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Environmental engineering

#### RESEARCH REGARDING THE INFLUENCE OF THE VARIETY AND CULTURE CONDITIONS ON CERTAIN FRUIT QUALITY FEATURES FOR SEVERAL CHERRY VARIETIES

Albulescu Carmen Maria Andrada<sup>1</sup>, Hoza Dorel<sup>1\*</sup>

<sup>1</sup> University of Agronomical Sciences and Veterinary Medicine Bucharest, Faculty of Horticulture; 59 Mărăşti Bvd, 011464, Bucharest, Romania, \* correspondence author. E-mail: dorel.hoza@gmail.com

Key words: fruit caliber, average weight, soluble dry substanc

#### ABSTRACT

Cherries are important for the consumers for the moment of appearance, being the first fresh fruit that appear on the market, and for their quality, with important implications in the human health. The current case study presents several aspects related to the influence of the culture area on fruit quality. From the study of several varieties, during a time span of two years, it could be noticed an influence of the culture area and the climatic year on certain fruit quality features. The recorded differences were different both in terms of fruit size, average weight and of the capacity to accumulate soluble dry substance. The same variety had different behaviors in different areas and during different climatic years, which leads to a correct choice regarding the assortment to be used in order to capitalize at maximum the pedo-climatic potential of the culture area.

#### INTRODUCTION

Cherries are important for the consumers for the moment of appearance, being the first fresh fruit that appear on the market, and for their quality, with important implications in the human health (Hoza 2015). The obtained fruit production depends on the used rootstock; generally, small rootstocks lead to a larger production (Edin et al., 1996; Hrotko et al., 2009; Cantin et al., 2010). Fruit quality is influenced by a series of factors among which the following can be named: rootstock used (Jimenez et al., 2004; Cantin at al., 2010), degree of orchard intensity (Lang et al., 1997; Balmer 1998), crown shape used in the plantation (Budan and Gradinariu 2000) and the culture area that ensured conditions more or less proper for cherry fructification (Asănică et al., 2011). Recently, the fruit size has been on the focus and in order to obtain high caliber fruit standardization works are performed related to the fruit load, using chemical substances that determine a poorer bud differentiation (Lenahan et al., 2006) or applying chemical or physical methods to ensure the flowers would be sparse.

#### MATERIAL AND METHODS

The research was conducted between 2015 and 2017 on fruit from several traditional locations for cherry culture: UASVM Bucharest, Istrița Buzău farm, SCDP lași, Moara Domnească farm and two other private farms from Călărași and Vrancea counties. The assortment was not the same; during the first year 17 varieties were analyzed while during the second year 27 varieties were used. Measurements were

made related to fruit size, average weight and content of soluble dry substance. In order to measure fruit size (height, large diameter, small diameter) the calipers was used and for measuring the average weight the precision scale with two decimals was used. Soluble dry substance was measured using the HI 96801 refractometer.

#### **RESULTS AND DISCUSSIONS**

During the first year, the behavior of the trees was very different from one area to another and, in general, the fruit were smaller than the ones from the second year. Average fruit size did not vary for the same area; fruit caliber, expressed through the large diameter, had values below the varieties' potential, the values recorded being similar for the majority of varieties, with several exceptions where the values were larger: Biggareau moreau, Ferm Red and Giant that exceeded 24 mm and Lapins with under 20 mm (table 1).

Table 1

Location	Variety	Height	Large	Small	Average
		(mm)	diameter	diameter	wieaht
		()	(mm)	(mm)	(a)
Moara D.	Stella	20.62	20.44	17.94	5.7
	Biggareau moreau	20.94	24.00	19.96	7.2
SCDP laşi	lva	20.62	22.12	18.66	5.6
-	HC 883515	20.32	22.28	19.06	6.3
	Cociu	19.6	21.9	18.32	5.1
	Ludovic	20.74	22.56	19.34	5.8
	Average	20.47	22.22	18.88	5.70
UASVM	Giant	20.74	22.56	19.34	6.4
Bucharest	New Star	20.86	22.72	20.7	6.0
	Katalin	20.46	22.98	20.38	6.8
	Lapins	19.38	19.66	18.24	5.0
	Kordia	22.14	22.14	18.74	5.8
	Van	19.34	21.1	18.5	5.3
	Average	20.49	21.86	19.32	5.88
Istriţa	Ferm Red	20.94	24.38	19.2	7.2
Buzău	New Star	17.94	20.78	18.18	5.0
	Giant	24.58	26.5	22.32	10.1
	Kordia	20.22	20.54	17.48	4.7
	Early Red	18.32	22.9	19	5.4
	Average	20.40	23.02	19.24	6.48
General average		20.45	22.37	19.14	6.19

Influence of the variety and culture area on fruit size (2015)

Average fruit weight did not follow an hierarchy according to the biological potential, but was influenced by the location, with the maximum value obtained for the variety Giant at Istrița, Buzău County, of more than 10 g. and with the minimum value obtained for the variety Kordia, also at Istrița, of only 4.7 g.

Fruit caliber, as well as weight, is extremely important for capitalization; fruit with diameter larger than 30 mm are capitalized at very good prices. This year, there were no such fruit obtained.

The influence of the culture location was highlighted by the comparison of three varieties: Kordia, New Star and Giant, cultivated in two locations (fig. 1). It can be

noticed that for the fruit obtained within the plantation from UASVM Bucharest the values recorded for fruit size are similar among the three varieties, but for the Istrița plantation the values were very different, the behavior of the trees being strongly influenced by the area.



Fig. 1 Influence of the culture area on the fruit size

During the second year of experiment, several varieties from 5 locations were tested and. as first impression, it resulted that the climatic year was better, the fruit being larger in all centers of origin (table 2).

Fruit height was over 24 mm for 5 of the tested varieties, while the large diameter exceeded 27 mm for some varieties, without having any production standardization works applied in order to determine this caliber. Among the varieties with potential to generate large fruit one can mention Early Red, Summit, Giant and Ferm. Fruit size was influenced by the culture area, without any correlation between them, which means that when establishing the assortment one has to choose among the varieties that best respond to the local conditions. Average weight was good, for many varieties it exceeded 9 g, or even 10 g for three varieties (Grace Star, Summit and Early Red), while the variety Giant produced at Istrita exceeded 12 g, being the highest value recorded during the experiment.

The climatic year influenced the capacity of the varieties to accumulate soluble dry substance. Comparing the values recorded for several varieties during the two years, it could be noticed that there is no unitary influence for all varieties, as they react differently to the climatic conditions of the year. Generally, the values recorded for the first year of culture, when the fruit were smaller, were higher; however, at Istriţa, two varieties produced larger fruit during the second year, by 19.6% for the variety Ferm Red and 5.3% for Kordia (table 3).

