the evolution of male butterflies was recorded during November 12-December 17, reaching a maximum of males on 28 November (figure 5) compared with females that were encountered in forestry plantations in the period 20 November-3 December, with a maximum of 3 screenshots on 28 November.

The analysis of the data recorded in the two stationary taken into study it can be concluded that in forestry plantations of oak, male butterflies have a longer duration than females in each of the two years study compared to females whose number was much smaller.



Figure 5. Evolution of catches of winter moth and mottled umber in 2012, in Colibasi area

The above data show the population level since that time. Thanks to them we can highlight the population by sex, early abstraction, development and completion of the work. In order to avoid the appearance of forecast errors has been cleansing of insects trapped in the ring and glue their refresh at regular intervals or whenever necessary.

Another way in which he made the forecast in the pupa stage. In such a case in September, six trees evenly spread under their scarce have been conducted six surveys in soil every 1 m square, at a depth of up to 10 cm.

Pupas found included to calculate the average healthy pupas one square meter. This method is more difficult, since because of a lack of water in the soil is very hard and the work was executed with difficulty.

In the light of changing climate conditions, remains in the study time of hatching of caterpillars and tracking the evolution of these defoliaters.

## CONCLUSIONS

In forestry plantations of the Arges County, were frequently reported in two species: *Operophtera brumata* 1.- winter moth and *Erannis defoliaria* CI. - mottled umber.

The methods used for catching adult butterflies have prevented the migration of pests, have low population density and the biological reserve of damaging the following year, and the frequency and intensity of the attack.

In the station of the area studied following catches of adults with the help of the sticky ring have found a higher frequency of male butterflies.

In the oak forests of two stationary studied butterflies, the males had a life expectancy greater than that of females, in the two years of observation.

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# ANALYSIS OF THE GARLIC LANDRACES USING THE FLOWCYTOMETRY METHOD

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Key words: garlic, landraces, Flow cytometry

### ABSTRACT

Plant genetic resources are one of the most valuable natural resources (Ramanatha and Hodgkin 2002), providing the genetic diversity necessary for both farmers and breeders to obtain new cultivars either with high yield, or better quality, or more adapted to abiotic stress, or more resistant to pest and pathogens. Therefore, conservation and use of plant genetic resources plays an important role in agriculture, food security, and forestry.

Biological material consisted from 6 garlic landraces (Allium sativum L.) collected from Timis and Arad, the landraces were cultivated during 4 years in the experimental field Timisoara and Cenad.

The FlowCytometry analysis revealed a normal cell cycle for all garlic landraces regardless of ex situ conditions. The number of cells analyzed by FlowCytometry varied between 5000-10000 being enough for cell cycle analysis. This indicated again a stable genome in garlic landraces and that the ex situ conditions have no effect on plant growth. The cytological data obtained from each garlic landrace indicate a normal mitosis with no chromosomal aberration.

## **INTRODUCTION**

Genetic diversity is a fundamental component of biodiversity as it is the basis of species diversity and ecosystem diversity. Genetic diversity can be defined as the range of genes within a species and can be studied at the individual, population and species level ( Lowe et al. 2004).

The flow cytometric method was introduced in 1980 by estimating the nuclear DNA, and was aplyed in researching (Dolezel & Bartos, 2005). The flow citometry allows the exactly measurements of the high number of nucleys

### MATERIALS AND METHODS

Biological material consisted from 6 garlic landraces (*Allium sativum* L.) collected from Timis and Arad County (table 1), landraces were cultivated for the 4 years in the experimental field Cenad and also in Timisoara.

Nuclei have been extracted from 0,5 cm² leaves according to manufactures specifications (Fig. 1).Samples were processed in the FCM and data were recorded and analyzed using manufacturer software.

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In order to analise a sample, 2 ml tampon coloring solution (Staining Buffer), will be mixed wit 12 µl stoc PI (propidium iodide) and 6 µl solution stoc RNA-za; the resulting coloring solution can be used 24 hr if it is maintained at 4°C.

General	General characteristics of Allium sativum landraces at in situ collection time									
No.	Catalog No.	Catalog No. Collection site/Address								
			collection							
1	1252	Mărăuş, nr. 18, jud. AR	2003							
2	1484	Căpâlnaș, jud. AR	2004							
3	1763	Sebiş nr. 5, jud AR	2004							
4	1231	Cenad,nr.1259, jud TM	2002							
5	1235	Chizătău, nr. 80, jud TM	2002							
6	1480	Valcani, nr. 556, jud TM	2004							

Table 1



Figure 1. FlowCytometer used in our research (The Partec CyFlow SL part of Genetic Sciences Timisoara)

## **RESULTS AND DISCUSSIONS**

FlowCytometry is one of the methods commonly used in determination of DNA content and analysis of cell distribution during cell cycle progression. During mitosis four phases can be distingueshed: G1,G2,S (DNA synthesis) and M (mitosis). In case of G2 and M that contain the same amount of DNA, the differentiation is not possible based only on DNA.

The FlowCytometry technique used in garlic landraces followed the manufacturer indications in order to calculate the procentage of cell undergoing different cell cycle phases. The cell cycle is very important in plant growth and father development. Miss regulation of this process leads to abnormalities and poor development and as a consequence a poor yield. The resulting citograms are presented for each landrace.

The analise of the celular cicle, of Maraus garlic landrace, shows a 12,25% of the cells being in sintesis phase (S), with 24,68% of the cells being in G2/M and a group of cells being stationary G0/G1, (Figure 2).

The FCM analise of the Capalnas landrace from Arad county, shows an distribution of the cells in the cellular mithotic cicle at high level, with a variability 7,09% in G1 period, and 6,18% in G2 period, 11,87 % in sinthesis phase. (Figure 3) The FCM analise is rounded on this landrace being possible a dublets showings (merged cells that appear single), this will produce a high data variation. (Petcov 2011).



Fig.2 Citograms of cells distribution in the garlic landraces Maraus, Arad county



Fig.3 Citograms of cells distribution in the garlic landraces Capalnas, Arad county



Fig. 4 Citograms of cells distribution in the cycle of the garlic landraces Sebis , Arad county

Fig. 5 Citograms of cells distribution in the cycle of the garlic landraces Cenad, Timis county

The FCM analize shows a normal citogram (Fig.4), with a higher cell number in sinthesis phase. The mithosis phase cells are less procents, due the fact thet in the synthesis phase occurred some kind of blocage. The datas shows that the cells can not pass in the mithosis phase- but no popiploidy reveals

The FCM analise for the Cenad landrace, Timis county (Fig.5), shows an normal distribution of trh cells in the cellular cycle, being relate with the citograms average of the cell cicle that were noticed on the other landraces. A high activity of the cells in mithosis is showing up, with an normal cells distribution (Petcov 2011).

The FCM analise (Fig.6), shows a normal distribution of the cells in the cellular cicle, 15,69% of cells being in sintesis phase (S), and 1,89 procent a average of the cells in G1/G2 phase of mithosis, reveals also the efficiency of marking and preparing the samples, taking in consideration the total number of the cells comparing with the G0 cell procent.



Fig. 6. Citograms of cells distribution in the cycle of the garlic landraces Valcani, Timis county



Fig. 7. Citograms of cells distribution in the cycle of the garlic landraces Chizătău, Timis county

The FCM analise (Fig.7), shows a normal distribution of the cells being in the mithotic cell cicle a 5,66% of the cells in the sinthesis phase (S), and doubling the DNA quantity, and 1,88 procent in G1/G2 M period. It shows a hight activity in mithosis phase with a normal cells distribution

### CONCLUSIONS

The cell cycle in garlic landraces analyzed by FlowCytometry is normal although in some landraces the variability was higher then 2%, variations that can be the effect of nuclei isolation technique.

The FlowCytometry analysis revealed a normal cell cycle for garlic landraces regardless of *ex situ* conditions. This indicated again a stable genome in garlic landraces and that the *ex situ* conditions have no effect on plant growth. The cytological data obtained from each garlic landrace indicate a normal mitosis with no chromosomal aberration.

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# THE INFLUENCE OF CLIMATIC FACTORS ON THE PHYSIOLOGY OF PLANT SPECIES GROWN ON SANDY SOILS

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key words: photosynthesis rate, transpiration rate, Q-leaf, diurnal variation

#### ABSTRACT

Harsh conditions on sandy limits the number of species and varieties grown on these soils very poor in high humus. Temperatura air at  $35-42^{\circ}$ C in July and August, reduced soil moisture in the absence of precipitation and low humidity to 25% negative influence metabolism plant. By choosing appropriate species and varieties and directing agrotechnical factors (irrigation, fertilization, protection, grafting) can increase the photosynthetic efficiency of plants and their production.

Research has focused on diurnal variation of transpiration rate and leaf photosyntesis under the influence of climatic factors and agrotechnical in the area. The results obtained will elect resistant and drought-tolerant varieties with high productivity and promote the culture of sandy soils.

#### **INTRODUCTION**

Research on the behavior and influence of potato varieties agrotechnical (irrigation and fertilization) were made in other potato growing areas (Berindei, 1997; Chichea, 2000).

Photosynthesis rate varies according to climatic factors and agrotechnical action. Photosynthesis the diurnal variations due to concomitant action of physiological parameters, represented by external factors and internal factors. Among the external factors, the intensity of photosynthesis is limited by  $CO_2$  concentration, temperature and light (Acatrinei, 1991).

Transpiration rate varies depending on the humidity of the soil, air humidity and air temperature. Among the external factors influencing the process of photosynthesis and transpiration are important action photosynthetic active radiation incident on the leaf surface and air temperature. Photosynthetically active radiation have a role in the induction of photosynthesis, as well as sweating by making the photoactive movement of the stomatal opening and leaf temperature rises (Burzo et al., 2004).

Temperature, solar radiation intensity along is the main external factor affecting the processes of photosynthesis and transpiration. Atmospheric and soil drought limits plant metabolism deployment stress falls in thermal-hydric (Petcu, 2008).

#### MATERIAL AND METHODS

In the present study aimed to influence of diurnal variation agrotechnical on physiological indices in terms of climatic factors in the sandy soils.

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Agrotechnical factors studied were: species (watermelons and potatoes); variety; biofertilizer watermelons; densite potato. Determinations were: photosynthesis rate of machine - L. C. PRO +; leaf transpiration rate; photosynthetic active radiation; climatic factors: temperature, precipitation, relative humidity

## **RESULTS AND DISCUSSIONS**

The experience watermelons performed on grafted on rootstock variety Crisby F1 mace. The planting was used 11 plant solution concentration 0.5% and during the vegetation period 4 treatments with solutions of 0.5%. Substances biostimulating were Rizophus, Viostar, Fertiacil GZ, Bionat.

Bioactive fertilization substances influenced the speed of the physiological processes in plants watermelons grown on sand. Photosynthetic active radiation was influenced by light intensity during the day exhibited a diurnal variation.

At 9 hour values were between 1041-1421 mmoli/m²/s , at 12 hour between 1656-1833, and 15 hour between 1232-1555 moli/m²/s.

Diurnal maximum was recorded at noon and in variants fertilized with Rizophus.

Photosynthesis was influenced both by climatic factors of the area as well as substances used to fertilize plants. Photosynthesis rate showed a diurnal variation with values between 10.65 -28.97 micromoles  $CO^2/m^2/s$  at 9 hour , between 19.85 - 28.90 micromoles  $CO^2/m^2/s$  at 12 hour , and 15 hour between 12.39 - 21.48 micromoles  $CO^2/m^2/s$ .

Note that the rate of photosynthesis at midday in elevated recordings where variants fertilized with Viomix, Fertiacil and Bionat where they iregistrat and high productions between 104 - 113 t / ha.

Unfertilized low values recorded in both photosynthesis rate at 9 hour 10,23 micromol  $CO^2/m^2/s$ , at 15 hours 12.39 micromoles  $CO^2/m^2/s$ , influencing the watermelons production that was reduced to 10 t / ha. Bioactive fertilization substances positively influenced all variants leaf index and photosynthetic rate compared to the unfertilized control.

Photosynthesis rate recorded high values in variants fertilized with bioactive substances positively affect the producțion watermelons (104-113 tones/ha).

Temperature increased from 26.1°C during determinations (9hour) to 34.2°C (at 15hour). Thus low relative humidity below 30% has increased drought increasing atmospheric evaporation through sweat leaf level (Table 2). Diurnal variation of leaf transpiration rate follows the air temperature rises during the day. The transpiration rate at 9 hour values ranged between 1.62 - 4.28, at 12 hours between 2.35 - 4.82, at 15 hour befwen 4.07 - 5.90 milimoles H₂ O /m²/s. Diurnal maximum was recorded in the variant fertilized with bionat evaporated water is harnessed very effectively increasing the rate of photosynthesis and the production of watermelons.

Were studied in this experiment three varieties (Tresor, Riviera and Carera) with three fractions of tubers (large tubercles, mall tubercles, tubercles cut) and two densities (43000 plants/ha, 63000 plants/ha) prezented in Table 3. The variants with small tubers and planting density of 43 000 plants/ha photo-synthesis rate values ranged from 14.6 micromoles  $CO_2/m^2/s$  the variety Riviera and the variety Carerra 16.9 micromoles  $CO_2/m^2/s$ . Rate photosynthesis influence was reduced and production of tubers was 16-18 tons / ha.

The density of 63 000 plants / ha photosynthesis rate values were between 19.2 micromoles  $CO2/m^2/s$  the variety Tresor and 21.0 micromoles  $CO2/m^2/s$  the variety Carerra. Increased density of plants per hectare increased photosynthetic efficiency and the production of tubers per hectare.

Table 1

Variant	Production,	Q leaf-PAR			Photosynthesis rate, micromoles CO ² /m ² /s		
	t/na	9 hour	12 hour	15 hour	9 hour	12 hour	15 hour
Unfertilized	10,0	1160	1795	1555	10,23	19,85	12,39
Rizophus	95,1	1041	1833	1232	24,65	21,18	17,61
Viomix	108,2	1397	1656	1454	28,97	28,90	16,99
Fertiacil	104,2	1309	1666	1426	19,92	26,67	15,45
Bionat	113,1	1421	1712	1477	26,85	28,94	21,48
Nitrozim	96,7	1209	1820	1523	21,43	20,97	20,53

Diurnal variation Q-leaf and photosynthesis rate (micromoli  $CO_2/m^2/s$ )

Table 2

Diurnal variation transpiration rate (milimoles  $H_2 O /m^2 /s$ )

Variant	Te	Temperatura air			Transpiration rate milimoles $H_2 O /m^2 /s$		
	9hour	12 hour	15hour	9 hour	12 hour	15 hour	
Unifertilized	26,1	26,7	32,1	2,29	3,86	4,07	
Rizophus	27,0	28,8	32,9	1,62	2,35	5,12	
Viomix	27,4	29,3	33,5	2,95	4,30	5,15	
Fertiacil	27,7	30,0	33,8	2,91	3,40	5,41	
Bionat	28,6	31,8	33,9	4,28	4,82	5,90	
Nitrozim	28,9	32,2	34,2	3,35	3,91	5,53	

In variants with large tubers and planting density of 35000 plants/ha rate of photosynthesis ranged between 16.4 t - 28.2 micromoles  $CO_2/m^2/s$  and the density of 65000 plants/ha values between 19.4 - 29.9 micromoles  $CO_2/m^2/s$  (maximum in variety Carerra).

In variants cut tubers and planting density of 35 000 plants/ha values ranged from 11.2 - 19.0 micromoles  $CO_2/m^2/s$  and the density of 63 000 plants/ha from 19.9 - 26.0 micromoles  $CO_2/m^2/s$ .

Carerra variety recorded maximum photosynthetic which positively influenced the production of large tubers (30.8 tons/ha). Increasing density per hectare of photosynthetic potential infuence the varieties studied. Leaf transpiration rate of the following values: the variety Tresor between  $3.56 - 5.01 \text{ mmoli } \text{H}_2\text{O/m}^2/\text{s}$ ; the variety Riviera between  $3.75 - 5.71 \text{ mmoli } \text{H}_2\text{O/m}^2/\text{s}$ ; the variety Carerra between  $3.74 - 5.82 \text{ mmoli } \text{H}_2\text{O/m}^2/\text{s}$ .

Because the rate of photosynthesis performed strongly in the same variables, evaporated water was harnessed efficiently and productively.

Table 3

Variety	Tubers Density		Photosynthesis rate, micromoles CO ² /m ² /s	Transpiration rate, milimoles H ₂ O /m ² /s
	Small	43 000 plants/ha	15,9	3,56
	tubers	63 000 plants/ha	19,2	3,74
	Large	43 000 plants/ha	18,6	4,06
Tresor	tubers	63 000 plants/ha	21,3	4,40
	Cut tubora	43 000 plants/ha	16,9	3,86
	Cut tubers	63 000 plants/ha	25,0	5,01
	Small	43 000 plants/ha	14,6	3,75
	tubers	63 000 plants/ha	19,6	3,92
Large		43 000 plants/ha	16,4	4.02
Riviera	tubers	63 000 plants/ha	19,4	4.21
	Cut tubors	43 000 plants/ha	11,2	3,75
	Cut tubers	63 000 plants/ha	26,0	5,71
	Small	43 000 plants/ha	16,9	3,74
	tubers	63 000 plants/ha	21,0	5,20
	Large	43 000 plants/ha	28,2	5,75
Carerra	tubers	63 000 plants/ha	29.9	5,82
	Cut tubora	43 000 plants/ha	19,0	4.,01
	Cut tubers	63 000 plants/ha	19,9	4,21

Influenced density plants on physiological parameters of potato

The high density increased water consumption and transpiration foliar inregistered high values in all varieties.