The influence of the area on fruit size can be better shown through the comparison between existing varieties in different locations. Thus, for the varieties Giant and Ferovia, the best area for culture proved to be Istrița, Buzău County, for the variety Summit Vrancea area, while for the variety Early Red the best area is Bucharest (UASVM) (fig. 2).

Table 2

Influence of the variet	/ and culture area on	the fruit size (2017)
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	Variety	Height	Large	Small	Average		
Location	,	(mm)	diameter	diameter	weight		
			(mm)	(mm)	(g)		
Moara	Giant	22.27	25.62	20.07	7.51		
D.		22.21	25.05	20.07	7.51		
Călărași	Grace Star	23.90	26.81	26.54	10.10		
	Early Red	24.69	27.28	21.98	9.53		
	Media	24.30	27.04	24.26	9.82		
Vrancea	Ferovia	24.31	24.89	20.61	8.19		
	Summit	24.28	27.53	21.15	10.17		
	Biggareau Burlat	21.66	24.51	19.85	9.53		
	Average	23.42	25.64	20.54	9.30		
	Katalin	23.24	22.98	19.81	6.95		
	Rubin	21.06	23.95	19.80	6.92		
	Lapins	21.56	22.36	19.52	7.34		
	Uster	21.66	21.74 18.43		6.51		
UASVM	Van	20.93	23.27	18.65	6.38		
Bucha-	Ferm Red	22.79	24.06	24.06 19.32			
rest	Early Red	22.51	25.13	20.89	10.70		
	Summit	18.53	18.23	16.08	5.32		
	Giant	20.66	24.11	20.23	7.38		
	Kordia	24.06	23.71	19.24	7.45		
	Skeena	21.51	23.27	19.69	6.75		
	Ferovia	21.20	21.16	17.92	6.28		
	Average	21.64	22.83	19.13	7.29		
	Kordia	23.20	23.58	19.79	7.81		
Istriţa	Van	21.62	25.44	20.39	7.67		
Buzău	Lapins	20.85	23.02	19.29	6.17		
	Ferovia	22.90	25.62	20.70	8.41		
	Giant	24.91	28.91	23.00	12.34		
	Summit	23.20	27.36	22.50	9.74		
	Ferm Red	23.96	27.54	21.21	9.00		
	Early Red	22.36	26.86	22.10	7.54		
	New Star	22.75	25.59	20.81	9.21		
	Average	22.86	25.99	21.09	8.65		
Experiment average		22.90	25.43	21.02	8.51		

The largest difference between the values during the two years were recorded for the variety Giant, of 30.27%.



Fig. 2 Influence of the culture area on fruit weight (g)

Table 3

million of soluble dry substance (%
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Location	Variety	2015	2017	Diffe	erence
				absolute	relative
UASVM	Katalin	15.9	12.84	3.06	19.25
	Lapins	14.3	12.48	1.82	12.73
	Van	17.1	14.82	2.28	13.33
	Kordia	15.5	12.82	2.68	17.29
	Average	15.7	13.24	2.46	15.67
Istrita	Giant	18.3	12.76	5.54	30.27
	Ferm Red	15.1	18.06	-2.96	-19.60
	Early Red	15.0	13.44	1.56	10.40
	New Star	16.6	15.77	0.83	5.00
	Kordia	14.7	15.48	-0.78	-5.31
	Average	15.94	15.10	0.838	5.26

#### CONCLUSIONS

From the present study, the following conclusions can be drawn: the varieties have reacted differently from one year to another and from one location to another, depending on their biological features; the climatic year influenced the fruit size and content of soluble dry substance; establishing the assortment has to be done according to the manner in which the variety responds to the climatic conditions of a certain area, in order to obtain high quality and superior capitalization possibilities.

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## ANNALS OF THE UNIVERSITY OF CRAIOVA

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#### OPTIMIZATION OF ECOLOGICAL FACTORS AND THE INFLUENCE OF LIGHT ON THE RATIONAL USE OF RESERVE CARBOHYDRATES IN PERIOD DURING FORCING CUTTINGS

Bădulescu Adriana<sup>1</sup>, Ficiu Lidia<sup>2</sup>

<sup>1</sup> National Research & Development Insitute for Biotechnology in Horticulture Stefanesti, bădulescuadriana18@yahoo.com
<sup>2</sup> Institute of Research & Development for Viticulture and Vinification Valea Călugărească, ficiulidia@yahoo.com

Key words: grafted cuttings, ecological factors, carbohydrates

#### ABSTRACT

In most countries of the world, where viticulture has millenary traditions but not only, diseases caused by phytoplasms, viruses, viruses, bacteria and pathogenic fungi cause irreparable damage to vineyards. The production of viticulture propagating material of higher biological categories represents the peak area of knowledge regarding the vegetative multiplication of the vine. This involves addressing new techniques of genetic selection (in the framework of a technically acceptable protocol for officially accepted clonal selection), phytosanitary status verification by complementary methods (technical protocol for phytosanitary selection), the use of rapid multiplication techniques (especially in vitro biotechnologies) and the conservation of the vine propagation material (biological maintenance) in the greenhouse, the classification of the activities in the certification schemes and the unanimously accepted classification schemes.

#### INTRODUCTION

The new varieties and valuable clones as well as the rootstock cuttings used in the experiments were derived from the nucleus of the isolate, which is the national collection of viticulture germplasm made of deviated material by heat therapy and in vitro culture (Tita and al., 2004). The biological category of the material used for grafting was the initial propagation material. The choice of SO4-4 rootstock was primarily due to its superior behavior on a variety of soil types and, secondly, to the great vigor of growth it imparts to grafting varieties (Tita I. and al., 2001; Corbean.D. and al., 2009). However, it is a difficult rootstock in the vine school in the sense that it roots harder, and the triggering of calogenesis at the grafting point is delayed by 1-2 days compared to other rootstocks (Tita I., 2004). The technological improvement aimed at experimentation was the reduction of carbonate consumption from the biological reserve of cuttings grafted during the forcing period and after the greening of the forced cuttings (Hamdan A-J.S. si colab., 2010).

#### MATERIALS AND METHODS

The preparation of the grafting material was done using the classic technology. The grafting was performed mechanically, with DPA 5, which cuts the inverted U-shaped cuttings, and the stratification was made in resinous sawdust, in forcing boxes having the frame and the metal bottom, and the walls of plastic foil.

The special complexity of the issues pursued necessarily implied a series of experiments, on technological phases, all with a view to improving graft growth and stimulating early organogenesis in order to reduce the consumption of carbohydrates in the rootstock cuttings reserve.