#### CONCLUSIONS

Bioactive fertilization substances of watermelons positively influenced all variants leaf index and photosynthetic rate compared to the unfertilized control. Diurnal maximum was recorded in the variant fertilized with Bionat evaporated water is harnessed very effectively increasing the rate of photosynthesis and the production of watermelons.

Density plants of potato per hectare increased photosynthetic efficiency and the production of tubers per hectare. Carerra variety recorded maximum photosynthetic which positively influenced the production of large tubers (30.8 tons / ha). The high density increased water consumption and transpiration foliar inregistered high values in all varieties.

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# UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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# SOME PHYSIOLOGICAL CHANGES INDUCED BY THE ACTION OF DITHANE M 45 FUNGICIDE IN BLEAK (ALBURNUS ALBURNUS L.) AND PERCH (PERCA FLUVIATILIS L.)

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Keywords: bleak, perch, Dithane, oxygen consumption

#### ABSTRACT

In this paper we study the action of the Dithane M 45 fungicide (the active substance is the mancozeb), under two concentration (2.5 and 5 mg /l water) on some physiological parameters (oxygen consumption, breathing frequency, glycaemia and the number of erythrocytes) on bleak - Alburnus alnurnus (Linnaeus 1758) and perch - Perca fluviatilis (Linnaeus 1758) at two thermic intervals (6-8°C and 18-20°C). The Dithane had an inhibitory effect on oxygen consumption and breathing frequency for the bleak and perch. In the concentration of 2.5 mg/l, the Dithane produces, after two weeks of immersion, a significant decrease of the glycaemia values and the number of erythrocytes.

#### **INTRODUCTION**

Pesticides derived from chemical syntheses are the best examples of compounds whose use is risky because they spread all over the environment. The contamination of surface waters by pesticides used in agriculture is a problem of worldwide importance (Sibley & Kaushik 1991). Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are significant (Hazarika & Das 1998, John 2007).

The water environment is much more complex chemically and is subject to intense variations under the action of external factors, rather than the air environment. By their immersion in the water environment, pesticides affect a wide range of non-target organisms such as invertebrates and fish (Otludil et al. 2004).

The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on toxicity data and effects of pesticide preparations on non-target organisms. Fish are among the group of non-target aquatic organisms (Velisek et al. 2009); the use of pesticides in agriculture resulted in, among other things, the decline of fish populations (Grant 2001). Fish are useful bioindicators to evidence environmental degradation (Fausch et al. 1990).

First registered in the United States in 1948, mancozeb is marketed by the trade names Dithane, Manzeb, Nemispot and Manzane. Mancozeb is a fungicide belonging to a subclass of carbamate pesticides called dithiocarbamates (US EPA 1987).

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Mancozeb affects the nervous system through its main metabolite - carbon disulfide (inhibits enzyme activity by complexing with metal-containing enzymes). Mancozeb is moderately to highly toxic to fish and aquatic organisms. Reported 48-hour LC50 are 9 mg/L in goldfish, 2.2 mg/L in rainbow trout, 5.2 mg/L in catfish, and 4.0 mg/L in carp; the reported 72-hour LC50 for mancozeb in crayfish is greater than 40 mg/L; the 48-hour LC50 is 3.5 mg/L in tadpoles (DuPont de Nemours 1983).

This study was carried out to analyze the impact of lethal and sub-lethal concentrations of mancozeb, the active ingredient of Dithane M 45, on some physiological parameters (oxygen consumption, breathing frequency, glycaemia and the number of erythrocytes) of bleak (*Alburnus alburnus L.*) and perch (*Perca fluviatilis L.*).

## MATERIAL AND METHODS

This study was performed with the approval of the local Committee of Bioethics according to the Romanian law 205/2004 art.7, 18, 22 and regulation number 143/400/2002 for care and use of animals for research purposes.

The concentrations of Dithane M 45 used in all experiments and treatments were established by conducting preliminary survival tests. Two concentrations (2.5 and 5 mg/l water) and a control were used in the tests.

Determinations were made on two fish species: bleak (*Alburnus alburnus*) and perch (*Perca fluviatilis*) caught in the surrounding lakes of Pitesti city. After 14 days of adaptation in the lab (in glass aquariums with a capacity of 100 l, under natural photoperiodic conditions and the oxygen dissolved in water was not below 80% - by using water aeration devices), when they were fed *ad libitum*, the fish were separated in lots. For the low temperature samples, the fish were put in refrigerators, in 25 l aquariums, using artificial lighting (10 hours/day). The solutions in the aquariums were changed every 24 hours using semi-static tests.

The lots that met the necessary criteria (less than 5% mortality rate during the first week, and no mortality rate in the second week of acclimatization, if the mortality rate in the first week was between 5 and 10%) were divided into experimental samples (about 10 samples/experimental lot). Dead fish were immediately removed and their number was counted. Each lot was a witness for itself, the oxygen consumption and the respiratory rate being established under standard conditions.

The first experiment was carried out with bleak individuals and the second experiment with perch individuals. For each experiment fishes was separated in two lots (at two thermic intervals: 6-8°C and 18-20°C), each lot being subdivided in three sub lots (control group and fish exposed to 2.5 and 5 mg Dithane M 45/l water). During the period of exposure feeding was stopped in order to avoid the additional influence of this factor.

The following physiological parameters were investigated: the lifespan, the energetic metabolism, the breathing frequency, the number of erythrocytes and the concentration of plasma glucose.

The energetic metabolism, expressed by the oxygen consumption, was determined by using the closed respiratory chamber method (the oxygen dose in the water was established by using the Winkler chemical method) (Picoş & Năstăsescu 1988). The oxygen consumption and the respiratory rate were determined for all experimental samples, at intervals of 24, 48, 72, 96, 168 and 336 hours (where allowed by the surviving animals).

The blood glucose level and the number of red blood cells were determined after two weeks of exposure to 2.5 mg Dithane M 45/l water; fish were slaughtered after the

interval to get blood intake. The blood glucose concentration was determined by means of Accutrend[®]GCT using a drop of blood in a very short time. The blood was sampled from the caudal artery using the method recommended by Picos and Năstăsescu (1988). The number of red blood cells was determined using Thoma counting chambers, following the method described by Picos and Năstăsescu (1988) with blood from the caudal artery.

The results were interpreted statistically using SPSS 13.0 program for Windows, in accordance with the specialized studies. The independent t-test for the significance threshold p<0.05 was used to compare between two variables.

# **RESULTS AND DISCUSSIONS**

The experimental group showed significantly lower (p<0.05) values of plasma glucose, number of erythrocytes, oxygen consumption and breathing frequency compared with the control group.

Dithane M-45 fungicide reduced the energy metabolism (figure 1) and the respiratory rate (figure 2) - a stronger effect on the perch at room temperature variations.



Figure 1. The influence of Dithane M 45 fungicide upon oxygen consumption on bleak and perch

Dithane M 45 produced, since the first hours of exposure, a significant reduction in the oxygen consumption of bleak and perch, reducing directly proportional to the toxic concentration and duration of exposure. No significant correlation was found after calculating the Pearson correlation coefficients between the oxygen consumption and the respiratory rate of bleak and perch exposed to 5 mg Dithane M 45/l water.

Decreased oxygen consumption and changes in respiratory rate under the action of some pesticides (Dithane M 45, Reldan, Tilt) has also notice in prussian carp (Marinescu et al. 2004, Ponepal et al. 2009 a,b).

Physiological adaptations to stress in fish causing alterations in blood chemistry standards; hematological parameters are commonly used in fish as secondary stress indicators (Wedemeyer et al. 1990).



Figure 2. The influence of Dithane M 45 fungicide upon breathing frequency on bleak and perch

The average number of red blood cells decreased after 14 days from the fish immersion in Dithane M 45 solutions, both at room temperature and in cold samples (figure 3a). The determination of glucose concentration in blood plasma is widely used for the assessment of fish stress (Martinez-Porchas et al. 2009). The blood glucose decrease after two weeks from the immersion of fish in fungicide solutions (figure 3b).



Figure 3. The influence of Dithane M 45 fungicide (2.5 mg/l water) upon a. number of erythrocytes; b. glucose blood concentrationin bleak and perch

A decline in RBC was observed in *T. mossambica* after exposure to sumithion and sevin (Koundinya & Ramamurthi 1979), in *C. carpio* after 48 h exposure to cypermethrin (Reddy & Bashamohideen 1989), and in *A. testudineus* exposed to azodrin (Santhakumar et al. 1999). On the other hand, Atamanalp et al. (2002) observed a significant increase in the erythrocyte (RBC) count in *O. mykiss* exposed to cypermethrin; similar findings were also observed in *C. idella* after exposure to fenvalerate (Shakoori et al. 1996) and in *H. fossilis* after exposure to deltamethrin (Kumar et al. 1996).

Perches recorded the strongest hypoglycemic effect. The low blood sugar level as a secondary response of fish to stress induced by pesticides, suggested its faster release from the blood and its use by the tissues. Decreased blood glucose concentration has also notice in rats after mancozeb exposure (Kerchrid et al. 2007).

The acute test (96 hours) records mortality in all experimental variants; absolut mortality was recorded in bleak experiment at 5.0 mg/l fungicide. The findings of this study showed that the perches were more sensitive to Dithane M-45 fungicides, the effect being stronger at low temperatures.

# CONCLUSIONS

Exposure to Dithane M 45 resulted in significant differences (p<0.05) of plasma glucose and number of erythrocytes between the experimental and control groups. The experimental samples regarded the presence of respiratory and hematological changes in bleak and perch adapted to different temperatures (6-8 °C and 18-20 °C) and revealed differences in the reactivity of this species to changing environmental conditions.

Fish exposed to Dithane M 45 showed significantly (p<0.05) lower levels of plasma glucose and number of erythrocytes after 14 days.

Therefore the use of pesticides should be controlled to prevent possible contamination of the aquatic environments. The response to pesticides may differ in some fish species, and therefore further work is needed to determine the responses of different species.

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# WEST COAST COMMUNITIES WITH PHOLIURUS PANNONICUS AND PLANTAGO TENUIFLORA FROM OLTENIA

Răduțoiu D.1

Keywords: flora, vegetation, halophilic communities, Oltenia.

#### ABSTRACT

The most significant salty areas in Oltenia are found in Danube Meadow at Gighera- Dolj County, in Jiu River Meadow between Bechet and Filiaşi with altitudes of 30 -170 m.s.m, and also small patches of halophilic vegetation in Olt River Meadow between Corabia and Ganeasa. Also, in Oltenia Plain are found insignificant areas with halophilic species between Plosca and Dunăreni, Ionele –Urzicuța, Calafat, Radovan, Seaca de Câmp, Plenița. In northern Oltenia are known the halophilic lands from Ocnele Mari – Ocnița, at altitudes of 350-450 m.s.m.

The halophilic surface identified at Osica de Sus, with the coordinates: 44°15'06"N 24°22'30"E is framed to: West-pontic communities of Pholiurus pannonicus and Plantago tenuiflora. This meadow is analyzed both in terms of flora and vegetation.

#### INTRODUCTION

Osica de Sus Village is placed in the middle part of Olt County. Is made up of Osica de Sus, Vlăduleni, Greci, Peretu, Ostrov and Tomeni villages. Osica de Sus is placed in Oltet River Meadow.

The relief of Osica de Sus Village is a part of Caracal Plain, a component which is integrated in the comprehensive ensemble of Romanian (Aur, 1996).

The analyzed territory consists of alluvial material, with different textures from sandy to oozy. The meadows met on salty soils in this locality area are used as grasslands, and after improvement works can become arable.

The climate of our area is temperate-continental, influenced by the dry air waves from the east, characterized by severe winters and dry summers.

Data about halophilic flora in Romania are found in numerous papers: Buia & al., 1960, 1961, Doltu & al., 1979, Mihai, 1969, Pop I., 1968, Prodan, 1922, Sanda, 1967, Şerbănescu, 1963, Țopa, 1939, 1954.

About the studies of flora from the salty areas in Oltenia, are mentioned those made by Al. Buia, M. Păun, I. Şerbănescu, D. Cârțu etc.

Syntheses papers focused on the study of flora and vegetation of the salty lands in Romania has been made by E. Topa (1939, 1954).

Almost 40 years ago, Al. Buia et al. (1961), reported in this area, in the meadows, on low salty soils, numerous species such as: *Trifolium ornithopodioides, T. subterraneum, T. echinatum, T. angulatum, Medicago arabica, M. hispida* şi *Scorzonera cana (Podospermum canum).* 

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Between 1990-1999 the studies on the salty lands in Oltenia were resumed by the team from Botany discipline at the University of Craiova. After the studies, a part of the species mentioned by Al. Buia and the collaborators were not found again (Popescu Gh. & al., 2000).

#### MATERIAL AND METHODS

The research methods were different, some as floristic studies, others as vegetation. Data from the field were processed in the laboratory and the results are presented in this paper. Species determination was made using the specialty literature in the country (Beldie, 1977, 1979; Ciocârlan, 2009, Dihoru, 1990, 2002) and abroad (Tutin & al. 1964-1980) and the phytosociological framing and the reporting to the habitat types in Romania were made using valuable specialty papers (Topa, 1939, 1954; Sanda & al., 2006; Doniță & al. 2005). The families with representatives are noted in systematic order and the species in alphabetical order.

#### **RESULTS AND DISCUSSIONS**

## FLORA

After the field inspection and the checking of the vegetal material in the laboratory, a floristic list of 94 taxa was made:

Phylum Spermatophyta, Magnoliopsida Class, Ranunculaceae Family: Myosurus minimus L., Ranunculus sardous Cr., Ranunculus sceleratus L., Cerastium dubium (Bast.) Guépin, Dianthus guttatus Bieb., Gypsophila muralis L., Spergularia maritima (All.) Chiov., S. rubra L. J&C Presl., Chenopodiaceae Family: Atriplex patula (L.), A. prostrata Boucher ex DC., A. tatarica L., Camphorosma annua Pallas, Chenopodium glaucum L., Salicornia europaea L., Suaeda maritima (L.) Dumort., Plumbaginaceae Family: Limonium latifolium (Sm.) Kuntze, L. tomentellum (Boiss.) Kuntze, Polygonaceae Family: Polygonum aviculare L., P. neglectum Bess., P. patulum Bieb., Rumex conglomeratus Murray, R. crispus L., R. palustris Sm., R. stenophyllus Ledeb., Rosaceae Family: Potentilla reptans L., Fabaceae Family: Lotus tenuis Waldst. & Kit., Melilotus albus Medik., M. dentatus (Waldst. & Kit.) Pers., Trifolium angulatum Waldst. & Kit., T. campestre Schreb, T. dubium Sibth, T. echinatum Bieb., T. fragiferum L. subsp. bonannii (C. Presl) Soják, T. hybridum L., T. pratense L., T. repens L., T. resupinatum L. subsp. suaveolens (Willd.) Ponert, T. retusum L. Höjer, T. striatum L., Trigonella procumbens (Bess.) Rchb., Lythraceae Family: Lythrum hyssopifolia L., Apiaceae Family: Bupleurum tenuissimum L., Peucedanum latifolium (Bieb.) DC., Malvaceae Family: Althaea officinalis L., Brassicaceae Family: Cardaria draba (L.) Desv., Diplotaxis muralis (L.) DC., Erysimum repandum L., Lepidium perfoliatum L., L. latifolium L., L. ruderale L., Rorippa austriaca (Cr.) Bess., R. sylvestris (L.) Bess. subsp. kerneri (Menyh.) Soó, Sisymbrium polymorphum (Murray) Roth, Tamaricaceae Family: Tamarix ramosissima Lebed., Gentianaceae Family: Centaurium pulchelum (Sw.) Druce, Boraginaceae Family: Heliotropium supinum L., Lamiaceae Family: Mentha pulegium L., Plantaginaceae Family: Plantago tenuiflora Waldst. et Kit., P. major L., Asteraceae Family: Achillea collina Becker ex Rchb., Artemisia pontica L., A. santonica L., Aster tripolium L. subsp. pannonicus (Jacq.) Soó, Bidens cernua L., Inula britannica L., Matricaria recutita L., Pulicaria vulgaris Gaertn, Scorzonera cana (C.A. Mey.) Griseb., Taraxacum bessarabicum (Hornem.) Hand.-Mazz., Tragopogon graminifolius DC., Liliopsida Class, Butomaceae Family: Butomus umbellatus L., Juncaceae Family: Juncus bufonius L., J. compressus Jacq., Cyperaceae Family: Bolboschoenus maritimus (L.) Palla, Carex distans L., C. divisa Huds., Cyperus pannonicus Jacq., Schoenoplectus tabernaemontani (C.C. Gmelin) Palla, Poaceae Family: Agrostis stolonifera L., Alopecurus pratensis L., Beckmannia eruciformis (L.) Host, Bromus commutatus Schrader, Catabrosa aquatica (L.) P. Beauv., Crypsis alopecuroides (Pill. & Mitterp.) Schrad., Cynodon dactylon (L.) Pers., Elymus repens (L.) Gould, Festuca arundinacea Schreb., F. pseudovina Hackel, Hordeum geniculatum All., Pholiurus pannonicus (Host) Trin., Phragmites australis (Cav.) Steudel, Poa bulbosa L., Puccinellia distans (L.) Parl., Puccinellia convoluta (Horrnem.) Hayek subsp. pseudobulbosa (E.I. Nyárády) Borza.

By analyzing the spectrum of bioforms (Fig.1) is observed that the annual species and the hemicryptophytes have a very high share comparing to the rest. This proves that these salty meadows in Osica de Sus are still forming. The hemicryptophytes, although ranked the second, have a much higher share as individuals number, these giving the physiognomy of the place.



Fig. 1. Bioforms spectrum (orig.)