The study of the quality of the grapevine material grafted during the most critical period for symbionts was carried out in the following experimental variants:

 $V_1$  - witness, forcing into unlighted rooms

V<sub>2</sub> - forcing into artificially lighted rooms

V<sub>3</sub> - forwarding in non-illuminated rooms and forcing in natural light

#### **RESULTS AND DISCUSSIONS**

The experiments during the grafted period consisted of:

- determination of the physiological state of grafted cuttings (total water, soluble carbohydrates and starch);

- protection against dehydration, oxidation of sections and pathogenic infection of the grafting point.

These determinations were followed by complete rehydration of graft and rootstock cuttings, initial amounts of soluble carbohydrates in carbohydrates and starch, which were the starting points in the assessment of consumption during the period of forcing grafted cuttings.

The experiments during the forcing of the grafted cuttings followed:

- optimal control and management of environmental factors;

- reduction of electric and thermal energy consumption;
- early and concurrent activation of bumper partners' bindings;
- stimulation of early coughing and early vascularization of the grafting point;

- stimulation of chlorophyll synthesis in young shoots of graft shoots;

- the influence of natural light on reducing the consumption of carbonated carbohydrates and on the endogenous production of auxins involved in both the vascularization of the grafting point callus and the early rhizogenesis decalcification.

The evolution of carbon hydrate consumption was carried out by repeated determinations at the beginning of the vegetation phases specific to forcing grafted cuttings (debulking, beginning of callus formation, beginning of shoots, raising of shoots, development of callus and beginning of vascularization of grafting area) (Bartoloni G., and al., 1996).

For the correct observation of the phenophases of growth and growth of the shoots in the buds, the top of the grafted cuttings (the grafting point and the grafts) was not covered with sawdust as can be seen in figure 1.





Fig. 1. Forcing cut grafted cuttings into metal casting forcing rooms and hot glass tables in greenhouse

In rooms with or without artificial illumination, the baskets are placed on the rasters in a single row. Natural light penetrates only through the windows and only affects the baskets in their immediate vicinity. For the rest of the baskets artificial lighting is needed.

Early chlorophyll assimilation is more important for the subsequent development of vines than root nutrition during the same period. The intensity of photosynthesis is closely correlated with the intensity of the forcing spaces and the temperature level. The highest values of photosynthesis occur at 30 ° C and 12,000 lbs.

Determinations on the amounts of carbonated carbohydrates at 10 days, 16 days, 32 days after grafting show that this technology does not consume more than 57% of the initial reserve so that a large enough amount remains to repair the organs removed at the time classification of forced cutters (Tita I. and al., 2001, 2002, 2003).

In the 7 days of exposure to natural light, the assimilating pigments were synthesized as follows:

- chlorophyll A - from 0.867 to 1.475 mg / g of dry matter representing an increase of 58.78%;

- chlorophyll B - from 0,153 to 0,365 mg / g of dry matter, representing an increase of 41,92%;

- carotenoid pigments - from 0.383 to 1.020 mg / g of dry substance representing an increase of 37.55%, as shown in Figure 2



Fig. 2. Accumulation of assimilable pigments in light shoots

Analyzing the consumption of carbohydrates, from the removal of the cuttings from the storehouse to the end of the forcing into light, there are periods when the total carbohydrate consumptions are minimal and periods of intense respiratory and organogenic processes resulting in maximum carbohydrate consumption cutlery reserve.

Table 1

Force	Content in		Total H of C	Available	Number of		
technology	Carbohydr	ates at	consumption	carbonates in	good		
	the End of Force		the End of Force		(%)	cuttings (%)	cuttings to
	Total	Starch			plant in the		
	glucosides				field		
	-				(%)		
Classic	7,2	0,8	73,6	26,4	72		
With artificial							
lighting	7,6	1,2	52,8	47,2	76		
With natural							
light on hot	8,5	2,1	47,8	52,2	83		
meals							

Content in carbohydrates at the end of forcing by the three foil techniques

Thus, during the pre-harvesting period (8-10 days after grafting), the total carbohydrate drops from 11.7 g to 9.8 g, resulting in a consumption of about 2 g / 100 g dry material, and during the pre-emergence period in natural light (leaves and green shoots), the consumption is only 0.1 g within 4 days.

In these two periods the consumption is minimal, in the first part only the processes of calogenesis and deforestation are carried out, and in the second part, the leaves being intensely chlorophylised, the photosynthesis process reduces the consumption of the symbionts reserve.

The consumption of carbohydrates is influenced by exposure to light.

Thus, from the grafting to the greening of the shoots (14 days) the consumption of carbonated hydrates is 5.1 g, and after the greening of the leaves and until the planting in the vine school (15 days) the consumption is only 1.1 g although the energy needs are maximum in part II of the force.

Percentage, at the end of forcing cuttings, by driving environmental and technological factors, significantly reduces the consumption of carbohydrates: 56.4% of carbohydrates are consumed in the first part of the forcing and only 8.6% in part II; thus prior to planting in the vineyard school in the rootstock cuttings remain 52.2% of the initial carbonate content, which represents a great advantage for the restoration of the vines after planting in the school and for the subsequent development of the grafted vines (Figure 3)

#### CONCLUSIONS

By making a comparison between the cutter force technologies in the three experimental variants, the classic V<sub>1</sub> (without illumination), the improved V<sub>2</sub> and the V3 experienced at INCDBH Ştefăneşti (8-10 days darkness and forced natural light in greenhouses, on tables warm) we can see the following:

► the highest total carbohydrate content (8.5 g / 100 g of dry material) was recorded in cuttings forced into natural light by the technique used at INCDBH Ştefăneşti;

► the minimum carbonate consumption (47.8% of the initial content) was recorded in the same experimental variant, which provides the highest content in carbohydrates (52.2%), available in rootstocks for organ recovery (roots and shoots) destroyed during planting in the vineyard school



Fig. 3. Evolution of Carbohydrate Consumption during Forcing on Merlot 8 VI / SO4-4.