The analyze of the geoelements spectrum (Fig. 2) revealed that the Eurasian species represent almost half of the total (43 species), followed at distance by the Pontic species (11 species), Circumpolar (10 species) and by the European (9 taxa). Mediterranean species and atlantic-mediterranean have both two representatives, while the rest (Central-European; Panonic; Tropical Africa; Ukraine, Romania; Panonic; Russia, Romania, Yugoslavia and adventive) with one representative.



Fig. 2. Geoelements spectrum (orig.)

## VEGETATION

Natural vegetation in the research area occupies today some small areas because of the anthropogenic factor that reduced continuously the spread area through deforestation and grubbing for the agriculture or made changes in the floristic composition, structure and dynamics of the tree and herbaceous associations through an irrational exploitation of the forests and grasslands overgrazing.

The halophilous vegetation from these meadows is framed to *Puccinellio-Salicornietea* Ţopa 1939; *Puccinellietalia limosae* (Soó 1968) Géhu et Rivas Martinez 1982; *Puccinellion limosae* (Klika 1937) Wendelbg. 1943, 1950: *Pholiuro – Plantaginetum tenuiflorae* (Rapaics 1927) Wendelbg. 1943.

The phytocenosis of these associations are met in the meadows from Oltet River Meadow, in places with high humidity during the spring.

The presence of *Beckmannia eruciformis* species in high number explains the stationary conditions where these phytocenosis are found.

The predominance of *Pholiurus pannonicus* species (Table nr. 1) led to the separation of *pholiuretosum* subassociation (Bodrogközy 1956) Soó 1964, fact mentioned by Sanda & al., 2006 too. From this side of the country the authors give two quadrates from Tâmburești.

In the salty places from these meadows are met some rare elements such as: Limonium tomentellum, Dianthus guttatus, Cyperus pannonicus, Iris halophila or Tragopogon graminifolius.

Table 1

Quadrat	1	2	3	4	5	6	7
Covering (%)	95	95	90	85	90	90	95
Exposition ( ⁰ )	-	-	-	-	-	-	-
Altitude (m.s.m.)							
Area (m ² )	60	60	50	40	50	50	60
The characteristic s	species	of the	associa	ation			
Pholiurus pannonicus	4	4	3	3	3	3	4
Plantago tenuiflora	+	+	1	+	1	+	1
Puccinellio-Salicon	rnietea	& Puc	cinellie	etalia li	mosae		
Limonium tomentellum	+	+	+	+	+	+	+
Dianthus guttatus	+		+		+		+
Puccinellia distans	+		+	2		1	+
Mentha pulegium		+				+	+
Matricaria recutita	+			+			+
Cerastium dubium			+			+	
Lepidium ruderale					+		+
Beckmann	nion er	uciforn	nis				
Beckmannia eruciformis	+	+	+	+	+		+
Ranunculus sardous	+	+			+	+	+
Festucio	n pseu	dovina	e				
Trifolium angulatum			+		+	+	
Trifolium retusum			+				+
Vari	aesynt	axa					
Scorzonera cana		+	+		+	+	

Pholiuro - Plantaginetum tenuiflorae (Rapaics 1927) Wendelbg. 1943

Poa bulbosa	+	+	+		+		
Polygonum aviculare	+	+		+			
Cynodon dactylon		+	+		+		
Gypsophila muralis	+			+			
Rorippa sylvestris subsp. kerneri			+			+	
Bromus commutatus							+
Elymus repens			+				+
Alopecurus pratensis		+		+			

If we report the halophilic meadows met at Osica de Sus to the habitat types in Romania (Doniță and colab. 2005) we can say that these are included to the West-Pontic Communities with *Pholiurus pannonicus* and *Plantago tenuiflora*. On this occasion the chorology of this habitat type in Oltenia is improved and not only to this.

# West-Pontic Communities with Pholiurus pannonicus and Plantago tenuiflora

**Spread**: Oltenia (Sadova, Tâmburești, Piscu), Dobrogea (Eforie), Muntenia (salty lands from Baragan Plain), **Osica de Sus** (Olt County).

Areas: At national level the areas occupied by these west-pontic communities are estimated to 10 ha.

**Stations**: Altitude: 10–100 m; Climate: T = 11-10,50C; P = 450-550 mm. Relief: plane field with some small depressions (Fig. 3). Rocks: sands, in Oltenia, loess in the rest area of the country. Soils: solonetzs, salty chernozem, marine sands (Doniță & al., 2005).



Fig. 3. Meadow pysiognomy (orig.)

**Structure:** *Pholiurus pannonicus* was found in salty places, where the humidity at soil level is kept for a longer time. It prefers the slightly alkaline soil. The annual plants are dominant inside the phytocenosis, more frequent being: *Plantago tenuiflora, Hordeum marinum, Myosurus minimus, Cerastium dubium, Gypsophila muralis.* 

All these plant species realize the inferior level of these phytocenosis. The superior one, high of 25–35 cm, is realized by perennial plants: *Puccinellia distans, Alopecurus pratensis, Artemisia santonicum, Limonium tomentellum, Beckmannia eruciformis.* 

**Conservation value:** high – at national level, but we can say that through the presence of the rare species *Limonium tomentellum*, the conservation value of this habitat is very high. That is why we consider that these areas deserve to be protected in the future.

#### CONCLUSIONS

In terms of floristic we can say that were identified 95 taxa met on these salty lands from Osica de Sus. Some of these are rare (*Dianthus guttatus*, *Cyperus pannonicus*, *Iris halophila* sau *Tragopogon graminifolius*) or very rare (*Limonium tomentellum*) in Romanian vascular flora.

In terms of phytosociological we can say that the salty lands from Osica de Sus are framed to: *Pholiuro – Plantaginetum tenuiflorae* (Rapaics 1927) Wendelbg. 1943

At national level the conservation value of this habitat type is high.

The importance of these salty lands is higher than in other regions in the country because of the *Limonium tomentellum* presence, very rare taxa in the spontaneous flora of our country.

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# UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

## Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

Vol. XVIII (LIV) - 2013

## CONTRIBUTIONS TO SOME TAXA CHOROLOGY OF THE ROMANIAN FLORA (II)

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Keywords: chorology, taxa, Oltenia, Romania.

#### ABSTRACT

The present paper continues our completion activity in some taxa chorology with different sozological rates in Romania spontaneous flora. The knowledge of these taxa widespread has a special importance, being known that some species have the limit area on Romania territory. To the analyzed taxa is specified the sozology at national level, the ecological conditions where these are growing, origin and coenotic background where were found and the new identification stations.

# **INTRODUCTION**

The first data referred to some taxa chorology in Romania spontaneous flora are found in the paper written by Grecescu (1898). Subsequently, Prodan (1939) uses terms as: sporadic, very sporadic, etc., without emphasis on the chorolgy aspect.

With the establishment of IUCN (The International Union for Nature Conservation) appeared lists with rare species, endangered and endemic, which were added the sozological status.

In our country are known 4 national red lists and a Red Book of the Vascular Plants in Romania.

# MATERIAL AND METHODS

During numerous field trips, in this side of the country, the presented taxa have been found, noticed and photographed. The determination was made using the specialty literature from Romania and abroad (Săvulescu et al., 1952 -1976; Tutin et al., 1964 –1980; Beldie, 1977, 1979; Ciocârlan, 2009). Where necessary, the material was compared to the one existing in the University of Craiova Herbarium (CRA) to remove any doubt referred to the identity of the analyzed taxa.

The authors' abbreviations were done according to Brummitt & Powell (1992).

The localities where these species were found are accompanied by the locality code according to Lehrer & Lehrer (1990) and by the GPS coordinates.

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## **RESULTS AND DISCUSSIONS**

Acanthus balcanicus Heawood & I. Richardson – Fam - Acanthaceae

Sozologic characterization: Is known as endemic species for Balcanic Peninsula (Dihoru & Negrean, 2009). Data referres to the species presence in different places in the country are known from 4 counties (AB, CS, DJ, MH). At national level is a vulnerable taxon.

Ecology and geoelement. It prefers the dry, thermophile places, with a rocky sublayer made of bushes or forest borders. Balc.

Coenology. Orno-Cotinetalia.

New locations: Localities Milovan - GQ01 (Lehrer & Lehrer, 1990); 44°21'42"N 23°33'51"E and Sopot (Dolj county) – FQ92 (Lehrer & Lehrer, 1990).

Azolla filiculoides Lam. – Fam. Azollaceae

Sozologic characterization: Vulnerable fern in Romania spontaneous flora. Is known from the south and south-east of the country (Dihoru & Negrean, 2009).

Ecology and geoelement. It is a strictly linked aquatic plant. Adventive, naturalized in the West, Central and South Europe, (Dihoru & Negrean, 2009).

Coenology. Lemnion

New locations: In a canal with water at the periphery of Gostavăţu locality (Olt County) locality – LJ08 (Lehrer & Lehrer, 1990); 44°04'20"N 24°32'23"E.



Fig. 1. Azolla filiculoides to the edge of a canal, parallel with Olt River; fig. 2 - detail (orig.)

## Clematis integrifolia L. - Fam. Ranunculaceae

Sozologic characterization: Few locations are known from Oltenia (Bistret, Desa, Gighera). As a proof we have the sheets from the University of Craiova Herbarium (CRA). At national level is sporadic (Ciocârlan, 2009).



Fig. 3,4. Clematis integrifolia -general aspect; fig. - flower detail (orig.)

Ecology and geoelement. In xerophylous places, grass, on the Danube protection levee (Fig. 3,4). Euras. Cont.

Coenology. Festucetalia valesiacae.

New locations: Danube Meadow, near Rastu Vechi – FP86 (Lehrer & Lehrer, 1990); N 43°53'03"; E 23°17'02". Altitude about 30 m.s.m; (Leg. et det. D. Răduțoiu, 03. VI.2012).

*Erophila praecox* (Steven) DC. – Fam. Brassicaceae

Sozologic characterization: So far, it is an unrated species (not analyzed as sozology).

Ecology and geoelement. Partially grass places, humid during the spring (Fig. 5), easy ruderalised. Eur.

Coenology. Festuco-Brometea.

New locations: Localities Borăscu, Miluta (Gorj County) – FQ75 (Lehrer & Lehrer, 1990); N 44°41′40″; E 23°17′3″. Altitude about 350 m.s.m; (Leg. et det. D. Răduțoiu, 01.IV.2013).



Fig. 5. Erophila praecox in Miluta locality (orig)

Hesperis pycnotricha Borbás et Degen - Fam. Brassicaceae

Sozologic characterization: It is known from Romania spontaneous flora 2005 (Costache, 2005) from The Inferior Basin of Motru River. In the last specialty determination books is mentioned as very rare (Ciocârlan, 2009).

Ecology and geoelement. Grass places, at the edge of the bushes (Fig. 6). Pont. balc.



Fig. 6. Hesperis pycnotricha from Brănești (orig.)

Coenology. Arction lappae.

New locations: Brănești locality, Capu Dealului Village (Gorj County) – FQ94 (Lehrer & Lehrer, 1990); N 44°39'6"; E 23°27'18". Altitude about 110-130 m.s.m; (Leg. et det. D. Răduțoiu, 10.V.2013).

# Ruscus hypoglossum L. – Fam. Liliaceae

Sozologic characterization: Data referred to this species chorology are sporadic. In the specialty literature from Romania is known in 5 counties (Ciocârlan, 2000). The plant is declared as natural monument in Romania. Ecology and geoelement. It was found in oak forest (*Quercus dalechampii*) (Fig. 7). Pont. Balc. Pan.

Coenology. Querco-Fagetea.

New locations: Bolboși locality (Gorj County) – FQ75 (Lehrer & Lehrer, 1990); N 44°45'; E 23°13'. Altitude about 330 m.s.m; (Leg. et det. D. Răduțoiu, 16.III.2013).



Fig. 7. Ruscus hypoglossum from Bolboşi (orig.)

Salvia aethiopis L. – Fam. Lamiaceae

Sozologic characterization: It is a taxon rarely included in the red national lists. Few places from Oltenia are known.

Ecology and geoelement. It is encountered in ruderal meadows (Fig. 8), fallows or bushes. Mesotrophic, xerophyte and sub thermophile species. Pont. Medit.



Fig. 8. Salvia aethiopis from Rastu Vechi (orig.)

Coenology. Sisymbrion, Festucion rupicolae. New locations: Rastu Vechi (Dolj County) – FP76 (LEHRER, 1990).

#### Trapa natans L. – Fam. Trapaceae

Sozologic characterization: At European level this species is endangered (according to the Convention form Berna). In Romania is frequent in the plain area.

Ecology and geoelement. Is met in stagnant or slowly flowing waters. In the new location is invasive (Fig. 9), being a real danger for both the aquatic fauna and fishermen or swimmers, when this plant make fruits. Euras. Cont. (Submedit.)

Coenology. Nymphaeion.

New locations: In a lake which water is coming from a canal attached to the Olt River, at the periphery of Gostavăţu locality (Olt County) – LJ08 (Lehrer & Lehrer, 1990); 44°04′20″N 24°32′23″E.



Fig. 9. Trapa natans in a lake at the periphery of Gostavățu locality (orig.)

## Turgenia latifolia (L.) Hoffm. – Fam. Apiaceae

Sozologic characterization: This species is mentioned in Romania specialty literature (Ciocârlan 2009) as a sporadic taxon, but in Oltenia, is known from a few locations (Buia & Mihăilă-Popescu, 1952).

Ecology and geoelement. It was identified in ruderal places at the edge of an oat culture (Fig. 10). Euras.



Fig. 10. Turgenia latifolia at the edge of an oat culture in Bolboși locality (orig.)

Coenology. Caucalidion.

New locations: Bolboși locality (Gorj County) – FQ75; N 44°45'; E 23°13'. Altitude about 250-300 m.s.m; (Leg. et det. D. Răduțoiu, 01.VI.2013).

#### CONCLUSIONS

In the present paper new contributions are added to the chorology of 9 taxa with different sozology rates. Some of them are endangered at European level (*Trapa natans*), others are sporadic for Romania Vascular Flora and some other category species have an insufficient area, known in our area (*Erophila praexox*).

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# PHYTOSANITARY CONDITION OF TOMATO CROP AFTER APPLICATION OF ECOLOGICAL CONTROL SCHEMES

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Keywords: ecological cultivation of tomatoes, ecological combat, pathogens.

#### ABSTRACT

Year after year, crops are affected by numerous diseases and pests, registered significant losses in potential volume of production. The life cycle of phytopathogenic agents and plant resistance to diseases and pests are influenced by environmental factors (temperature and humidity). Tomato crops during the vegetation period are subject to attacks of different pathogens, of

which the most important and most damaging are: Phytophthora infestans, Cladosporium fulva, Leveillula taurica, Botrytis cynereea.

In organic greenhouse INCDBH Ştefăneşti Arges a treat scheme was applied to prevent diseases caused by different pathogens. It was noted that application of the first treatment has an important preventive role. Prevention and control is done by using plant extracts. The substances based on copper are only those accepted in ecological cultivation.

#### **INTRODUCTION**

The damaging pests are a major limiting factor in the production of tomatoes. Pest includes the pathogens which produce the plants disease and animal pests (nematodes, insects and mammals) that cause damage to the cultivated plants.

The diseases can be classified into two classes. The first one includes diseases caused by microorganisms or infectious particles that include fungi, bacteria, and viruses. These diseases are contagious and can rapidly spread from the infected plant to the healthy one, especially when the culture and environmental conditions are favorable (Mitrea & Necula 2004).

Another class includes non-infectious diseases, due to stressors, physical or chemical, such as environmental factors, excess or deficiency of nutrients etc. Non-infectious diseases can not spread from plant to plant but may be affected plants a fairly uniform distribution and extended when the culture was exposed to a major stress factor.

For the correct diagnosis of a disease it is extremely important to distinguish between infectious and non -infectious even when symptoms are similar (Blancard 1988).

The pathogens can cause significant crop decreasing but they can also concentrate in the products dangerous compounds for the consumer's health especially toxic microorganisms, from witch the food (Iacob 2006).

Pests cause damage either by reducing crop quality or by reducing the amount of production by destroying flowers, fruit, or even whole plants (Ceausescu et al. 1980).

For tomato crop in greenhouses and solariums it is used F1 hybrids with large resistance to diseases, and pests adapted to lower light conditions in some periods, pronounced precocity, uniform fruit shape and size, uniform color and resistance transport (Ciofu et al. 2003). Planting varieties with high biological resistance is probably the most effective control of pathogens especially for getting organic products. Unfortunately, there are varieties of tomato immune culture but were created strains which are resistant or tolerant to one or more specific pathogens (Ciofu et al. 2009).

The tomato plants are attacked by various pathogens, including *Phytophthora infestans, Cladosporium fulva, Leveillula taurica Botrytis cinerea* that are more dangerous. Favorable environmental conditions, specific pathogen infestation can affect organs or plant in its entirety and cause damage, and in extreme cases, total destruction of tomato crops (Pārvu 2010).

#### MATERIAL AND METHODS

The studied biological data is the germplasm collection made up of 36 tomato varieties and biotypes created at INCDBH Stefanesti Arges, as well as other varieties used as control variant to testing the biological endurance to pathogens specific to crop tomatoes. A comparative study was conducted on the tolerance or resistance to tomatoes specific pathogens. The aim of the study was to identify biotypes with durability for organic crops to help farmers interested in than the period of conversion to organic farming the land.

Both the seeds and the seedlings were produced in organic greenhouse INCDBH Stefāneşti-Arges. Inspections during environmental certification were performed by an accredited firm for this purpose.Organic greenhouse horticultural crops destined green has a usable area of 249 m², has heating and is grown in two production cycles: cycle 1: tomatoes, peppers, lettuce seed, cycle 2: lettuce, green onions and spinach.The greenhouse is located on the first terrace of the meadow Arges. The soil is alluvial and sandy loam texture.

The tomatoes assortment included determined and undetermined biotypes, figures 1 and 2, cultural structure are presented in table1.

Table 1.

	Structure organic greenhouse crops in 2013
Rows	Species / Variety
R1	Pepper (California orange, red, peppers)
R2	Tomatoes Arges 11 (variety certificate)
R3	Tomatoes Argeș 123 (variety certificate)
R4	Tomatoes Argeș16 (variety certificate)
R5	Tomatoes Argeș20 (variety certificate)
R6	Tomatoes Ștefănești 22 (variety certificate)
R7	Tomatoes Ștefănești 24 (biotype currently under official testing)
R8	Tomatoes Costate 21( variety certificate)
R9	Tomatoes Gloria, Marzarno, Ștefănesti 20 N, Ștefănesti 20D, Beautiful violet
R10	Tomatoes Ox heart, Louis Sevrasca, Ideal, Chery different types
R11	Different biotypes of grape vines from seeds, lettuce and spinach seed

Tomato crop varieties containing 5 creations, approved in the last two years, two varieties approved in 2012 under a variety of official testing at ISTIS four biotypes in the clonal selection phase, 24 varieties of different origin, used as a control and / or genetic material for breeding.



Figure 1. Variety Ștefănești 21 with growth driven (original)



Figure 2. Variety Costate 21 with indeterminate growth (original)

#### **RESULTS AND DISCUSSIONS**

In the spontaneous flora grow different plant species that have the ability to synthesize fitoalexines as a defense against pests.

Fitoalexines can be extracted by various simple methods (infusion, decoction, infusion simple or alcohol etc.) and used in solutions with different effects: stimulating the growth and/or fruiting, repellent (repulsive), bactericidal, fungicidal, insecticide, nematicids, acaricids.

For profitable vegetable crops in the period of transition to organic farming is useful to conduct studies to identify species of local wild flora that contain fitoalexines important to identify the extraction method and how it can be used to prevent infestation or combating harmful organisms.

Equally important is the use of several methods that have the effect of maintaining pest populations below the economic damage threshold. Combining technological factors with the biological and physical, can ensure a good crop is healthy green.

To prevent and control harmful organisms were used alcoholic extracts obtained from plants growing in the wild flora around organic greenhouse. The extracts were made from both the dry and the plants in the green plant.

Extracts obtained by infusion and cold soak are natural products that have various effects: repellent, insecticide, growth promoter, plant growth and fruitfulness. By infusing affected cell membranes and a good part of vacuole contents into solution so that plant extracts have a stimulating effect of growth and fruitfulness.

The use of alcohol in a concentration of 2% extract stimulates fitoalexines broadcasting poorly soluble in water.

Combining therapeutic plants has the effect of reducing the number of treatments and the increasing number of harmful organisms which ensures protection.

The aim was to use extracts prevent primary infection, to maintain populations of pests below economic damage and control of phytopathogenic agent (table 2.).

# Table. 2.

Date of treatment	The treatment	The role of treatment
24.05.2013	Soak plant	Fighting the Trialeurodes V., and aphids
28.06.2013	copper sulphate 0.6%	Prevenirea patogenilor foliari
30.06.2013	alcoholic extract	Prevention of foliar pathogens
15.07.2013	copper sulphate 0.6%	Preventive and curative role

The treatment scheme used to control pathogens in organic greenhaouse.

Alcoholic macerate was obtained by grinding plants following quantities: 300 gr sumach (*Rhus typhyna*) 50g fern (*Dryopteris Fleece-mas*), 50g basil (*Basilicum officinalis*) 100g horsetail (*Equisetum arvense*), 100g nettle (*Urtica dioica*).

The plants were infused into 10 liters of water, after cooling, alcohol was added to a concentration of 2%, was allowed to soak for at least 48 hours. Then, the preparation obtained was filtered and used immediately. The extract was used after removal of trap plants, preventive and curative for insect pathogens were identified as adhesive traps and trap plants the greenhouse whitefly (*Trialeurodes vaporariorum*) and aphids.

Alternating cupric products with plant extracts was more effective in preventing infections compared with use of a product in a given period and the other in another period Bordeaux mixture was used in the period of maximum flowering and fruit after tying the 3 floor. High humidity in the second part of June has favored installing gray mold on one side of the inflorescence but other pathogens infestation was prevented. Assessment of plant health of the plants was carried out by visual analysis in a period of 14 days after application of the scheme of treatment and the results are shown in table 3

#### Table. 3

Varietis	Phytophthora	Cladosporium	Leveillula	Botrytis
	infestans	fulva	taurica	cynereea
Argeș 11	Absent	Absent	Absent	Absent
Argeș123	Absent	Absent	Absent	Present
Argeș16	Absent	Absent	Absent	Absent
Argeș20	Absent	Absent	Absent	Absent
Ştefăneşti 22	Absent	Absent	Absent	Absent
Ştefăneşti 24	Absent	Absent	Absent	Absent
Costate 21	Absent	Absent	Absent	Absent
Gloria, Marzarno,	Absent	Absent	Absent	present 20% of
Stefănesti20N,				inflorescences
Frumoasa violetă				the upper floors
Inima boului,	Absent	Absent	Absent	present 20% of
Lodovic				inflorescencesthe
Sevrasca,				upper floors
Angheloiu				
Different varieties	Absent	Absent	Absent	Absent
of tomatoes Cherry				

Assessment of plant health of tomato varieties cultivated in ecological system

#### CONCLUSIONS

Good grinding plant extracts used in the preparation of compounds useful in the solution diffusion allowed a treatment plant.

Cherry type varieties are more resistant to specific pathogens of tomato crops.

Alternating cupric products with plant extracts was more effective in preventing infections compared with use of a product in a given period and the other in another period. Alcoholic extracts 2% proved insecticide effect better than soak drinks.

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# THE RESPONSE OF PTERIDOPHYTE SPORES AND GAMETOPHYTES TO THE PRESENCE OF HEAVY METALS IN THEIR CULTURE MEDIA

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Keywords: pteridophytes, heavy metals, germination, gametophyte.

#### ABSTRACT

The aim of this research was to study the influence of heavy metals on the germination process of pteridophyte spores and on gametophyte differentiation in the native ferns Asplenium scolopendrium, Athyrium filix-femina and Polypodium vulgare so as to identify the species that are tolerant to metals. The presence of heavy metals in the nutrient medium in which pteridophyte spores were grown significantly influenced the germination process, except for the experimental variants with lead. Gametophyte differentiation took place much more slowly and in some cases it was interrupted at early stages.

# INTRODUCTION

Pteridophytes are non-target plants currently used in ecotoxicological studies. Some species can tolerate the presence of heavy metals in their environment, as they have the ability to hypereraccumulate them.

Hyperaccumulation of heavy metals or metalloid is a rare phenomenon in terrestrial higher plants. To date, some 400 taxa of hyperaccumulator species have been identified (Zhao et al., 2002).

For example, the fern *Pteris vittata* may grow in soils that are contaminated with arsenic, which can be accumulated into their biomass in high amounts (3.280 - 20.000 ppm) (Ma et al., 2001).

*Azolla filiculoides* grown in nutrient media containing 8-15 ppm of different heavy metals was found to contain about 10.000 ppm cadmium, 1.990 ppm chromium, 9.000 ppm copper, 9.000 ppm nickel and 6.500 ppm zinc (Sela et al., 1989).

The bioaccumulation of metals allows the use of the species in phytoremediation processes. The term "heavy metals" refers to elements with densities greater than 5.0 gcm⁻³ and usually indicates metals and metalloids associated with pollution and toxicity; however, the term also includes elements that are required by organisms at low concentrations (Adriano, 2001).

Thirteen trace metals and metalloids (Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl, Zn) are considered priority pollutants (Sparks, 2005).

The aim of this research was to study the influence of heavy metals on the germination process of pteridophyte spores and on gametophyte differentiation in the

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native ferns *Asplenium scolopendrium*, *Athyrium filix-femina* and *Polypodium vulgare* so as to identify the species that are tolerant to metals.

# MATERIAL AND METHODS

**Biological materials:** spores collected from *Asplenium scolopendrium*, *Athyrium filix-femina* and *Polypodium vulgare* (Vâlsan Valley, Argeş county, Romania).

**Experimetal variants.** In this experiment we used 10 and 100 ppm solutions of: PbCl₂, CoCl₂, CdCl₂, CoSO₄ and NiSO₄. The solutions were prepared in Knop (1865) mineral medium. The control variant was Knop solution.

**Spore germination and gametophyte differentiation.** Spores were spread on the surface of the culture media. The culture vessels were closed with cover, sealed with Parafilm, and were placed in the growth room at 25°C during the day and 15°C during night, under a 16-hour photoperiod (8 hours of darkness).

After 14 days we randomly selected 100 spores for which we determined the germination percentage. The experiment was done in triplicate.

The average and standard deviation was calculated for each experimental variant. Microscopic observations on gametophyte differentiation were made after 3 and 17 weeks after the spores had been sown.

#### **RESULTS AND DISCUSSIONS**

Spore germination in *Asplenium scolopendrium* was influenced in all tested experimental variants (figure 1). For the 10 ppm concentration, the germination percentage decreased in the following order: Control; PbCl₂; CoSO₄; CoCl₂; NiSO₄; CdCl₂.

For the same concentration mentioned above, between the control variant and the variant with  $CdCl_2$ , we registered a difference in germination percentages of 60.33 units.

The same influence was also noticed in the case of the 100 ppm concentration, with the germination percentages obtained being reduced in the same order, but, except for the variant with  $PbCl_2$  (100 ppm), the germination percentages were lower.

For the 100 ppm concentration, there is a difference of 67 units between the germination process obtained for the control variant and that obtained for the variant with CdCl₂.



Figure 1. The influence of heavy metals on spore germination in *Asplenium scolopendrium*.

In *Athyrium filix-femina* the germination percentages decreased as follows: Control; PbCl₂; CoCl₂; CoSO₄; NiSO₄; CdCl₂, both for the 10 ppm concentration and for the 100 ppm concentration (figure 2). The germination percentages were higher than those obtained for *Asplenium* scolopendrium. Thus, we registered a germination percentage of 59.66% for the 10 ppm concentration of the variant with  $CdCl_2$  and a percentage of 47% for the 100 ppm concentration.

There was a difference of 35.34% between the control variant and the variants with CdCl₂ in the case of the 10 ppm concentration and a difference of 48% for the 100 ppm concentration. These differences are much lower than those obtained for the previous species.

In *Polypodium vulgare*, for the variants with 10 ppm concentration, the germination percentages were not significantly different from the control variant (except for the variant with  $CdCl_2$ ). At a100 ppm concentration, for the variants with  $CoCl_2$ ,  $CoSO_4$  and  $NiSO_4$  the germination percentages were significantly lower than those obtained in the case of the control variant. Spores did not germinate in the variant with  $CdCl_2$  (figure 3).



Figure 2. The influence of heavy metals on spore germination in *Athyrium filix-femina*.



Figure 3. The influence of heavy metals on spore germination in *Polypodium vulgare*.

After 3 and 17 weeks, respectively from the initiation of cultures, the gamethophytes of the 3 tested species were at different differentiation stages (Table 1-3). Thus, for the experimental variant with NiSO₄ of *Asplenium scolopendrium*, the

gametophyte was still at the stage of germinated spores (necrotic after 17 weeks), while the

variant with  $PbCl_2$  was at the same stage as the control variant (prothallic filament, prothallic blade after 3 weeks and branched chordate prothallia with gametangia after 17 weeks) (Table 1).

In *Athyrium filix-femina*, the gametophyte in the experimental variants with PbCl₂ differentiated much faster than in the control variant and after 17 weeks the sporophyte was formed on the gametophyte (Table 2).

Table 1

Experi	mental	Stage* of gametophyte differentiation		
variants		3 weeks after culture initiation	17 weeks after culture initiation	
	Control	prothallic filament, prothallic blade	branched chordate prothallia with gametangia	
10ppm	PbCl ₂	prothallic filament, prothallic blade	branched chordate prothallia with gametangia	
	CoCl ₂	prothallic filament (3-4 cells)	prothallic blade with necrotic chlorocytes and male gametangia	
	CoSO ₄	prothallic filament (3-4 cells)	prothallic blade with necrotic chlorocytes	
	NiSO4	germinated spores	necrotic germinated spores	
	CdCl ₂	prothallic filament (1-4 cells), prothallic blade	chordate prothallia with gametangia	
100ppm	PbCl ₂	prothallic filament, prothallic blade	branched chordate prothallia with gametangia	
	CoCl ₂	prothallic filament (1-3 cells), prothallic blade	branched prothallic blade with necrotic chlorocytes	
	CoSO ₄	prothallic filament (2-3 cells)	prothallic blade with necrotic chlorocytes	
	NiSO ₄ germinated spores		necrotic germinated spores	
	CdCl ₂	prothallic filament (1-3 cells)	prothallic blade with necrotic chlorocytes	

Differentiation of gametophyte in Asplenium scolopendrium

*stage identified for most gametophytes

Among the species that tolerate Pb in their environment and accumulate it are those in the species *Athyrium*. *Athyrium yokoscense* accumulates Pb in the sporophytic tissues (Nishizono et al., 1987) and in the gametophyte (Kamachi et al., 2005).

For the variant treated with  $NiSO_4$  (100 ppm), the gametophyte was the most affected. It remained at the stage of germinated spores, as in the case of the previously mentioned species.

In *Polypodium vulgare*, the variants with  $PbCl_2$  treatment (10 ppm and 100 ppm) evolved similarly to the control sample. After 3 weeks, in the variant with  $CdCl_2$  treatment (10 ppm), the gametophyte was at the stage of spores and two- or three-celled prothallic filament; at the 100 ppm concentration, except for the variant with  $PbCl_2$ , the gametophyte was either at the stage of germinated spores (the variants treated with  $CoCl_2$  and  $NiSO_4$ ) or ungerminated spores (the variants treated with  $CoSO_4$  and  $CdCl_2$ ).

The latter were at the same stage even after 17 weeks (Table 3).

# Table 2

	Differentiation of gametophyte in Amyrum jutx-jemina				
Experi	mental	Stage* of ga	ametophyte differentiation		
variants		3 weeks after culture	17 weeks after culture initiation		
		initiation			
	Control	prothallic blade $\rightarrow$ chordate	chordate prothallia with gametangia		
		prothallia			
10ppm	PbCl ₂	prothallic blade, chordate	chordate prothallia with gametangia and		
		prothallia	sporophyte		
	CoCl ₂	prothallic filament (3-4	branched prothallic filament with male		
		cells), prothallic blade	gametangia, some of them necrotic		
	CoSO ₄	prothallic filament (3-5	branched prothallic blade with male		
		cells), prothallic blade,	gametangia		
	NiSO ₄	prothallic blade	branched prothallic blade with male		
			gametangia		
	CdCl ₂	prothallic blade	chordate prothallia with male gametangia		
	PbCl ₂	prothallic blade, young	chordate prothallia with gametangia and		
100ppm		chordate prothallia	sporophyte		
	CoCl ₂	prothallic blade	branched prothallic filament with male		
			gametangia		
	CoSO ₄	prothallic blade	necrotic prothallic blade		
	NiSO ₄	germinated spores	necrotic germinated spores		
	CdCl ₂	short prothallic filament	necrotic prothallic blade		

# Differentiation of gametophyte in Athyrium filix-femina

*stage identified for most gametophytes

#### Table 3

Experi	mental	Stage* of gametophyte differentiation			
variants		3 weeks after culture initiation	17 weeks after culture initiation		
	Control	prothallic filament $\rightarrow$ prothallic blade	chordate prothallia with female gametangia		
10ppm	PbCl ₂	prothallic filament $\rightarrow$ prothallic blade	chordate prothallia		
	CoCl ₂	short prothallic filament	necrotic prothallic filament		
	CoSO ₄	prothallic filament	prothallic blade (some branched)		
	NiSO ₄	prothallic filament	chordate prothallia		
	CdCl ₂	spores, prothallic filament (2-3 cells)	necrotic prothallic filament		
100ppm	PbCl ₂	prothallic blade	chordate prothallia with female gametangia		
	CoCl ₂	germinated spores	necrotic prothallic filament		
	CoSO ₄	spores	spores		
	NiSO ₄	germinated spores	necrotic germinated spores		
	CdCl ₂	spores	spores		

# Differentiation of gametophyte in Polypodium vulgare

*stage identified for most gametophytes

# CONCLUSIONS

All three fern species tested manifested tolerance to the presence of lead in their culture media. Both germination process and gametophyte differentiation were similar to the control variants. These species can be tested for their ability to bioaccumulate lead.

All the other metals tested affected the germination of spores and the subsequent development of gametophytes.

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#### UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

# Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# HARMFUL ENTOMOFAUNA FROM THE BOTANICAL GARDEN "A. BUIA", CRAIOVA

Stan Cătălin ¹Țucă Ovidiu Andrei¹, Mitrea Ion¹

Key words: harmful entomofauna, botanical garden

#### ABSTRACT

From our observation regarding the harmful entomofauna from the Botanical Garden "Al. Buia", Craiova, there has been identified a number of: 137 species, sistematicaly framed in 10 ordine, belonging to 2 Clases : ARACHNIDA, INSECTA and 2 species of harmful mollusks from GASTROPODA class. Most species belonged to Coleoptera order (43 species), followed by Lepidoptera order (28 species), Homoptera order (13 species), Homoptera and Diptera each with 13 species, Heteroptera order (12 specii).

#### **INTRODUCTION**

A. Buia Botanical Garden Craiova is located in the southwestern of Craiova, between 440 18 N latitude and 23048 E longitude, on an area of about 17 hectares.