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# FAVORABILITY OF CLIMATIC FACTORS FROM SANDY AREAS (OLTENIA, ROMANIA) REGARDING THE WALNUT CULTURE

Birsanu Ionescu Mariana<sup>1,\*</sup>, Cosmulescu Sina Niculina<sup>2</sup>

<sup>1\*</sup>University of Craiova, Horticulture Faculty, Doctoral School of Plant and Animal Resources Engineering, Romania; e-mail: mary.yonescu@yahoo.com

<sup>2</sup>University of Craiova, Horticulture Faculty, Department of Horticulture & Food Science, A.I.Cuza Street, 13, Craiova, Romania; e-mail: sinacosmulescu@hotmail.com

Key words: walnut, climatic factors, sandy areas, favorability

#### SUMMARY

Walnut grows in a varied areal, but there are few areas where it yields high and constant production, which demostrates that, besides its hereditary attributes, the ecological factors play an important role on quantity and quality of walnut production. This study summarizes the favorability of climatic factors on walnut culture; factors existing in sandy area of the left bank of Jiu River. In this respect, there were used data registered during the last 55 years (1961-2016) and statistically processed. The area that represents the aim of this study has the following attributes: the average annual temperature of the analyzed period was  $11.51^{\circ}$ C, the average temperature during the hottest month was  $27.40^{\circ}$ C (registered in July 2012), the average temperature during the coldest month was  $-7.58^{\circ}$ C (registered in January 1985), the average temperature during the coldest month (January) was  $-1.42^{\circ}$ C, while the hottest one (July) of the analyzed period was  $23.38^{\circ}$ C; the amount of annual rainfall was 514.47 mm and the average value of aridity index was 54.99 during the same period. The sandy area on the left bank of Jiu River, situated in the South-West of Oltenia Region, in Romania, corresponds mostly to ecological requirements of walnut, which determined a high diversity regarding this tree species in the area. During some passed years, climatic factors have not favoured the growth and fructification of the tree.

#### INTRODUCTION

Despite the fact that walnut grows in a varied areal, there are few areas where it yields high volume and constant production. This fact demostrates that, besides its hereditary attributes, ecological factors play an important role on quantity and quality of walnut production. For the fruit trees to be successful on sandy soils, climatic factors and soil have the highest influence. Environmental factors have a direct influence on plants, that is their growth and development depends on climatic characteristics (Cosmulescu & Gruia 2016). Plants need a specific index of warmth during their entire period of growth, in order to develop their vital processes: breathing, sweating, photosynthesis, water absorption. Temperature exerts conditions on evolution of assimilation, breathing and sweating processes, the going through different phases of growth and fruit setting, the latent life of trees during winter rest etc. Temperature is also very important during flowering period (Cosmulescu et al. 2010a,b). For the success of fruit tree farming in Southern area of Oltenia region, rainfall is also extremely important as far as the amount is concerned, as well as the period of the year when it occurs. Prentovic et al. (2014) showed that walnut production depends

mostly on weather conditions, especially during fertilization and fruit growth, but it also depends on quantity of pollen dispersed in the air. A study by Lee & Sumner (2016) regarding the impact of climatic changes on walnut culture showed that it is one of the most vulnerable and the projections indicated that some species' production can be affected during the years with unusual warm winters. Luedeling & Gassner (2012) show that high temperature influenced the blooming of walnut and the appearance of leaves. Autumn frosts could affect annual growth, consequently with the loss of wood guality and causing even the death of the tree in the end (Guàrdia et al. 2013). There is a considerable uncertainty as far as the magnitude of potential impact of climate change on walnuts is concerned. Walnut could be negatively affected by climate changes due to its disadvantageous sensitivity to drought, and frost damage manifested in the areas where the tree grows today, taking into account the predicted temperature increase and extreme weather phenomena (Gauthier & Jacobs 2011). Xiao et al. (2013) show that walnut has great requirements regarding temperature and humidity indices. The predominant climatic factors which affected the growth and development of walnut in the Sichuan Province were: average annual temperature, cumulated temperature of  $\geq$ 10°C, low annual temperature, the annual sunlight, annual rainfall, average temperature in January and July. Han et al. (2012) conducted a comprehensive analysis of climate and soil factors, which combines climatic conditions and biological characteristics of walnut. The appropriate ecological indices for walnut plantations were summarized as follows: 400~2800 m altitude, 5.5~8.5 value of soil pH, ≥60 cm soil thickness, ≥10°C optimal temperature, 3000~6500°C sum of annual temperatures,  $9 \sim 18^{\circ}$ C annual temperature,  $\geq -15^{\circ}$ C extreme annual temperature, 400~1200 mm annual quantity of precipitation, 300 hours/year sunshine duration, ≥-1°C average temperature in January, ≤28°C average temperature in July.

This study aims to analyze the favorability of sands on the left bank of Jiu River (in Oltenia, Romania) for walnut culture.

#### MATERIAL AND METHOD

**Material.** The weather data recorded by the Romanian National Meteorological Institute for Meteorological Station in Bechet were used for the analysis of favorability of climate factors regarding walnut culture in the sandy areas on the left of Jiu river. Data recorded and processed during 55 years, 1961-2016, were used. Two time periods were used for analysis and comparison: 1961-2013 and 2007-2016.

**Method.** Climate data from the Meteorological Station in Bechet were used to calculate ecoclimatic indices for light, temperature and rainfall. Favorability of climatic resources in the sandy area on the left side of Jiu River for walnut culture was evaluated. In order to estimate the evolution trend of climatic parameters the regression analysis was used, the quantification of causal relations being processed by linear equations. To analyze the space-time variability of meteorological parameters, the standard deviation was used and the correlation analysis was used to measure the intensity of the link between the variables.

#### **RESULTS AND DISCUSSIONS**

Generally, ecometric indices are numerical aggregates that provide information on characteristics of a bioclimatic phenomenon at a given location (Linster 2003) and they are methods of analysis used to highlight the relationship between climatic favorability or restrictiveness of a territory and its biotic envelope.

**Temperature factor analysis.** The average annual temperatures in Bechet area between 1961 and 2013 varied between 10.26°C (1969) and 13.07°C (2007) with fairly large differences from one year to the next. According to previous studies,

walnuts grow and develop in climatic zones where average annual temperatures are between 9 and 10°C. Xiao et al. (2013) consider that temperature, including the average temperature in January, the average temperature in July, the average annual temperature and the sum of temperatures  $\geq$ 10°C, had a major impact on walnut distribution in Yunnan Province (China). Thus, temperature is the primary dominant factor. Related to the average annual temperature, Bechet area is suitable for walnut growth (Ionescu Birsanu & Cosmulescu 2016).

According to climatic data of the last 10 years (2007-2016), there is a tendency to increase the average annual temperature, following the model of a straight line whose equation is  $y = 0.459 \times +9.403$  (Figure 2). Between 1961 and 2013, the temperature increased steadily, the trend being linear and the confidence interval  $\pm 0.4^{\circ}$ C (Figure 1). The variation limits for the average annual temperature of the reference period (1961-2013) were between 10.26°C (1969) and 13.07°C (2007).



Figure 1. Evolution of air temperature (annual averages) over the period 1961-2013



Figure 2. Evolution of air temperature (annual averages) for the period 2007-2016

Comparing the last 10 years (2007-2016) with the period 1961-2013, one can notice that the lower limit for the average annual temperature increased from 10.26°C (1969) to 11.40°C (2010) and the upper limit was maintained (13.07°C). In comparing the average annual temperature for the period 2007-2016 and the average temperature between 1961 and 2013, the differences vary between 0.19°C (2010-2014) and 1.6°C (2007), the annual averages of the period 2007-2016 exceeding the average of the reference range. 2011 represents an exception of these measurements. Comparing the average monthly temperatures of the period 1961-2013 with the 2007-2016 period, there is an increase of 0.08°C (in May), 1.36°C (in August) and 2.25°C (in February), while for the average annual growth the rate is 0.73°C (Table 1). The results obtained at the temperature factor analysis in the sandy area on the left side of Jiu River, the South-West Oltenia Region reveal the tendency of temperature rise over the last years. This growth trend is also supported by Ionita et al. (2015) for the analysis of summer temperatures in Romania, Della Marta et al. (2007) for the summer waves in Western Europe, Cosmulescu et al. (2010, 2015) and by Busuioc et al. (2015). Globally, Alexander et al. (2006) report significant changes in the extreme temperatures associated with the heating trend, especially those related to the daily minimum temperatures during the period 1951-2003 and a generalized and significant increase in the amount of rainfall. Overall findings of Alexander et al. (2006) are not representative for Europe if the entire 20<sup>th</sup> century is taken into account, reporting a small difference between the average daily and daily temperature trends (Moberg et al. 2006).

Table 1

Average monthly temperatures (°C)						Average							
Period									(°C)				
	I	II		IV	V	VI	VII	VIII	IX	Х	XI	XII	(°C)
1961-2013	1.46	0.7	5.94	12.28	17.8	21.4	23.36	22.56	17.8	11.5	5.5	0.3	11.47
2007-2016	-0.59	2.95	7.12	13.01	17.8	22.0	24.44	23.92	18.69	11.2	6.41	1.44	12.2
Differences	-2.05	2.25	1.18	0.73	0.08	0.62	1.08	1.36	0.89	-0.2	0.91	1.14	0.73

Monthly and annual average temperature during 1961-2013 and 2007-2016

Klein Tank & Konnen (2003) reported similar results between 1946 and 1999, respectively a small difference between the average daily and minimum daily temperature trends. In the Mediterranean Basin, extreme temperatures are in line with global trends: the minimum temperature decreases and the maximum increases (Efthymiadis et al. 2011). For walnut culture, Xiao et al. (2013) reported the following optimal temperatures in Yunnan: 9.19°C average temperature in January, with variations between 6.23-12.16°C, 21.42°C July average temperature, with variations between 19.08-23.76°C and average annual temperatures of 16.36°C. Solar et al. (2008) determined how winter and spring temperature could affect the walnut (*Juglans regia* L.). Phenological models showed that the average air temperature in the two months (March and April) was important for the estimation of deforestation.

**Rainfall analysis.** From the analysis of data on the amount of rainfall recorded in Bechet area during period 1961-2013 it is observed that the highest values of annual average are registered in 2005, with an average value of 811.83 mm. The lowest rainfall were recorded in 2000 (288.24mm), considered to be dry in terms of rainfall. The multi-annual average (1961-2013) of rainfall in Bechet area is 516.92 mm, being below the lower limit of walnut need (minimum 600 mm annual rainfall). During the last 10 years (2007-2016) annual average values ranged between 349 mm (2016) and 686 mm (2010). Also, the multiannual rainfall average in this area over the last 10 years (508.73 mm) is below the lower limit of walnut need. Botu et al. (2014) investigated the horticultural performance of 10 native varieties and eight varieties of walnuts introduced from other countries cultivated in the Northern region of Oltenia. It was found that the environment contributed with 24.44 and 29.1%, respectively, and the G x E interaction with 12.22 and 14.6%. For a synthetic image of the space-time variability of rainfall variance, we calculated the deviations of averages of annual rainfall quantities registered during 2007-2016 compared to the annual rainfall averages in the reference period 1961-2013. In Table 2, one can see that positive values of deviations of annual rainfall quantities were recorded in 2007 (20.97 mm), 2009 (91.52 mm), 2010 (169.59 mm), 2014 (100.89 mm) and negative values in most of the years: 2008 (-72.05 mm), 2011 (-139.84 mm), 2012 (76.66 mm), 2013 (818.82 mm), 2015 (69.99 mm), 2016 (167.54 mm).

Lately there have been drought problems in many agricultural areas. According to the National Meteorological Agency (NMA) in the high risk area of drought and desertification, the climate is warm and dry, with annual average temperatures above 10°C, and the average annual rainfall is between 350-550 mm, of which the amount between 200-350 mm is during vegetation period (April to October).

#### Table2

	Sum of annual average	Sum of average rainfall from	Difference
Year	rainfall (mm)	1961 to 2013 (mm)	(mm)
2007	537.55	516.58	20.97
2008	444.53		-72.05
2009	608.1		91.52
2010	686.17		169.59
2011	376.74		-139.84
2012	439.92		-76.66
2013	497.76		-18.82
2014	617.47		100.89
2015	446.59		-69.99
2016	349.04		-167.54

Difference between annual average rainfall and average rainfall from 1961 to 2013

Based on these criteria, an analysis of Bechet area was made on the risk of drought manifestation. Annual average temperatures exceed 10°C. For rainfall, the sum of annual rainfall was also calculated from April to October. From this point of view, the years 1983, 2002, 2007 and 2016 were years of high risk of drought, the sum of rainfall being below 350 mm, and the average annual temperatures of 11.57°C, 12.72°C, 13.07°C and 12.16°C, respectively. A parameter that expresses the correlation between climate, vegetation and the waters of a territory is considered aridity index, established by Martonne. It expresses the relationship between multiannual rainfall and multiannual average temperature. This index makes it possible to determine the aridity of a region for characteristic periods (a year or a month), being an expression of the restrictive nature that climate conditions impose on certain plant formations (Satmari 2010). The analysis of aridity indices by Martonne and UNEP reveals the obvious tendency of aridization in S-V Oltenia over the last three decades, and since 1980 a semiarid climate has been established in the area, with the aim of escalating aridisation (Pravalie et al. 2013). According to climatization of the region, according to the provisions of Balteanu et al. (2013) Southern Oltenia is characterized by a temperate continental climate of transition with Mediterranean influences. The average annual temperature is 10°C (over 11°C in the Danube Meadow), precipitation ranging from 500-600 mm / year, air humidity around 78-80%, specific climatic characteristics for the sandy, southern plains, its individuality being due to aridity and drought phenomena. The annual average evapotranspiration potential is over 700 mm/year (Sandu et al. 2008; Dragota et al. 2011). The space distribution of the values of Martonne aridity index on the territory of Romania has been the object of several researches (Dumitrascu 2006; Paltineanu et al. 2007a,b; Pravalie 2014). These researches show that the lowest (extreme) values, below 20, are in eastern Dobrogea below 25 in the Danube (Oltenia), in the East of the Romanian Plain and West Dobrogea; also in the Plain of Oltenia and the Center of the Romanian Plain the values are between 25-30. For plant culture, the amount of rainfall in the growing season is important. The lack of rainfall from a certain period can not be compensated for by surplus rainfall in another period. Between 1961 and 2016, in Bechet area there were months of very low, insignificant rainfall: 0.14 (October 1969), 0.25 (January 1989) or months with rainfall over the multiannual average of the month (August 2005 -200.19mm, October 2007 - 106.99 mm, September 2014 - 111.50 mm). Correcting with the literature and the requirements of the fruit tree species, it is found that the area is suitable for crops with low requirements (at least 500 mm annual rainfall, peach, apricot, almond), but also to medium-sized species (minimum 600 mm annual rainfall: pear- tree, walnut, cherry, cherry, summer apple varieties). Average rainfall of 700 mm or above is exceptional (year 2005-811.83), so it is necessary to irrigate the crops of