The garden was designed and works with the following sectors: plant systematics, floral provinces of the globe, floristic provinces of Romania, greenhouses, ornamental nursery, Rosarium, cultivated plants (D. Radutoiu, 2001)

In anthropogenic ecosystems in the natural control of harmful entomofauna occur internal factors of these populations represented by populational polymorphism, which involves differentiation of the populations individuals on special categories different as phenotypic and genotypic; the interrelationships between individuals of different morpheme and the functions performed by these morpheme. The exogenous population factors, are as in other organisms, represented by abiotic factors: physical, chemical, mechanical and biotic ones: predators, parasites, pathogens, also called "natural enemies", antagonists, as well food and competition (Toncea I., 2011)

Surveillance of these pests is absolutely necessary for the preparation of forecast and warning programs, to implement timely control treatments (Tuca O., 2012).

#### MATERIALS AND METHODS

Observations were conducted during March 2012 - July 2013 in the the Botanical Garden of Craiova A. Buia.

To determine the structure of the harmful entomofauna were made collection of material using various means and methods: directly by hand from plants or soil, frame metric, soil surveys and soil surface collected with entomological net, visual inspection, collection with sticky traps for flying insects, analyzing samples with binocular magnifier

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glass directly in the field or laboratory. For as little influence on the ecosystem we preferred to capture picture with the camera.

After collecting of biological material was made the material collected was analyzed and determined with the binocular magnifier glass using the Identification Manual (Panin L. 1951, Chatened du Gaetan 1990, Chinery M. 1998, Godeanu S.P. 2002,).

# **RESULTS AND DISCUSSION**

The harmful entomofauna identified comprised a number of 137 species (Table 1), sistematically framed in 10 ordine, belonging to 2 Clases: *ARACHNIDA*, *INSECT* and 2 species of harmful mollusks from *GASTROPODA* class.

Table nr.1

Nr. crt	Class/Order	Species
1		Panonychus ulmi Koch.
2	ARACHNIDA /ACARI	Tetranychus urticae Koch.
1		Gryllus campestris L.
2		Gryllus desertus L
3		Ephippiger ephippiger Fieb.
4	INSECTA/ ORTHOPTERA	Caliptamus italicus L.
5		Dociostaurus maroccanus Thunb.
6		Tetigonia viridisima
7		Locusta migratoria L.
8		Gryllotalpa gryllotalpa
1	INSECTA/	Forficula auricularia L.
	DERMAPTERA	
1	THYSANOPTERA	Haplothrips tritici
2		Limothrips cerealium Hal.
1		Lygus pabulinus L.
2		Lygus pratensis L.
3		Dolycoris bacarum L.
4		Pentatoma rufipes L.
5		Eurygaster integriceps L.
6	INSECTA/	Eurygaster maura L.
7	HETEROPTERA	Eurygaster austriaca Schr.
8		Aelia acuminata L.
9		Aelia rostrata Boh.
10		Graphosoma lineata
11		Pyrocoris apterus
12		Nezara viridula L.
1		Quadraspidiotus perniciosus Comst.
2		Cicada plebeja
3		Ceresa bubalus L.
4		Eulecanium corni Bch.
5		Psylla mali Schmdt.
6		Aphis pomi De Geer.
7	INSECTA/ HOMOPTERA	Aphis fabae L.
8		Eriosoma lanigerum Hausm.
9		Aulacaspis rosae
10		Macrosiphon rosae
11	4	Myzodes persicae
12	4	Hyalopterus pruni
13		Myzus cersi
1	INSECTA/	Vespa vulgaris L.
2	HYMENOPTERA	Vespa germanica L.

Harmful entomofauna identified during research in Botanical Garden Al. Buia
Nr. crt	Class/Order	Species
3		Hoplocampa minuta L.
4		Hoplocampa testudinea L.
5		Cenhus promaeus L
1		Melolontha melolontha I
2		Melolontha hippooastani E
2		Delenheilte Gille E
5		
4		Anomala solida Er.
5		Anomala vitis F.
6		Anomala dubia Scop.
7		Amphimalon solstitialis L.
8		Rhyzothrogus aequinoctialis Herb.
9		Anoxia orientalis L.
10		Lethrus apterus Laxum.
11		Phyllopertha horticola L.
12		Agelastica alni L.
13		Melasoma populi L
14		Chrvsomella violacea
15		Byctiscus betulae L.
16		Otiorrhynchus ligustici L.
17		Otiorrhynchus sulcatus F
18		Cetonia gurata Oliv
10		Chrysomella mentharsti
20		Chrysometia meninarsti
20		Ruguloscolylus lugulosus Ralz.
21	NICECTA (COLEODTEDA	Zabrus tenebriotaes Goeze.
22	INSECIA/ COLEOPTERA	Anisopita segetum Herb.
23		Anisoplia austriaca Herb.
24		Anisoplia agricola Poda.
25		Agriotes obscurus L.
26		Opatrum sabulosum L.
27		Crioceris quatordecempuctata L.
28		Crioceris asparagi L.
29		Crioceris duodecempunctata L.
30		Lilioceris lilii L.
31		Anthonomus pomorum L.
32		Epicometis hirta Poda.
33		Oxythyrea funesta Poda.
34		Rhynchites bacchus L
35		Galerucela luteola Mull
36		Lyta vesicatoria L
37		Entomoscelis adonidis Pall
38		Cassida nebulosa L
30		Subcoccinella 24 punctata 1
40		Rothynoderes nunctiventris I
41		Sitona linoatus I
41		Hypera variabilis Herb
42		Hypera variabilis nero.
43		Anoxia vilosa F.
1		Aporta crataegi L.
2		Sphynx convolvuli L.
3		Pieris brassicae L.
4		Pieri napi L.
5		Pieris rapae L.
6		Hyphantria cunea Drurry.
7		Hyponomeuta malinella Zell.
8		Arginis pahia L.
9	DIGD CTL /	Cydia pomonella L.
10	INSECTA/	Laspeyresia funebrana
11	LEPIDOPTERA	Euproctis chrysorrhoea L.

Nr. crt	Class/Order	Species
12		Malacosoma neustria L.
13		Operophtera brumata L.
14		Hibernia defoliaria Cl.
15		Noctua pronuba L.
16		Amphipyra tragopoginis L.
17		Acronicta rumicis Hb.
18		Euxoa nigricans L.
19		Phlogophora meticulosa L.
20		Helicoverpa armigera
21		Zeuzera pyrina L.
22		Vanessa polychloros L.
23		Vanessa urticae L.
24		Vanessa io L.
25		Agrotis segetum Den et Schif.
26		Loxostege sticticalis L.
27		Plusia gamma L.
28		Cameraria ohridella Deschka & Dimic
1		Haplodiplosis marginata
2		Contarinia tritici
3		C. medicaginis
4		Oscinella frit
5		Chlorps pumillionis
6	INCECTA / DIDTED A	Lyriomyza trifoli
7	INSECIA/ DIFTERA	L. solani
8		Phytomyza nigra
9		Delia brassicae
10		D. antiqua
12		D. florilegea
12		Phormia reginia Meig.
13		Rhagoletis cerasi L.
1	MOLUSCA/	Helix spp.
2	GASTROPODA/	Limax spp.

As it can be observed from the Table 1 data most species belonged to *Coleoptera* order (43 species), followed by *Lepidoptera* order (28 species), *Homoptera order* (13 species), Homoptera and Diptera each with 13 species, *Heteroptera order* (12 specii).

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## Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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## ENTOMOFAUNA FROM THE PLENITA (DOLJ) AREA

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Key words: entomofauna, beneficial species, harmful species

#### ABSTRACT

Importance of the entomofaunistic study lies in highlighting and determine the distribution of insect species in a given area, of particular importance in achieving ecological studies in highlighting the biodiversity of a given area.

The study was conducted in from May to October in Plenita (Dolj) area. In this area there is a strong correlation between areas of vegetation (which provides food and shelter) and territorial distribution of entompfauna. During our research we have captured a total of 54 species belonging to 8 orders and 40 families

### **INTRODUCTION**

Insects are the largest systematic unity of the animal kingdom, with a million extant species, that is several times more than all other living taxa together, is still a very conservative estimate, and their real number is for sure many times more. They are incomparably diverse in terms of their size, structure and way of life. (Rasnitsyn, A.P., 2002). Insects are extremely diverse and important to ecosystems (Wiggins 1983; Finnamore 1996). They have permeated the diverse and essential natural processes that sustain biological systems, making up over 75% of known species of animals. Indeed, our present ecosystems would not function without insects and arachnids.

They can live in just about any situation or climate. Certain insects are helpful to us by producing products we can use (for example, honey), by pollinating our crops, or by attacking pest insects. The pervasive ecological importance of this great variety of insects makes them valuable to assess disturbance or environmental impacts of various kinds (Lehmkuhl et al. 1984; Rosenberg et al. 1986) through assessments of mortality, sublethal effects, population changes, and modifications in community structure. Knowledge of arthropods also is essential to conserve or manage ecosystems, because a skewed focus only on large and conspicuous organisms misrepresents ecosystem dynamics (Kremen et al. 1993; Finnamore 1996).

The diversity and ecological importance of insects makes them very valuable for studies of biodiversity. However, the same overwhelming diversity means that valid and useful results will only be obtained if studies are properly planned. The attention being paid to the study of biodiversity has led to increasing interest in assessing the diversity of insects and their relatives, because these groups dominate terrestrial and freshwater ecosystems and are valuable indicators of their health.

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### MATERIAL AND METHODS

The study was conducted in 2012 during the months of May to October in Plenita (Dolj) area. In this area there is a strong correlation between areas of vegetation (which provides food and shelter) and territorial distribution of entompfauna. During our research we have captured a total of 54 species belonging to 8 orders and 40 families

The collection of the insects has been made different according to the morphological characteristic features, in particular the way they they move. Thus the entomological material was divided into groups insects that move by flying (Lepidoptera, Diptera, Odonates, Hymenoptera) and the group of insects that move by walking (Beetles, Heteroptera, Orthoptera and larvae from different species). To determine the structure of the harmful and beneficial populations were made collection of material using various means and methods: directly by hand from plants or soil, frame metric, soil surveys and soil surface collected with entomological net, visual inspection, collection with sticky traps for flying insects, analyzing samples with binocular magnifier glass directly in the field or laboratory (Stan, C. et al.2012).

Collecting the biological material has been made every two weeks, after that the entomological material was analyzed and determined with the binocular magnifier glass using the Identification Manual (Godeanu, 2002).

### **RESULTS AND DISCUSSIONS**

During our research we have recorded a total of 54 species belonging to 8 orders and 40 families. Most species of insects has been framed in to the Coleoptera order (16), followed by the lepidopteran species 13 species and Heteroptera order with eight species. The orders *Orthoptera*, *Hymenoptera* and *Diptera* has been represented by four species each, while the Neuroptera order has been represented only by two species.

From all the 54 species 27 species are harmful and 6 species are beneficial, the rest of 21 are indiferent.

Table 1

	Entomofauna from Plenita area 2012				
Nr.	Species	Family	Order		
1	Cantharis rustica F.	Cantharidae	Coleoptera		
2	Carabus ulrichi Sch.	Carabidae	Coleoptera		
3	Poecilus cupreus L.	Carabidae	Coleoptera		
4	Chrysolina fastuosa Sc.	Chrysomelidae	Coleoptera		
5	Leptinotarsa decemlineata Say.	Chrysomelidae	Coleoptera		
6	Phytodecta fornicata Brügg.	Chrysomelidae	Coleoptera		
7	Trichodes apiarius L.	Cleridae	Coleoptera		
8	Adalia bipunctata L.	Coccinellidae	Coleoptera		
9	Hippodamia variegata Goeze	Coccinellidae	Coleoptera		
10	Thea punctata L.	Coccinellidae	Coleoptera		
11	Agriotes lineatus L.	Elateridae	Coleoptera		
12	Hydrous piceus L.	Hydrophilidae	Coleoptera		
13	Lucanus cervus L.	Lucanidae	Coleoptera		
14	Meloe proscarabeus L.	Meloidae	Coleoptera		
15	Opatrum sabulosum L.	Tenebrionidae	Coleoptera		
16	Potosia cuprea Fabr.	Scarabaeidae	Coleoptera		
17	Gryllus campestris L.	Gryllidae	Orthoptera		
18	Tettigonia viridissima L.	Tettigonidae	Orthoptera		

Nr.	Species	Family	Order
19	Dociostaurus maroccanus Thngb.	Acrididae	Orthoptera
20	Gryllotalpa gryllotalpa L.	Gryllotalpidae	Orthoptera
21	Graphosoma lineata L.	Cantharidae	Heteroptera
22	Coreus marginatus L.	Coreidae	Heteroptera
23	Tanymecus dilaticollis Gill.	Curculionidae	Heteroptera
24	Dolycoris baccarum L.	Pentatomidae	Heteroptera
25	Eurygaster maura L.	Pentatomidae	Heteroptera
26	Eurydema ornata L.	Pentatomidae	Heteroptera
27	Aelia acuminata L	Scuteleridae	Heteroptera
28	Pyrrhocoris apterus L.	Pyrrhocoridae	Heteroptera
29	Aphis pomi De Geer.	Aphididae	Homoptera
30	Myzus cerasi Fabr.	Aphididae	Homoptera
31	Macrosiphum rosae L.	Aphididae	Homoptera 3
32	Apis melifera L.	Apidae	Hymenoptera
33	Formica rubra L.	Formicidae	Hymenoptera
34	Vespa germanica L.	Vespidae	Hymenoptera
35	Vespa vulgaris L.	Vespidae	Hymenoptera
36	Agrotera nemoralis Sc.	Crambidae	Lepidoptera
37	Sitotroga cerealella Ol.	Gelechiidae	Lepidoptera
38	Lycia zonaria Den & Sch.	Geometridae	Lepidoptera
39	Autographa gamma L.	Noctuidae	Lepidoptera
40	Mamestra brassicae L.	Noctuidae	Lepidoptera
41	Orthosia stablis Den. & Schif.	Noctuidae	Lepidoptera
42	Pieris brassicae L.	Pieridae	Lepidoptera
43	Pieris rapae L.	Pieridae	Lepidoptera
44	Adoxophyes reticulana	Tortricidae	Lepidoptera
45	Zygaena purpuralis Brün.	Zygaenidae	Lepidoptera
46	Loxostege stiticalis L.	Crambidae	Lepidoptera
47	Cydia pomonella L.	Torticidae	Lepidoptera
48	Hyphantria cunea Drury	Arctiidae	Lepidoptera
49	Chrysoperla carnea St.	Chrysopidae	Neuroptera
50	Ascalaphus macaronius L.	Ascalaphidae	Neuroptera
51	Musca domestica L.	Muscidae	Diptera
52	Calliphora vicina Robineau-Desvoidy	Calliphoridae	Diptera
53	Lucilia viridis Robineau-Desvoidy	Calliphoridae	Diptera
54	Sarcophaga carnaria L.	Sarcophagidae	Diptera



Figure 1. The structure of the entomofauna from Plenita area 2012

#### CONCLUSIONS

Following our research in Plenita area, during 2012, we found that the structure of the entomofauna consists in 54 species.

From the total of 54 species 17 species are harmful among these some with high economical importance such as: Agriotes lineatus L., Dociostaurus maroccanus Thngb., Gryllotalpa gryllotalpa L., Mamestra brassicae L., Autographa gamma L., Mamestra brassicae L., Pieris brassicae L., Pieris brassicae L., Pieris rapae L.

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## RESEARCHES REGARDING THE ECOLOGICAL CONTROL OF THE PEST TRIALEURODES VAPORARIORUM ON TOMATOES GROWN IN GREENHOUSES

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*Keywords*: greenhouse whiteflies (Trialeurodes vaporariorum), herbal extracts, organic combat.

#### ABSTRACT

The greenhouse whitefly is an important pest of tomato crops in greenhouse and can produce significant losses if we do not act in time to fight or maintain under its economic damage threshold. Extracts were obtained from plants that were provided from spontaneous flora. The extracts were as soak cold infusion and alcoholic extract. The methods for controlling the action of Trialeurodes vaporariorum were: "Trap plants" method; Mechanical methods (removal Fragaria leaves attacked by pests); Colored traps; Application of extracts with insecticide role. Because the pest was observed only on these plants and the tomatoes were not affected, the "trap plants" method was the most efficient. Therefore, the activity of pest intensity and frequency were observed only to Fragaria, although alcoholic extract was applied to all plants in the greenhouse environment.

## **INTRODUCTION**

Cultivated plants as some spontaneous, but beneficial, must be protected by special technological measures of self-defense due to their relatively low capacity and increasing trend towards adverse actions of abiotic and biotic factors.

For survival cultivated plants must compete weeds of about 30,000, of which 1,800 can cause serious economic losses, withstand attacks over 50,000 fungi, which causes more than 1,500 diseases, nematodes, of which 15,000 in 1500 causing severe damage and over 800,000 care10000 insects can sometimes cause devastating effects. Damage caused by these crops biotic factors varies between approximately 24% and 45%, regardless of the technology of cultivation and usually these crop losses contribute more or less all types of pests.

Each group of harmful organisms has some characteristics which give them a high capacity to adapt to different phenomena in the natural environment or culture, including increased resistance to pesticides, and common peculiarity such as fecundity, ecological plasticity, multiple possibilities of spreading, upward trend of the attack (Toncea& Stoianov 2002).

*Trialeurodes vaporariorum* is an important pest of vegetables grown in greenhouses, affecting especially tomatoes, cucumbers and ornamental plants (http://agroromania.manager.ro).

Greenhouse white flies have 3-4 generations per year, sometimes more, depending on environmental factors. In summer, flies can migrate from greenhouses on various plants in the field, where it multiplies until autumn. At lower temperatures, some of the insects returning and infecting the culture in greenhouse again (Roşca et al. 2011).