large species of fruit (quince, late varieties of apple, plum) with high and very high need for water (fruit shrubs). Walnut requires areas with average annual rainfall of at least 600 mm. The data analysis shows that in the Bechet area, only 10 of the 55 calendar years analyzed correspond to walnut requirements. Throughout the vegetation period there are phenophases in fruit growing which require an appropriate level of humidity. One of these is the growing period of shoots and/or the fruit, when the requirement of the trees for water are high. The period of intense growth of shoots and fruits is May - July. For a good development of these phenophases, we are interested in the amount of rainfall during this period. The analysis of the average precipitation in May - July, in the last 55 years (1961-2016) shows great variability, from 22.24 mm (1985) to 108.63 mm (1970), and in the last 10 years from 28.19mm (2007) to 89.19mm (2010). The average rainfall for May in the first and second interval of the month is 57.19mm, respectively, 64.27 mm, for June 61.55mm, respectively 71.43 mm, and for July 52.85mm, respectively, 46.45mm. The amount of rainfall from May to July differs from one year to the next, the variation limits in the first interval (1961-2013) being as follows: between 11.2mm (2000) and 111.01mm in May (1967); between 9.57mm (2003) and 126.66mm in June (1966); between 6.35 (1965) and 165.56mm in July (1970). In the second range (2007-2016), the variation limits are: between 32.80 mm (2008) and 107.14 mm in May (2010); between 25.82 mm (2007) and 117.35 mm in June (2010); between 10.11 (2007) and 102.09 mm in July (2009).

It is necessary to analyze whether rainfall was useful for growing and developing trees. Useful rain is considered to be over 10 mm. To ensure a sufficient wetting of soil with balanced hydrophobic characteristics, the amount of rainfall (in mm) of each month should be three times more than the average monthly temperature (Mihaescu, 1998). In order to highlight whether the rainfall was useful for the growth of shoots and fruits, the ratio between the annual average rainfall and the average annual temperature for the period 1961-2013 and May-July for Bechet area was calculated. According to the data obtained, not all the rainfall during the analyzed months and years were useful. Values smaller than 3, for the three analyzed months, were obtained in 1985 and 2000, being the years with the lowest aridity index (46.20 and 32.66 respectively). For other years, the variation limits for this report are very high: from 1.38 to 6.28 in 1961; from 3.70 to 7.37 in 1970; from 1.23 to 6.90 in 1980; from 2.92 to 7.24 in 1991; from 1.88 to 7.71 in 2004 etc. Of total values obtained after studies, for the 55 years, from May to July, about 34% situations have a value greater than 3, which means that only 34% of the analyzed cases have achieved sufficient wetting for the growth and development of fruit trees. Analyzing the values obtained for each month we can conclude the following: about 49% of the values obtained in May are over 3, about 40% in June and about 24% in July.

It is noted that irrigation is recommended for good growth and development of plants due to the fact that the unevenly distributed rainfall during the year does not provide the necessary moisture for certain vegetation phenophases. In order to mitigate the impact of climate change and to prevent land degradation, reconstruction of the forest protection belts created between 1970-1980 is a necessity as far as they are no longer able to protect the sandy lands (Achim et al. 2012).

#### CONCLUSIONS

The data from the recent studies reffering to the average temperature increase during the last years confirmed a value of 12.20°C (2007-2016) compared to 11.49°C between 1961 and 2013. Temperatures above 35°C (which cause thermal stress) were recorded each year in July-August between 2007 and 2016, with the exception of 2010 and 2014, the absolute maximum temperature (44.08°C) being reached in July 2007.

Temperatures below zero degrees were recorded in: April 1970 (-2.44°C), 1974 (-2.00°C), 1993 (-1.34°C), 1997 (-2.53°C), 2000 (-1.57°C) 2001 (-1.18°C), 2002 (-2.73°C), 2003 (-1.71°C), 2011 (-1.48°C), 2012 (-2.06°C) after starting in vegetation.

The multiannual average rainfall in the Bechet area is 514.47 mm, a value which is below the walnut limit (minimum 600 mm annual precipitation). In conclusion, the sandy area on the left side of the Jiu River corresponds largely with the ecological requirements of the walnut, which made the diversity of walnuts in this area to be quite large. Nevertheless, sometimes climatic factors are unfavorable to growth and fructification.

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#### EXTRACTS OF HERBS AND SPICES AS NATURAL ANTIOXIDANTS FOR IMPROVING THE FUNCTIONAL VALUE OF MEAT PRODUCTS

Boruzi Andrei Iulian<sup>1</sup>, Nour Violeta<sup>2\*</sup>

<sup>1</sup> "Dunarea de Jos" University of Galati, Romania <sup>2</sup> University of Craiova, Romania \*Corresponding author e-mail: vionor@yahoo.com

Key words: functional foods, extracts, oils, powders, herbs and spices, natural antioxidants

#### ABSTRACT

Lipid oxidation is one of the causes of deterioration in meat and meat preparations as it is accompanied by the appearance of a large number of undesirable changes in flavor, texture and nutritional value. The rate of lipid oxidation can be effectively reduced by the use of antioxidants. Synthetic antioxidants have been widely used in the meat industry, but consumer concerns about product safety and toxicity have prompted the food industry to look for natural sources. Natural antioxidants such as extracts of rosemary, oregano, sage, green tea, cloves, garlic, citrus peel and seeds, lotus, barley and Moringa oleiferia leaves can be used as alternatives to synthetic antioxidants because of their equivalent or even higher effect on inhibition of lipid oxidation.

#### INTRODUCTION

Consumers nowadays attach greater importance to all aspects that can help improve the quality of life; and diet, though not the only element that influences wellbeing and health, is one of the most important. The factors that favored this evolution include the current high impact on public opinion of media reports on the relationship between diet and health, increasing the life expectancy of the population (this generates high purchasing power consumers with higher health problems and are very motivated to participate in initiatives aimed at maintaining health), an attentive attention to disease prevention, etc.

Lipid oxidation is the major reaction that affects the flavor, color, texture and nutritional value of food (Kanner, 1994). Various synthetic antioxidants such as butyl hydroxytoluene (BHT), butylhydroxyanisole (BHA) and tert-butylhydroquinone have been used to prevent oxidative damage to food. However, synthetic antioxidants are not fully accepted by consumers because of health concerns. Therefore, some natural ingredients, including herbs and spices, have been studied, especially in Asian countries, as potential antioxidant products in meat and meat products (McCarthy et al., 2001).

Herbs and spices contain many phytochemicals that are potential sources of natural antioxidants including phenolic diterpenes, flavonoids, tannins and phenolic acids (Dawidowicz et al., 2006). These compounds have antioxidant, anti-inflammatory and anti-cancer activities. In food systems, they can improve the flavor, delay degradation of foods induced by lipid oxidation, inhibit the growth of microorganisms,

and play roles in reducing the risk of certain diseases (Achinewhu et al., 1995, Tanabe et al., 2002).

Among these spices, it is said that cloves have the strongest antioxidant capacity, followed by rose petals, cinnamon, nutmeg and other spices (AI-Jalay et al., 1987). In addition, spices have an antimicrobial capacity mainly due to phenolic compounds. Possible mechanisms for the antimicrobial effect of phenolic compounds include: modification of microbial cell permeability (Bajpai et al., 2008) interfering with membrane function, including electron transport, absorption of nutrients, protein and nucleic acid synthesis, and enzymatic activity (Bajpai et al. 2008), the interaction with membrane proteins that cause deformation in structure and functionality (Rico-Munoz et al., 1987) and substitution of alkyls in the phenol nucleus (Dorman & Deans, 2000) (Zhang et al., 2010).

#### **Rosemary extracts**

The rosemary extract contains high levels of phenolic compounds that lead to high antioxidant activity. Phenolic compounds are capable of regenerating endogenous tocopherol in the phospholipid bipolar lipoprotein (Rice-Evans et al., 1996). Sebranek et al. (2004) reported that extracts of rosemary added to pork sausages at 2500 ppm were the same or even more effective than BHA / BHT in delaying TBARS values in raw and chilled sausages during refrigeration and freezing. In addition, the addition of rosemary extracts has improved the color and freshness of pork sausages (Sebranek et al., 2004). Yu et al. (2002) added water-soluble extracts of rosemary in cooked turkey products and found they were effective in slowing lipid oxidation and preventing color loss evidenced by L-value decrease and increase in a\* during refrigeration. In the case of pork, the combination of oleoresins from rosemary with tocopherol effectively reduced volatile hexane without inducing any effect on the production of sulfur volatiles (Nam et al., 2006). Rosemary extracts have led to better color retention, evidenced by decreased metmyoglobin concentration and increased oxymyoglobin values on storage for 8 days in beef minced meat (Zhang et al., 2010).

#### Green tea

Catechins represent a predominant group of polyphenols present in green tea leaves, composed of four epicatechin compounds, epicatechin gallate, epigallocatechin and epigallocatechin gallate (Zhong et al., 2009). These tea compounds promote health by preventing lipid oxidation and providing antibacterial, anticancer and antiviral capabilities (Katiyar & Mukhtar, 1996; Yang et al., 2000). Tea catechins are known to reduce peroxide formation even more effectively than alpha-tocopherol and BHA in chicken and pig fat (Chen et al., 1998). Tea polyphenols may inhibit the formation of mutagenic agents known to be associated with breast and colon cancer (Weisburger et al., 2002).

Adding 300 ppm catechins from tea significantly reduced the TBARS values for beef, duck, ostrich, pork and chicken during refrigeration for 10 days. At the same concentration, tea catechins provided an antioxidant capacity of two to four times higher than alpha-tocopherol, depending on the meat of different animal species (Tang et al., 2001). Green tea extract reduced TBARS formation and the concentration of putrescine and tyramine in dried fermented sausages. The addition of green tea, however, had no significant effect on the pH, color, and overall sensory quality of sausages (Bozkurt, 2006). In the case of pork sausages, green tea powder could partially replace the nitrite, resulting in a lower TBARS value and a low volatile basic nitrogen content compared with samples prepared with nitrite only (Choi et al., 2003).

#### Cloves

Cloves (*Eugenia caryophyllus*) are known to have long-lasting antimicrobial activity due to their active ingredient - eugenol (Cort, 1974). Cloves oil at 0.5% and 1% inhibited the growth of *L. monocytogenes* in minced sheep meat. At 1%, the number of *L. monocytogenes* decreased by 1-3 log cfu/g in sheep meat (Menon & Garg, 2001). In ready-made chicken sausages, clove oil at 1% and 2% inhibited growth of *L. monocytogenes* during storage at 5 °C and 15 °C (Mytle et al., 2006). Clay oil was also effective in inhibiting other food-borne pathogens including *C. jejuni, S. enteritidis, Escherichia coli* and *Staphylococcus aureus* (Smith-Palmer et al., 1998). Cloves were able to prevent discoloration of raw pork during storage at room temperature and were the most potent antioxidant in the lipid oxidation delay of all spices and herbal extracts used, including cinnamon, oregano, pomegranate, and grape seed (Shan et al., 2009). In another study, adding cloves oil in combination with lactic acid or vitamin C could reduce lipid oxidation, maintain color and improve the sensory properties of buffalo meat during store sales (Naveena et al., 2006).

#### Garlic

Allicin is known as the main ingredient of garlic having antimicrobial activity against gram-positive and gram-negative bacteria. Allicin is produced enzymatically from its algin precursor through the intermediate product of allylsulfenic acid (Ellmore & Feldberg, 1994). Many studies have shown that garlic extract has been effective in reducing the growth of many pathogens including *S. aureus, S. albus, S. typhi, E. coli, L. monocytogenes, A. niger, Acari parasitus, Pseudomonas aeruginosa* and *Proteus morganni* (Kumar & Berwal, 1998; Maidment et al., 1999).

In refrigerated chicken meat, aqueous garlic extract inhibited the growth of microbial contaminants, including aerobic, mesophilic and feces coliforms on the surface of poultry carcasses (Oliveira et al., 2005). The addition of 1% and 3% garlic juice could lead to a decrease in the value of peroxide, TBARS, residual nitrite and total number of microorganisms in sausages during cold storage compared to the control group (Park & Kim, 2009).