A number of researchers (Hulspas & Lenteren 1989, Baumgartner & Yano 1990, Bi et al 2002), studied the life cycle of the pest and the influence of environmental factors (temperature, precipitation) and host plants on its development.

Keeping long for proper pest control, chemical control was the only alternative which led to disruption of ecosystems and the development of pest resistance.

In the last period are trying as an alternative to control the *Trialeurodes vaporariorum*, the use of essential oils from plants, because they contain many active ingredients. So has been made the laboratory tests where the used oils from the plant 53 (oil of eucalyptus, oil of oregano, oil of black pepper, peppermint oil, mandarin oil, basil oil, oil of rosemary, sage, of wormwood, hot pepper). Volatile oils were used in all stages of development of the pest (eggs, ninfa, adults).

Was noted that of these 23 essential oils (essential) have an efficiency of over 80% in the pest control at different stages of development. Peppermint oil has an efficiency of 83%.

Essential oils from plants, can be successfully used from the fly white the greenhouse many of which insecticedes having harmful effects on the environment and human rights (Won-II et al 2003).

#### MATERIAL AND METHODS

The research was made in an organic greenhouse INCDBH Ştefăneşti Arges, which is a collection of 36 tomato varieties with both types of growth:

- 5 varieties creations approved;

- 1 official variety testing under the ISTIS

- 4 biotypes undergoing clonal selection-

- 26 different varieties used to control and / or genetic material to improve.

Organic greenhouse for ecologic horticultural crops has a usable area of 249  $m^2$ , has heating and is grown in two production cycles: cycle 1: tomatoes, peppers, lettuce seed, cycle 2: lettuce, green onions and spinach.The greenhouse is located on the first terrace of the meadow Arges. The soil is alluvial and sandy loam texture.

Both the seeds and the seedlings were produced in organic greenhouse INCDBH Ştefăneşti-Arges.

Extracts has been made from plants from spontaneous flora. Extracts were, alcoholic extract, infusion and cold soak these are natural products that have various effects: repellent, insecticide, stimulating growth, development and fruition plants (fig. 1).

The purpose of the extracts was used to maintain the pest population below the economic damage. Both seeds and seedlings are certified organic.

The methods used to prevent and control the Trialeurodes vaporariorum, were:

- Mechanical method;

- Colored traps;

- Environmental control using insecticide effect of alcoholic extracts;

- Plant trap method.

With a magnifying glass determining the number of individuals and larvae of *Trialeurodes vaporariorum*.



Figure.1. Extracts macerated, infusions used to combat ecological (original)

## **RESULTS AND DISCUSSIONS**

On 05/20/2013, from work made in organic greenhouse, was observed the pest (*Trialeurodes vaporariorum*), in a row situated on the edge of strawberries used as trap plants.

In the same day was prepared an insecticide alcoholic extract (alcohol 2%) for pest control.

For the preparation of insecticides was used 300 g castor, fern 50g, 50g basil 100g horsetail, nettle.

It was left to soak until 24/05/2013, then filtered. Before applying the treatment was determined the number of individuals and larvae present on plants of *Fragaria* (table 1 and graphic 1).

Table 1

The total number of individuals and the larvae of the *Trialeurodes vaporariorum* before treatment

Variant	V1	V2	V3	V4	V5
The total number of	124	171	156	128	107
individuals					
The total number of the	44	53	28	16	13
larvae					



Graphic.1. Total number of individuals and the larvae of the *Trialeurodes vaporariorum* before treatment.

On July 20 was applied a different treatment consisting of peppermint oil level, tansy extract, sumac extract (table 2 and graphic. 2).

Table 2.

The number of individuals and the larvae of *Trialeurodes vaporariorum* after the first treatment.

The total number	V1	V2	V3	V4	V5
of individuals	Peppermint	Tansy	Witness	A mixture of the	Sumac
	oil	extract		three products	extract
The total number	5	46	78	23	1
of individuals					
The total number	1	9	18	2	0
of the larvae					

On 2.08.2013 shall apply a new treatment, and determine the number of individuals and larvae of *Trialeurodes vaporariorum* (table 3 and graphic 3).



Graphic. 2.. The number of individuals and the larvae of *Trialeurodes vaporariorum* after the first treatment.

## Table 3.

The number of individuals and the larvae of *Trialeurodes vaporariorum* after the second treatment.

The total number	V1	V2	V3	V4	V5
of individuals	Peppermint	Tansy	Witness	A mixture of the	Sumac
	oil	extract		three products	extract
The total number	5	46	78	23	1
of individuals					
The total number	1	9	18	2	0
of the larvae					

It can be noticed that the variants treated with peppermint oil level, sumac extract and mixture of the three products, the number of individuals of *Trialeurodes vaporariorum* decreased significantly, as the following graphics 4 and 5.

Additional methods were applied for the control- A mechanical method which consisted of manual removal of the leaves of strawberry (*Fragaria*) attacked early in the morning. Were carefully removed when the plants are still pest, harvesting was done in dark plastic bags which were then destroyed.



Graphic. 3.The number of individuals and the larvae of *Trialeurodes vaporariorum* after the second treatment.



Graphic 4. Number of individuals before and after treatment.



Graphic 5. The number of larvae after treatment.

#### CONCLUSIONS

It can be concluded that the methods used have had the desired effect, prevented migration reduced the population of pests, reducing the intensity of the attack phases detected and pest population was maintained below the economic damage threshold.

From the above data it appears that the products used have had the desired effect, and of these, sumac extract has a high efficacy in reducing and pest control. We applied these methods to combat, the obligation to take the risk of compromising the culture me not recorded in 2013, organic certification framework.

All products used have an effect on larvae of *Trialeurodes vaporariorum* because after their application is observed significantly reduce the number of this.

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## ASPECTS REGARDING THE ENTOMOFAUNA IN WINE ECOSYSTEM GALICEA

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Key words: entomofauna, Coleoptera, wine ecosystem.

### ABSTRACT

In the wine ecosystems, human intervenes permanently, often leading to serious consequences, especially by applying insecticides which is one of the main factors in the destruction of biodiversity.

In order to avoid these undesirable effects, it is necessary to know the entomofauna found in this ecosystem. This fact would allow a possible protection of helpful coleopterans and a milder diminishing of harmful coleopterans.

In order to determine the entomofauna structure of harmful and useful coleoptera during 2012, researches have been done in the vineyard ecosystem Galicea.

#### INTRODUCTION

Taking in consideration the position and the role of the coleopteran from different types of ecosystems, the current study was necessary in this type of vineyard ecosystems. (Mitrea I., 2005)

The helpful coleopterans, the entomophagous insects, represents a natural method of combating, they diminish the populations of harmful insect from the same order or from different orders, by consuming them. The entomophagous are insects which in the larva or adult stage, attack pests from a specific or several species, reducing their number. The entomophagous insects can be divided into two categories: predators and parasites. The parasites are usually specific to each host, they can keep a favorable report with it and larvae do not require foraging. The predators are difficult to grow in the laboratory and are less specific.

The harmful coleopterans are insects which consume various organs of vines, from root to stem, leaves, flowers and fruits. They attack both in the larva stage and in the adult stage, causing considerable damage (Rosca I. et al., 2011).

# MATERIALS AND METHODS

In order to identify the coleopterans collected, the researches have been conducted in 2012, in May, June, July and August, in the Vineyards Galicea. The entomofauna was collected using the method of Barber trap, placed on the ground level, near the vine trunk. The Barber traps were filled with formalin solution 4% or concentrated solution of NaCl

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(figure 1). Sampling the material from traps was done monthly. The collected entomofauna was analyzed and identified inside the laboratory with microscope and magnifying glass.

The identification was done by using the public Romanian fauna identifier (Panin, 1951). The resulting material was labeled and preserved in ethylic alcohol 70 % and glycerin or it was prepared on dry needles (figure 3).



Figure 1. Barber Trap



Figure 2. Material set on entomological needles

## **RESULTS AND DISCUSSION**

The collected entomofauna was subjected to a statistical analysis of population structure, and it was classified in harmful coleopteran and helpful coleopteran then both the useful and harmful species were distributed in families and species.

In the vineyard ecosystem Galicea in 2012, in all the months in which the research had been carried on: May, June, July, August, there were a total of 15 harmful species systematically arranged in 5 families (table no. 1).

The harmful coleopterans collected were classified in families: Rhynchitidae with 1 species, Scarabidae family with 9 species collected, Crisomelidae family with 1 species collected, Elateridae family with 3 species collected and Curculionidae family with 1 species collected. Regarding the abundance of damaging coleopterans, species ranked first are: *Byctiscus Betulae* 25 individuals, *Anomala dubia* 28, *Anomala vitis* 25 individuals, *Anoxia orientalis* 26 individuals and *Agriotes ustulatus* 27 individuals. The last positions ranged species: *Rhizotrogus aequinoctialis* 13 individuals, *Phyllopertha horticola* 15 individuals and *Otiorhynchus ligustica* 14 individuals.

Helpful species collected and identified during 2012 in the vineyard ecosystem Galicea were 11, systematically classified in two families (table no. 2).

Table no. 1

	in the vine yard ceosystem Ganeea					
N.	The meeter' name		No. of s	pecimens		Abunda
INO.	The species name	May	June	July	August	nce
	I.	Fam. Rh	nynchitidae			
1.	Byctiscus betulae	4	6	8	7	25
	II.	Fam. S	carabidae			
2.	Pollyphyla fullo	3	5	7	6	21
3.	Anomala solida	2	4	8	5	19
4.	Anomala vitis	3	5	9	8	25
5.	Anomala dubia	4	6	10	8	28
6.	Amphimallon solstitialis	3	4	7	6	20
7.	Melolontha melolontha	2	5	7	5	19
8.	Rhizotrogus aequinoctialis	1	4	5	3	13
9.	Anoxia orientalis	3	6	9	8	26
10.	Phyllopertha horticola	2	3	6	4	15
	III.	Fam. Cr	isomelidae	;		
11.	Adoxus obscurus	3	6	9	6	24
	IV	. Fam. I	Elateridae			
12.	Agriotes obscurus	3	5	9	7	24
13.	Agriotes lineatus	2	4	7	5	18
14.	Agriotes ustulatus	3	6	10	8	27
	V. ]	Fam. Cu	irculionida	e		
15.	Otiorhynchus ligustici	1	3	6	4	14
	Total	39	72	117	90	318

The structure and the abundance of the harmful coleopteran species encountered in the vineyard ecosystem Galicea

Table no. 2

The structure and the abundance of the helpful coleopteran species encountered in the vineyard ecosystem Galicea

			No. of	specimens	-	
NO.	The species' name	May	June	July	August	Abundance
		I. Fam. C	Coccinelid	ae		•
1.	Adalia bipunctata	1	3	5	-	9
2.	Coccinella septempunctata	2	4	7	5	18
3.	Adalia decempunctata	-	2	3	1	6
		II. Fam.	Carabida	e		
4.	Carabus ulrichi	2	4	7	6	19
5.	Carabus violaceus	-	3	8	7	18
6.	Carabus cancellatus	-	1	5	5	11
7.	Calosoma sycophanta	2	3	5	4	14
8.	Harpalus calceatus	3	6	8	7	24
9	Amara crenata	3	8	12	9	32
10	Cicindela germanica	2	7	10	8	27
11	Pterostichus nigera	-	2	9	7	18
	Total	15	43	79	59	196

Helpful coleopterans collected were classified in families: Coccinelidae 3 species and Carabidae family 8 species collected.

Regarding the abundance of helpful coleopterans, species ranked first are: *Amara crenata* 32 individuals, *Cicindela germanica* 27 individuals and *Harpalus calceatus* 24 individuals. The last positions ranged species: *Adalia bipunctata* 9 individuals, *Adalia decempunctata* 6 individuals and *Carabus cancellatus* 11 individuals.

## CONCLUSIONS

The total of harmful coleopterans collected in the vineyard ecosystem Galicea during 2012 was as follows:

- During the month of May 39 individuals of the harmful coleopterans were collected

- In June were collected a total of 72 individuals belonging to the Carabidae family and the Coccinelidae family

- In July the number of individuals was the highest, being represented by 117 individuals

- In August after the collections it was cumulated a total of 90 individuals.

- Total coleopterans collected in 2012 were 318 individuals.

The total of helpful coleopterans collected in the vineyard ecosystem Galicea during 2012 was as follows:

- During the month of May 15 individuals of the helpful coleopterans were collected

- In June were collected a total of 43 individuals belonging to the Carabidae family and the Coccinelidae family

- In July the number of individuals was the highest, being represented by 79 individuals

- In August after the collections it was cumulated a total of 59 individuals.

- Total coleopterans collected in 2012 were 196 individuals.

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## THE STRUCTURE OF THE HARMFUL AND USEFUL COLEOPTERA ENCOUNTERED IN THE FRUIT-GROWING ECOSYSTEM PODARI

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Key words: entomofauna, coleoptera, fruit-growing ecosystem

#### ABSTRACT

Having a high ecological plasticity (phenotypic plasticity), the Coleoptera are found in all ecosystems – from the polar regions to the equatorial forests. They are common in all kind of habitats; they feed on any kind of food.

Numerically the coleoptera are superior to other groups of beetles, being in most ecosystems more than 80% of all insect species existing.

The Coleoptera meet various useful functions for humans, are good indicators can serve as one of the main objects solving problems and integrated environmental monitoring.

In order to determine the entomofauna structure of harmful and useful Coleoptera during 2012, researches have been done in fruit crops from Pomicola Podari.

#### **INTRODUCTION**

From the total number of animal species inhabiting the Earth, the insects represent 70%. Every year the entomology experts discover and describe new species and according the latest information from literature, approximately 2 million species of insects are known in the world nowadays.

Taking in consideration the position and the role of the coleoptera from different types of ecosystems, the current study was necessary in this type of fruit-trees ecosystems. (Mitrea I., 2005)

The helpful fauna in the Coleoptera order found in fruit ecosystems is part of natural combating methods and has the role of reducing the pest populations belonging to the same order or to another order. The helpful fauna is influenced by the environmental factors and not directly by humans.

The entomophagous insects, how they are called, are insects which in the larva or adult stage, attack pests from a specific or several species, reducing their number. The entomophagous insects can be divided into two categories: predators and parasites. The parasites are usually specific to each host, they can keep a favorable report with it and larvae do not require foraging. The predators are difficult to grow in the laboratory and are less specific.

The pests identified in fruit growing ecosystem attack various organs of vegetation. Summarily, we found out that the most affected organs of vegetation are: aerial organs of plants, floral organs attacked by adults, buds or shoots and fruits. Larvae that

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attack fruits are generally harmful, consuming mesocarp and seeds (Perju T., B. Bobirnac, D Bob, 1976), (Rosca I, Oltean I., Mitrea I., și colab. 2011).

### MATERIALS AND METHODS

The researches have been conducted in 2011, in May, June, July and August, in the Fruit-growing Plantations Podari. The coleopteran entomofauna was collected using the method of Barber trap, placed on the ground level, near the strain. Traps were filled with formalin solution 4% or concentrated solution of NaCl (figure 1, 2). Sampling the material from traps was done monthly. The collected entomofauna was analyzed and identified inside the laboratory with microscope and magnifying glass.

The identification was done by using the public Romanian fauna identifier (Panin, 1951). The resulting material was labeled and preserved in ethylic alcohol 70 % and glycerin or it was prepared on dry needles (figure 3).



Figure 1. Barber Trap

Figure 2. Barber Trap



Figure 3. Material set on entomological needles

## **RESULTS AND DISCUSSION**

The coleopteran population collected in the Fruit-growing Plantations Podari, was identified and classified in harmful entomofauna and helpful entomofauna. The collected coleoptera were submitted to a statistical analysis regarding their structure and they were grouped in species and families.

In the fruit-growing ecosystem it has been identified both harmful and helpful coleoptera. After analyzing entomofauna it was noticed that harmful coleoptera are grouped into 4 families while useful coleoptera shows only 3 families (table no. 1, table no. 2).

Table no. 1

The structure of the harmful coleopteran species encountered in the fruit-growing ecosystem Podari

Nr	Families	Species
crt.		
1.	Curculionidae	Anthonomus pomorum
		Rhynchites bacchus
		Sciaphobus squalidus
2.	Cetoniidae	Oxythirea funesta
3.	Rutilidae	Epicometis hirta
4.	Rhynchitidae	Rhynchites aequatus

Harmful coleoptera fall into four families, of which Curculuionidae family shows 3 species identified, the remaining families: Cetoniidae, Rutilidae and Rhynchitidae show a single species identified.

Table no. 2

The structure of the helpful coleopteran species encountered in the fruit-growing ecosystem Podari

Nr	Families	Species
crt.		
1.	Carabidae	Calosoma inquisitor
		Abax ater
		Pterostichus melanarius
		Pterostichus oblongopunctatus
		Pterostichus versicolor
2.	Coccinelidae	Adalia bipunctata
		Adalia decempunctata
		Coccinella quinquepuntata
		Hypera campestris
		Hypera ripensis
		Stethorus punctillum
3.	Staphylinidae	Pilonthus decorul
		Oligota flavicornis

The useful Coleoptera are grouped in 3 families, they present a higher number of individuals: Carabidae family shows five individuals, Coccinelidae family owns six individuals and the Staphylinidae family just 2 individuals.

#### CONCLUSIONS

Regarding the total coleopteran entomofauna collected, we noticed that the number of useful coleopteran is higher than the number of harmful coleopteran.

In the case of harmful coleopteran we have a total of 6 species belonging to 3 families.

In the case of useful coleopteran there is an increase of the number of species to 13.

Tracking and summing up the results for each category of coleopteran we can conclude that the number of families found in plantation POMICOLA is 7 and the total number of species identified is 16.

#### ACKNOWLEDGMENT

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## Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

Vol. XVIII (LIV) - 2013

## PATHOLOGICAL ASPECTS OF THE DOG BONE SYSTEM CAUSED BY OSTEOSARCOMA

Carmen Vladulescu¹, Dr. Doru Safta²

Key words: osteosarcoma, skeletal system, lymph nodes.

#### ABSTRACT

Osteosarcoma is the most common primary bone tumor. In 12-15% it is of cranial origin. Osteosarcoma of the skull is different from those in other parts of the body or from those of the appendicular skeleton because they tend to be osteoblastic and present distinct border.

Osteoma is a benign tumor with relatively slow evolution. The radiography shows a formation with clear edges. These tumors can develop in the bones of the skull or jaw.

Osteosarcoma is by far the most common type of malignant bone cancer in dogs. This form of cancer affects dogs of all ages. It frequently appears with both males and females. Giant breeds such as St. Bernards, Great Danes, Pyrenean shepherd, are 60 times more likely to develop osteosarcoma than dogs that weigh less than 50kg.

#### INTRODUCTION

Large breeds such as Irish Setter and Boxer are 8 times more likely to develop the disease. Small breeds are rarely affected nearly.

Osteosarcoma occurs most often in the bones of the front legs followed, in order of frequency, in the hind legs, ribs and jaw. Often the first sign is the appearance of lameness in a mature dog that has not a related history of injuries and injury. Usually the sign is too little heeded until the leg or bone inflammation begins to be noticed. Pressure on the tumor causes pain and fractures can occur at the tumor site. Bone biopsy is recommended to determine the type of tumor, if the x-ray is not conclusive.

Current knowledge of the disease is not related to genetics or gender, but bone cancer occurs most commonly in dogs of large breeds. Some studies have shown a slight increase in the development of osteosarcoma among dogs that had an early bone stump.

Many are subtle signs of bone cancer. These can include swelling, lameness, and joint or bone pain. In some cases, dogs suffering from bone cancer appear tired or apathetic. Occasionally, dogs will exhibit either an increase in cell mass in the affected area on their body, or a painful inflammation around the tumor.

The veterinarian will use X-rays to view the volume, often using different angles to get an accurate picture. Other tests include biopsies, blood tests, bone scans and CT to visualize bony areas, and cell mass formed, where it was discovered.

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When the diagnosis is bone cancer prognosis is often bad and there are many side effects of treatment options.

Treatment is of surgical nature, consisting of removal of affected tissue as rigorous as possible, plus chemotherapy. After treatment, life expectancy is around 100-150 days, even up to 300 days in dogs. For these reasons, it is necessary a vet specialist consultation and radiological examination.

Because these tumors metastasize to the lungs it is very important to perform chest radiography before recommending surgery. The dog must be physically fully examined, including the complete blood analysis and fine needle aspiration biopsy of any enlarged lymph node swelling.

Chemotherapy is often used as a supplement to any surgical options to ensure that the disease does not spread to other areas of the dog's body, especially the lymph nodes. Chemotherapy besides amputation increases survival time of the animal but does not increase the degree of healing.

Radiotherapy can be taken into account especially if the disease is very advanced or metastatic.

Mandibular osteosarcoma treated by radiotherapy has a moderate responding. Radiation is used to decrease pain. Complete surgical removals of chondrosarcoma provide pain relief but should not be considered curative.

## MATERIAL AND METHOD

A Koolie dog, aged 7 years old came to the medical cabinet presenting a 6 cm tumor in the jaw.

To diagnose, the following were done:

- clinical consulting, signs of the disease being obvious since the entry of dogs in medical cabinet
- x-ray or computed tomography (CT)
- blood analyses.



Figure 1. Osteosarcome removed from the jaw

After hand feelings and x-ray examination of the jaw it has been concluded that surgical operation is needed in order to remove the tumor. Clinical diagnosis: jaw tumor.



Figure 2. Cytological aspects of tumor



Figure 3. Morpho-pathological aspects of tumor

As method of treatment was proceeded to extract the tumor by surgical operation. The tumor was removed and sent to the pathological anatomy laboratory to be diagnosed.

## **RESULTS AND DISCUSSIONS**

There were received and examined multiple small fragments with microscopic structure of stratified squamous epithelium that show subjacent islands nekeratinizat poorly differentiated invasive carcinoma with large areas of tumor necrosis, adjacent blades compact bone tissue invaded by tumor cells.

The dog presents advanced phases of the disease and one should treat the dog in order to improve its condition.

As methods of treatment chemotherapy and radiotherapy were used.

The affection of the dog's master is very important during the treatment.

Chemotherapy is often used as a supplement to any surgical options to ensure that the disease does not spread to other areas of the dog's body, especially the lymph nodes.

Radiotherapy can be taken into account especially if the disease is very advanced or metastatic.

## CONCLUSIONS

Treatment involves surgical removal of the osteosarcoma followed by prevention strategies.

Treatment is of surgical nature, consisting of removal of affected tissue as rigorous as possible, plus chemotherapy. After treatment, life expectancy is around 100-150 days even up to 300 days in dogs.

For preventing reasons, the dog must be periodically consulted by a vet in order to establish the status of its health.

For these reasons, it is necessary to vet specialist consultation and radiological examination even for seemingly without cause severe lameness.

There are currently no known ways to prevent bone cancer.

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## Seria: ✓ Biologie ✓ Horticultură

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## STUDIES OF METABOLIC COMPLICATIONS OF OVERWEIGHT AND OBESITY

Carmen Vladulescu1

Key words: metabolism, overweight, obesity, body mass index (BMI).

#### ABSTRACT

Obesity and associated or related comorbidities are major public health problems in the new millennium in most countries.

Worldwide 1.7 billion people are currently classified as overweight [Haslam and James, 2009].

Recent studies of the prevalence of obesity and related metabolic complications were furnished only for few countries. A more accurate quantification of the present prevalence of obesity and associated comorbidities is important for the rational allocation of resources.

A more accurate quantification of the present prevalence of obesity and associated comorbidities is important for the rational allocation of resources.

### **INTRODUCTION**

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health.

Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m).

The WHO definition is:

- a BMI greater than or equal to 25 is overweight
- a BMI greater than or equal to 30 is obesity.

BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and for all ages of adults. However, it should be considered a rough guide because it may not correspond to the same degree of fatness in different individuals.

Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity.

Overweight and obesity are linked to more deaths worldwide than underweight. For example, 65% of the world's population live in countries where overweight and obesity

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kill more people than underweight (this includes all high-income and most middle-income countries).

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended.

- Globally, there has been:
- an increased intake of energy-dense foods that are high in fat;
- an increase in physical inactivity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization.

Changes in dietary and physical activity patterns are often the result of environmental and societal changes associated with development and lack of supportive policies in sectors such as health, agriculture, transport, urban planning, environment, food processing, distribution, marketing and education.

Raised BMI is a major risk factor for diseases such as:

- cardiovascular diseases (mainly heart disease and stroke),
- diabetes,
- musculoskeletal disorders (especially osteoarthritis a highly disabling degenerative disease of the joints),
- some cancers (endometrial, breast, and colon). The risk for these diseases increases, with the increase in BMI.

Childhood obesity is associated with a higher chance of obesity, premature death and disability in adulthood. But in addition to increased future risks, obese children experience breathing difficulties, increased risk of fractures, hypertension, early markers of cardiovascular disease, insulin resistance and psychological effects.

## MATERIAL AND METHODS

Estimating the prevalence of DLP in Romania. Estimating the prevalence of Hyperuricemia in Romania. Estimating the prevalence of HTA in Romania.

CVR assessment in the population of Romania: SCORE diagram for the high-risk areas. Metabolic risk evaluation through FINDRISC score.

Estimating the prevalence of Metabolic Syndrome in the population of Romania.

PREDATORR Study is conducted according to the applicable ICH/GCP standards and WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (*Seoul, Korea, October 2008*).

Since 2009, CEBIS International holds an ISO 9001:2008 Certification for the Development of Clinical Research Projects, Project Management, Monitoring and Consulting in Clinical and Epidemiology Research.

CEBIS also holds an ISO 27001:2005 Certification regarding the Security and Management of Information implemented at the company level.

Assessment of the frequency of overweight and obesity and the frequency distribution on the pre-specified subgroups in the adult population from three administrative regions (South, South-West, Bucharest-Ilfov) of Romania

Evaluation of the **prevalence of different metabolic phenotypes of obesity and overweight** in the mentioned population group.

## **RESULTS AND DISCUSSIONS**

The present analysis comprises **972 subjects** (**569F/403M**; **55,4±14,1 years**) from **three administrative regions**:

- 38,8% from South region,

- 33,3% from South-West region,

- 27,7% from Bucharest-Ilfov County.

The results of the research are presenter in the following figures.

In figure number 1 it is presented the rate of obesity according to group ages and gender.

In figure number 2 it is presented the procentage of population who suffer from obesity and overweight.

In figures number 3 and 4 it is presented the BMI according to categories of gender and age groups.

In figures number 5 and 6 it is presented the variations of BMI according to the existences of abdominal obesity.



Figure 1. Demographic parameters according to age groups and gender



Figure 2. Anthropometric parameters of obesity and overweight



Figure 3. BMI according to categories of gender



Figure 4. BMI according to categories of age groups



Figure 5. Abdominal obesity categories according to gender/age groups



Figure 6. Variations of BMI according to the existences of abdominal obesity

#### CONCLUSIONS

The results indicated that there was significant relationship between all metabolic traits and BMI categories.

The presented preliminary data indicate a high frequency of overweight and obesity, in the studied regions (South, South-West, Bucharest-Ilfov). A higher frequency of obesity and overweight was registered after age of 40 years and in females.

Obesity, overweight and abdominal obesity phenotypes were associated with more pronounced insulin resistance, glucose metabolism impairment, dyslipidemia, arterial hypertension and hyperuricemia.

Knowledge of the **public health** in Romania must deal with the **detection** of metabolic diseases.

For all subjects, their role in the prevalence assessment of a major disease of the present has an outstanding contribution to the **improvement of the public health** from the perspective of society as a whole.

Providing primary prevention measures is very important in the future.

A more accurate quantification of the prevalence of metabolic disorders is important for a rational **allocation of human and financial resources**.

The final data are expected during the next year.

For further information please visit the study website: www.predatorr-study.com

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## Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

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# CONSUMER PROTECTION PROVIDED BY AGRICULTURAL TEHNOLOGIES AND LIMITING POLLUTION

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Keywords: grain technologies, environmental pollution.

## ABSTRACT

The existence, until 2002 of a highly polluted area in Zlatna, around the chemical plant, has made possible the release in the environment of various pollutants such as heavy metals more or less toxic which, due to their long lasting remanence, have negatively influenced the living organisms.

Mineral pollutants (lead, cadmium, copper) in soil are readily absorbed by plant roots and accumulate in leaves is a link between plants and humans, because contamination can be highlighted in plant tissues and organs of animals and in products intended for human. Combining heavy metals and minerals are converted into a block oligomineral, bodies such elements essential for life.

## INTRODUCTION

In this study we conducted an assessment of soil, water, air and vegetation in the polluted area of Zlatna and we used these data to apply in relation to a discussion of quantitative measurements of pollutants mineral grain (wheat, cor, oats).

Study and stretched objectives and highlighted the interrelation between increased tissue levels of pollutants investigated and cereals. (Popa M., 2005).

The pollutant minerals, interact with small amounts of grain in particulat environment conditions. (Bires J., 1995).

The evolution results on the grain mineral concentration of pollutants can say that they are stored and accumulate in concentration allowed in wheat, corn and oats. (Ripeanu M.D., 1979).

## MATERIAL AND METHOD

Determination were performed by Environmental Protection Agency Alba on grain samples collected from polluted area. Indicators which were monitored - heavy metals: lead, copper, zinc, cadmium accumulated in the upper soil layers. Interpretation of results was determined by comparing the maximum concentration allowed by the legislation in force and the normal concentration mentioned in the bibliography.

Determinations of grain mineral pollutants were carried out by atomic absorption spectrophotometer with Perkin Elmer.

This European Standard specifies a method for determination of lead, cadmium and copper in foodstuffs by atomic absorption spectrometry after burnt at 450 ° C.

Samples are burnt at 450°C with a gradual increase in temperature. The hydrochloric acid ashes are dissolved and the solution thus obtained is evaporated in a

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second. The final residue is re-dissolved in nitric acid and the content of metal is determined by flame atomic absorption spectrometry or graphite furnace.

Reactants: the concentration of the trace of the reagents and the water used must be low enough so as not to affect the results of the determination.

- 500 ml of concentrated hydrochloric acid are diluted in 1000 ml water to obtain 37% hydrochloric acid mass fraction, with a density of approximately 1,190 mg/m.
- 7ml of concentrated nitric acid are diluted with water to 1000 ml, in order to obtain at least 65% nitric acid mass fraction, density of about 1400 mg/m.
  Standard solution
- Lead standard solution: 1000 mg Pb are dissolved in 7 ml of nitric acid in a volumetric flask of 1 liter brought to the mark with water.
- Cadmium standard solution: 1000 mg of Cadmium are diluted in 14 ml of water + 7 ml of nitric acid in a volumetric flask of 1 liter brought to the mark with water.
- Copper standard solution: 1000 mg of copper are dissolved in 7 ml of nitric acid in a 1 liter volumetric flask. It is brought to volume with water.

Weigh an agreed quantity of sample into a crucible with an accuracy of 10 mg, depending on the type of sample. It works according to the type of oven.

Drying and burning in the scheduled oven: The crucible containing the sample is placed for analysis in an oven at an initial temperature of not more than 100 °C. The temperature increases at a maximum rate of 50 °C per hour to 450 °C. Allow the sample to stand overnight. If there is a risk of violent boiling, we need to ensure a burning temperature sufficiently low and the time of ignition sufficiently long.

Drying and burning in the un-scheduled thermostat furnace and ignition assembly (ceramic hob cooker and lamp glass cover + IR + sulphuric acid wash tank air purification): Place the crucible with the sample for analysis cap ceramic glass plate and allow the purified air that enters a glass tube to blow over the sample. Place the IR lamp at a distance of 30 cm to 40 cm from the sample and adjust the stove at 100 °C. Reduce distance as drying occurs until the sample is considered dry. The lamp should be just above the cap. Pre-burnt sample temperature is slowly increasing in step with IR light and hot plate. The final temperature of the ceramic plate must be about 300 °C. The time required for the pre-burning vary widely depending on the type of the sample. Place it in the oven between 200 and 250 °C and slowly increase the temperature to 450 °C not exceeding 50 °C per hour. Allow the sample to stand overnight.

Remove the crucible from the oven and let it cool. Moisten the ashes with 1 to 3 ml of water and evaporate on a water bath or on a hot plate. It is put back in the oven of no more than 200 °C and the temperature is increased gradually to 450 °C. Burn at 450 °C for one to 2 hours or more. Repeat this operation until the sample is completely burned, until the ash is white or slightly coloured gray. Add 5 ml of hydrochloric acid crucible in such a way that all the ashes coming into contact with acid. Evaporate the acid water bath or on the stove. The residue was dissolved in an exact volume (10 ml to 30 ml) of nitric acid. Turn the crucible carefully so that all the ashes into contact with the acid. Cover with a watch glass and allow the sample to stand for about an hour to two hours. Mix thoroughly with a solution of cruzet rod and transfer the contents into a plastic bottle.

The method used, the technique with flame or graphite furnace are chosen according to the concentration of the element to be analyzed. Flame AAS can be used whenever possible, as this technique is less sensitive to interference than graphite furnace AAS.

The metal content of the samples is derived from calibration curves made with 3 standards.

Table 1

The parameters for equipment flame AAS				
Metal	Flame	Wavelength nm		
Cu	Air-acetylene, oxidizing	324.7		

Additions method should be used every time, unless it proves superfluous. When using the method of additions is extremely important that measurements are carried out on linear. It is preferable to measure the area of the peaks rather than their height.

Table 2

		working	paramete	is for gra	pinte tun	lace AAS		
Metal	Wavele	Parameter	Stage 1	Stage 2	Stage 3	Stage 4	Sample	The
	ngth						volum	graphite
	nm							tube
								furnace
Pb	283.3	Temp. 0°C	130	450	1900	2500	20 µl	L'vov
		growth ,s	10	15	0	2	-	
		stop, s	30	10	4	2		
Cd	228.8	Temp. 0°C	130	350	1200	2500	10 µl	L'vov
		growth ,s	10	15	0	2	-	
		stop, s	30	10	4	2		

Working parameters for graphite furnace AAS

Calculation:

Measure the peak area when using GFAAS and flame AAS is used for the signal obtained. Plot the calibration curve and read off the concentration of the curve element. Calculate the W, the weight fraction of the element determined in mg / kg of the sample using the following formula: w = (a / b) * V / b

w- concentration of the sample in mg / kg, a- concentrations of the solutions to the sample in mg / l, b- average concentration in control solutions, in mg / l, V - volume of sample in ml, M - mass of the sample in grams.

### **RESULTS AND DISCUSSIONS**

Accumulation of heavy metals in grain variability is strongly determined by the type of metal, species and plant organ analyzed.

Table 3

Vegetation	Copper mg/kg	Lead mg/kg	Cadmium mg/kg
corn	9.4	6.4	0.2
grain	8.6	5.8	0.2
oats	8.5	5.6	0.2
СМА	20	15	0.4

Level concentrations, Cu, Pb, and Cd in corn, wheat, and oats in 2006



Figure 1. Level concentrations, Cu, Pb, Cd in corn, wheat, oats in 2006

In 2006, we determined values of Cu, Pb, Cd in corn, grain, oats below the maximum allowed concentration.

Table 4.

Vegetation	Copper m	ng/kg Lead mg	g/kgCadmium n	ng/kg
corn	9.2	6.4	0.2	
grain	8.5	5.7	0.2	
oats	8.5	5.4	0.2	
CMA	20	15	0.4	

Level concentrations, Cu, Pb, Cd in corn, wheat, and oats in 2007



Figure 2. Level concentrations, Cu, Pb, Cd in corn, wheat, oats in 2007

In 2007, we determined values of Cu, Pb, Cd in corn, grain, oats below the maximum allowed concentration.

Table 5

Vegetation	Copper mg/kg	gLead mg/kg	Cadmium mg/kg
corn	9.2	6.3	0.2
grain	8.4	5.7	0.2
oats	8.3	5.1	0.2
CMA	20	15	0.4

Level concentrations, Cu, Pb, Cd in corn, wheat, oats in 2008



Figure 3.Level concentrations, Cu, Pb, Cd in corn, wheat, oats in 2008

In 2008, we determined values of Cu, Pb, Cd in corn, grain, oats below the maximum allowed concentration.

### CONCLUSIONS

In the 2006-2008 values mineral pollutants (copper, cadmium, lead) derived from corn, wheat, oats were located below the CMA, but lead and cadmium exceeded in all years for normal plant tissues.

In 2006 we determined values for lead, copper and cadmium in corn, wheat, and oats. In the case of the determination of lead all the values obtained (6.4 mg/ kg maize, 5.8 mg/ kg of wheat and oats 5.6 mg/kg) are below the values of the CMA (Pb 10 mg/kg). In the case of the determination of copper all the values obtained, copper (9.4 mg/ kg maize, 8.6 mg/ kg of wheat and oats 8.5 mg/kg) is below the CMA (20 mg/kg). In the case of determination of cadmium all the values obtained (0.2 mg/kg maize, 0.2 mg/kg of wheat and oats 0.2 mg/kg) are below the CMA (Cd 0.4 mg/kg).

Cultivated cereals (wheat, corn, and oats) had amounts of heavy metals below the maximum permissible concentration in 2006.

In 2007, there were determined values for copper, lead, cadmium, corn, wheat and oats. In the case of the determination of lead all the values obtained (6.4 mg/kg maize, 5.7

mg/kg of wheat and oats 5.4mg/kg) are below the values of the CMA (Pb 10 mg/kg). In the case of the determination of copper all the values obtained (9.2 mg/kg maize, 8.5 mg/kg of wheat and oats 8.5mg/kg) is located below the CMA (20 mg/kg). In the case of determination of cadmium all the values obtained (0.2 mg/kg maize, 0.2 mg/kg of wheat and oats 0.2 mg/kg) are below the values of the CMA (Cd 0.4 mg/kg).

Grown grains (wheat, maize and oats) amounts of heavy metals were below the CMA in 2007.

In 2008 we determined values for copper, lead, cadmium in corn, wheat, oats. In the case of the determination of lead all the values obtained (6.3 mg/kg maize, 5.7 mg/ kg of wheat and oats 5.1 mg/kg) is located below the CMA (Pb 10 mg/kg). In the case of the determination of copper all the values obtained (9.2 mg/ kg maize, 8.4 mg/kg wheat and oats 8.3mg/kg ) is below the CMA (20 mg/kg). In the determination of cadmium all the values obtained (0.2 mg/kg maize, 0.2 mg/kg wheat and oats 0.2mg/kg)are below the values of the CMA (Cd 0.4 mg/kg).

Grown grain (wheat, maize and oats) amounts of heavy metals were below the CMA in 2008.

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# UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

Seria: ✓ Biologie ✓ Horticultură ✓ Tehnologia prelucrării produselor agricole ✓ Ingineria mediului

#### Vol . XVIII ( LIV ) - 2013

# CONTRIBUTION TO IMPROVING THE ROOTING YIELD OF CUTTINGS FROM ROMANIAN PLUM ROOTSTOCK ASSORTMENT

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Keywords: Prunus, rootstocks, clonal propagation.

#### ABSTRACT

Planting material, in general and fruit tree planting material in particular, play a crucial role in achieving profitable fruit growing.

Modern culture of stone fruits is dependent on the use of cultivars and rootstocks, whose influence is observed on the levels of production, fruit quality, degree of adaptability to the climatic and soil conditions, growth vigor, precocity, etc.

Given the advantages of clonal propagation of rootstocks, it was initiated a study to with the purpose of increasing the efficiency of rootstock rooting for six plum rootstocks from Romanian assortment (Miroval, Corval, Rival, Pinval, Oltval and Oteşani 11).

When testing clonal propagation by hardwood cuttings the following rootstocks were remarked: Corval (average 90.5% rooted cuttings); Miroval (average 89.5% rooted cuttings) Rival (average 63.0% rooted cuttings); Oltval (average 62.0% rooted cuttings).

Stimulation with bottom heating remains the favorable solution for clonal propagation by cuttings of plum rootstocks (an average 12.8% plus of rooted cuttings).

## **INTRODUCTION**

Profitable plum culture in Romania is achieved with productive and quality cultivars, rootstocks easy to propagate, high adaptability to environmental conditions of modern culture technologies..

Rootstock and cultivars variety are key elements in modernizing plum culture. Fortunately, the introduction of new rootstocks for vegetative propagation and improvement of their rooting provides the growers with necessary elements for modernization the plum culture.

In order to quantify the yield of rooting the biological material taken under study is necessary to evaluate the rooting ability and the capacity of growth and development after rooting.

### MATERIALS AND METHODS

The biological material taken under evaluation was represented by the following clonal rootstocks: Miroval, Corval, Rival, Pinval, Oltval and Oteşani 11 (as control).

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The experiment studied the influence of the planting mode on rooting of hardwood cuttings placed in cold tunnels with and without basal heating. The experiment was designed having the following planting treatments of hardwood cuttings:

 $V_1$  - on ridges;

V₂ - on flat bed, mulched with black polyethylene film;

V₃ - on flat bed, with bottom heating system for hardwood cuttings;

V₄ - on flat bed, without mulching or bottom heating system (control).

The trial design was randomized block with 6 plum rootstocks, 4 treatments and 4 replications of 100 hardwood cuttings per each plot.

Hardwood cuttings were harvested after leaves falling in autumn. The hardwood cuttings were trimmed to a length of 50-55 cm. At the base of the cuttings was performed a slant cut below the basal node, opposite to the node (cut length of about 2cm). At the top node of the cuttings a horizontal cut was performed. Cuttings were planted in November on a bed in a cold tunnel covered with polyethylene film. Bottom heating of cuttings was achieved using an electric cable resistance with thermostat.

Data was collected in order to evaluate the rooting capacity of the rootstocks and growth after rooting.

# **RESULTS AND DISCUSSIONS**

The hardwood cuttings were harvested and planted on the bed inside the cold tunnel covered with polyethylene film using 4 treatments ( $V_1$  - on ridges;  $V_2$  - on flat bed, mulched with black polyethylene film;  $V_3$  – on flat bed, with bottom heating system;  $V_4$  - on flat bed, without mulching or bottom heating system).

Both the ridges and black polyethylene mulch foil had the role to accumulate a larger amount of heat compared to the control.

Basal heated treatment of hardwood cuttings has the role to ensure the optimum temperature for rhizogenesis and root growth. This gives the possibility of increasing the rooting yield of rootstocks from more difficult to root fruit tree species. In the case of plum rootstocks, the vegetative propagation is relatively difficult.

Given these considerations and advantages of vegetative propagation of plum rootstocks, a study was initiated at SCDP Vâlcea in order to improve the propagation techniques. The 6 rootstocks tested (Miroval, Corval, Rival, Pinval, Oltval and Oteşani 11) emphasized different values in the rooting process of hardwood cuttings (Table 1).

The highest percentage of rooted cuttings has observed for Miroval and Corval (96 and 95%) when the base of the hardwood cuttings received caloric intake from the electric cable resistance displayed inside the bed (V₃). The lowest percentage was recorded in the case of Pinval rootstock (41%) in the V₄ control treatment (flat bed, without mulching or bottom heating).

It was found that Corval and Miroval rootstocks, which are *Prunus cerasifera* (Myrobalan) offspring recorded highest average values (90.5% and 89.5%) than the *Prunus insititia* offspring rootstocks (Rival - 63.0%; Oteşani 11 - 52.3% and Pinval - 46.0%) and Oltval interspecific rootstock (62.0%).

All the rootstocks tested for rooting ability of hardwood cuttings exceeded the control (Oteşani 11) with levels ranging from 9.7% (Oltval) to 38.2% (Corval). Pinval rootstock scored lower (6.3%) than Oteşani 11. These levels are due also to poor quality of the hardwood cuttings available for the test.

Analysis of hardwood cuttings planting types for rooting emphasized the fact that the  $V_4$  control treatment (flat bed, unmulched and unheated) recorded the lowest average

values (62.2%). The other three planting types recorded plus differences between 3.3% ( $V_2$ ) to 12.8% ( $V_3$ ).

The growth and development of the rooted cuttings are presented in Table 2. The number of shoots, shoots length and numbers of sylleptic shoots were tracked. The number of shoots per cutting did not exceed 4. Shoot length is variable depending on the rootstock used; from 15 cm in case of Pinval rooted cuttings in  $V_1$  and  $V_4$  treatments to 57 cm and 56 cm respectively in case of Corval rooted cuttings in  $V_3$  and  $V_2$  treatments. Only a few shoots with sylleptic ones (up to 2 pcs.) are recorded in case of Miroval and Corval rooted cuttings, the other rootstocks (Rival, Pinval, Oltval and Oteşani 11 have none or only one sylleptic shoot. A good development of rooted cuttings is determined primarily by the number and length of shoots, the leaf photosynthetic capacity in conjunction with the absorption capacity of the root system.

Comparing the 5 rootstocks under study with the control rootstock Oteşani 11, significant positive differences are observed in the case of shoot length range of 2.0 cm for Oltval rootstock and 21.0 cm for Corval. Relatively large positive differences present Miroval rootstocks (12.25 cm) and Rival (10.5 cm). Pinval rootstock recorded negative differences from the control rootstock (-13.0 cm). The planting mode of the hardwood cuttings for rooting influenced the length of annual growth. There were positive values in all three studied treatments compared with the control (V₄). The largest differences were recorded in case of  $V_3$  (+15.5 cm) and  $V_2$  (+10.17 cm).

Table 3 presents the growth and development of the root system of the cuttings harvested from the 6 rootstocks evaluated (Miroval, Corval, Rival, Pinval, Oltval and Oteşani 11). Data collected have revealed the following issues:

- all the cuttings from this study issue roots into the callus area from the basal cuts with the exception of Miroval rootstock cuttings which are issuing roots at the base but also along the cuttings;

- the number of roots thicker than 3 mm is much smaller than those of less than 3 mm in diameter;

- the average length of roots thicker than 3 mm varies from 17.5 cm in case of Pinval to 30.75 cm for Corval. Lower values than control rootstock Oteşani 11 (24.7 cm) are recorded for Oltval (20.5 cm.). Higher values than the control were observed in case of Miroval (29.5 cm) and Rival (25.7 cm);

- root length thicker than 3 mm in diameter is influenced by the mode of planting hardwood cuttings for rooting. Highest average value of 27.8 cm was recorded in case of cuttings planted on flat bed with bottom heat ( $V_3$ ). In case of the two other variants ( $V_1$  and  $V_2$ ), the differences are nonsignificant compared with the control treatment ( $V_4$ ).

## CONCLUSIONS

Evaluation of the capacity of vegetative propagation by hardwood cuttings highlighted the plum rootstocks Corval (average 90.5% rooted cuttings) and Miroval (average 89.5% rooted cuttings).

The Rival rootstock, which is offspring of *Prunus insititia* and Oltval, which is interspecific hybrid, emphasize high rooting capacity of hardwood cuttings (63.0% and 62.0%);

Stimulation of hardwood cuttings rooting through basal heating is the favorable solution for propagation by cuttings of plum rootstocks (an average gain of 8.8 to12.8% of rooted cuttings can be obtained comparatively with the other alternatives taken under study).

Basal heating of hardwood cuttings affect significantly positive the growth and development of roots and shoots especially in case of Corval, Miroval and Rival plum rootstocks.

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Behavior of Romanian new plum rootstocks on vegetative propagation through hardwood cuttings at University of Craiova - S.C.D.P. Vålcea

	Difference	compared with V4 (Control)	+4.0	+3.3	+12.8	1		
		Mean	66.2	65.5	75.0	62.2		
		Oteșani 11	49	48	65	47	52.3	1
ng rate (%)		Oltval	61	09	71	56	62.0	L.9+
Root	Rootstock	Pinval	47	43	53	41	46.0	-6.3
		Rival	61	63	70	58	03.0	+10.7
		Corval	91	89	95	87	90.5	+38.2
		Miroval	88	06	96	84	89.5	+37.2
Planting mode of hardwood cuttings for	rooting		On ridges (V1)	On flat bed, mulched with black polyethylene film (V ₂ )	On flat bed, with bottom heating system $(V_3)$	On flat bed, without mulching or bottom heating system (Control) (V ₄ )	Mean	Difference compared with control (Otesani 11)
z	0.		1	2	3	4		

Table 1

		sylleptic shoots	2	2		7	5		1			
npared		No. of annual growth with	+1,	+ 1,	0	-0,	+0,	1				
rence com	th Oteșani (control) (±)	Average shoot length (cm)	+12,25	+21,0	+10.5	-13,0	+2,0	I				
Diffe	wi	stoots fo. oV	$^{+1,0}$	+0.5	-0,5	- 1,75	+0.5	I				
		No. of annual growth with sylleptic shoots	1,5	1,5	0,25	0,0	0,5	0,25				
	Mean	Average shoot length (cm)	41,75	50,5	40,0	16,5	31,5	29,5				
		stoots or shoots	3,75	3,25	2,25	1,0	3,25	2,75				
	ithout ottom em	No. of annual growth with sylleptic shoots	1	1	0	0	1	0	0,5		I	
	at bed, wi hing or bo ating syst (Control) (V ₄ )	Average shoot length (cm)	37	39	34	15	19	23	27,83		,	
ooting	On fl mulc hea	No. of shoots	3	3	2	1	3	ю	2,5		ı	
gs for re	h stem	No. of annual growth with svots shoots	1	1	1	0	0	0	0,5		0	
od cutting	at bed, wii neating sy (V ₃ )	Average shoot length (cm)	50	57	51	20	47	35	43,33		+15,5	
Planting mode of hardwo	On fi bottom h	vo. of shoots	4	4	3	1	4	3	3,17		+0,67	
	shed ylene	No. of annual growth with sylleptic shoots	7	7	0	0	0	1	0,83		0,33	
	t bed, mulc ack polyeth film (V ₂ )	Average shoot length (cm)	43	56	40	16	40	33	38,0		+10,17	
	On fla with bla	No. of shoots	4	ю	2	1	3	ю	2,67		+0,17	
		No. of annual growth with sylleptic shoots	2	2	0	0	1	0	0,83		+0,33	
	On ridges (V1)	Average shoot length (cm)	37	50	35	15	20	27	30,67		+2,84	
		vo. of shoots	4	3	2	1	3	2	2,5		0	
Rootstock	1		Miroval	Corval	Rival	Pinval	Oltval	Oteșani 11 (Control)	Mean	Difference	compared	WILD V 4

Growth and development of rooted cutting of some Romanian plum rootstocks

Table 2

			1	1	1	r		1	1	
	pared I )	Average length of roots >3mm in diameter (cm)	+4.7	+6.0	+1.0	-7.2	-4.2	ı		
	ence com ith contro teşani 11 (±)	No. of roots <3mm in diameter	$^{+3.5}_{0}$	+4.0	+0.2 5	-3.25	+1.5	I		
	Differe wi (O	No. of roots >3mm in diameter	+2.25	+2.50	+0.25	-0.50	+0.25	ı		
		Average length of roots >3mm in diameter (cm)	29.50	30.75	25.75	17.5	20.5	24.75		
	Mean	No. of roots <3mm in diameter	14.75	15.25	11.5	8.0	12.75	11.25		
		No. of roots >3mm in diameter	4.5 0	4.7 5	2.5	1.7 5	2.5	2.2 5		
	ithout ottom em	Average length of roots >3mm in diameter (cm)	28	29	24	16	17	22	22.70	-
	at bed. w hing or b ating syst (Control) (V ₄ )	No. of roots <3mm in diameter	13	14	10	7	12	10	11.00	I
r rooting	On fi mulc) hea	No. of roots >3mm in diameter	4	4	2	2	2	2	2.70	I
uttings fo	vith iystem	Average length of roots >3mm in diameter (cm)	33	33	28	20	24	29	27.80	+5.10
dwood cı	Tat bed. v heating (V ₃ )	No. of roots <3mm in diameter	16	17	14	10	15	13	14.20	+3.20
de of har	On J bottom	No. of roots >3mm in diameter	5	5	3	2	3	3	3.50	+0.80
nting moo	Iched thylene	Average length of roots >3mm in diameter (cm)	30	31	28	18	22	26	25.80	+3.10
PI	t bed. mu ick polyei film (V2)	No. of roots <3mm in diameter	16	16	11	∞	12	12	12.5	+1.50
	On fla with bla	No. of roots >3mm in diameter	4	S	7	7	3	7	3.00	+0.30
		Average length of roots >3mm in diameter (cm)	27	30	23	16	19	22	22.80	+0.10
	On ridges (V1)	No. of roots <3mm in diameter	14	14	11	7	12	10	11.30	+0.30
	Č	No. of roots >3mm in diameter	5	5	3	1	2	2	3.00	+0.30
	Rootstock			Corval	Rival	Pinval	Oltval	Oteșani 11 (Control)	Mean	Difference compared with V ₄ (Control)

Growth and development mode of rooting system of cuttings from Romanian plum rootstocks

Table 3