#### Sage

Sage is commonly used in pork sausages. The main antioxidant compounds in sage include carnosol, carnosic acid, rosmadial, rosmanol, epirosmanol and methyl carnosate (Cuvelier et al., 1994). The addition of essential sage oil (3%) resulted in a decrease in TBARS in both raw and cooked pork meat by 75% and 86% respectively, whereas in raw beef and veal they fell by 57% and 62% % compared to the control (Fasseas et al, 2008). The sage extract, alone or in combination with sodium isoascorbate, reduced water and pH activity, reduced the number of mesophilic bacteria and coliforms in vacuum packed turkey meat but had a better taste in cooked sauces (Karpinska-Tymoszczyk, 2007). In high-pressure processed chicken, *Salvia* protected chicken breast from lipid oxidation during refrigerated storage for 2 weeks (Mariutti et al., 2008).

#### Oregano

Oregano is a traditional Mediterranean spice, and the essential oil of oregano obtained from the steam distillation process contains more than 30 compounds. Among the compounds, carvacrol and thymol contribute most to its antioxidant capacity (Vekiari et al., 1993). Pig meat and beef to which 3% essential oil of oregano was added showed lower levels of oxidation after 12 days of refrigerated storage (Fasseas et al., 2008).

Oregano oil could prolong the shelf life of fresh chicken meat by reducing the growth of microorganisms during storage in refrigeration. However, 1% of oregano oil could introduce a very strong unfavorable flavor to food, which would lead to low sensory quality (Burt, 2004, Chouliara et al., 2007). The oregano essential oil (0.05%, 0.5% and 1%) could delay the development of microorganisms and decrease the final number of alteration microorganisms under modified atmosphere conditions (Skandamis & Nychas, 2001; Zhang et al. 2010).

#### Lotus and barley leaves

Lotus (*Nelumbo nucifera*) is widely cultivated in Asia and grows in water (Kim & Park, 2008). It contains abundant dietary fiber, vitamins, riboflavin, potassium and copper (Chiang & Luo, 2007). It also contains antioxidant compounds such as ascorbic acid and phenolic compounds, carotenoids, flavonoids, phenolic acids, and tocopherols (Hertog, 1994). The free-radical scavenging activity of phenolic compounds from lotus leaves has been reported on by Park et. al. (2007), and the antioxidant activity of lotus leaf extract has been described by Choe et al. (2010).

In a recent study it has been confirmed that barley leaf extract has antioxidant activity, reducing power, free-radical scavenging activity, and superoxide dismutase-like activity (Choe et al., 2010). It has been demonstrated via test results that in cooked ground pork during refrigerated storage for 10 days the additions of lotus leaf powder (LP) and barley leaf powder (BP) had positive effects on oxidative and color stability. The ground pork made with addition of LP and BP had lower TBARS values, POVs and CD compared to control (–) during storage. The addition of BP had an inhibitory effect on lipid oxidation when compared to the control (–) over the 10 day period. The addition of LP and BP has shown no significant differences in the overall acceptability. Widely used vegetables in most Asian countries, lotus and barley leaf extracts, appear to be useful ingredients in ground pork, providing a source of natural antioxidants that are safe and enhance the oxidative stability of the processed meat (Choe et al., 2011).

#### Moringa oleiferia leaves

A good source of phenolic compounds and a very potent antioxidant activity is found in mature *M. oleiferia* leaves (MOL). Cooked goat meat patties could be protected against lipid oxidation during refrigerated storage by incorporating 0.1% extract of MOL (100 mg/100 g meat). The MOL extract was more effective than BHT in maintaining low TBARS number of precooked chilled goat meat patties. Frozen ready-to-eat meat products are available in Indian cities, but freezing is quite expensive leading to limited market. Therefore, MOL-treated meat products that can be stored at chilled temperatures would be beneficial for the manufacturer as well as the consumer. A potent source of phenolics and an immense nutraceutical value for the development of functional meat products of commercial interest are the unutilized mature *Moringa* leaves (Das et. al 2012).

#### Pomegranate

Native from Iran, pomegranate (*Punica granatum*) is now also cultivated in several provinces in China. Pomegranate rind and seeds are byproducts obtained during processing of pomegranate juice. It is reported that pomegranate rind, pomegranate juice, and pomegranate seeds possess significant antioxidant activity due to polyphenolic compounds (Fischer et al, 2003). The use of pomegranate juice, pomegranate seed powder, and pomegranate rind powder has been recently investigated as natural antioxidant in chicken and goat meat (Naveena et al, 2008; Devatkal et al, 2010).

#### CONCLUSIONS

Meat and meat products are fundamental components of our diet, contributing significantly to the intake of various nutrients and substances such as proteins, fats, fatty acids, cholesterol, sodium, nitrites, etc., which have various health implications. Meat and meat products could be protected against lipid oxidation by incorporating extracts, oils or powders from herbs and spices in order to maintain their color and to improve their sensory properties.

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#### CHARACTERIZATION OF THE ON-FARM PLUM COLLECTION FROM PLENIŢA - DOLJ COUNTY AND THE DEVELOPMENT OF GENETIC RESOURCES IN ROMANIA

Botu Mihai<sup>1,2\*</sup>, Pamfil Doru<sup>3,4</sup>, Botu Ion<sup>1</sup>, Hârţa Monica<sup>3,4</sup>, Scutelnicu Anca<sup>2</sup>, Giura Simona<sup>2</sup>, Popa Mihai<sup>5</sup>

<sup>1</sup>University of Craiova, Faculty of Horticulture, Department of Horticulture and Food Science, Craiova <sup>2</sup>University of Craiova – SCDP Vâlcea, Rm. Vâlcea <sup>3</sup>University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Horticulture, Department of Horticulture and Landscape, Cluj-Napoca <sup>4</sup>University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Life Sciences Institute, Research Centre for Agricultural Biotechnology, Cluj-Napoca <sup>5</sup>S.C. Secerişul S.R.L., Pleniţa \* Correspondence author. E-mail: stpomyl@onix.ro

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#### ABSTRACT

During 2014-2017 period, regeneration of a part of national plum collection from UCv-SCDP Vâlcea have been carried out. Through GERMPLUM project, an on-farm plum collection consisting in 141 accessions of those regenerated has been established in Pleniţa – Dolj county at S.C. Secerişul S.R.L. The 71 original and 70 foreign accessions from the on-farm collection belong to 13 species of Prunus genus, 5 interspecific hybrids and 2 others. P. domestica (51.8% of the accessions), P. insititia (22.7%), P. salicina (7.1%) and P. cerasifera (5.0%) hold the share in the collection.

The plum accessions consist in 96 improved cultivars (34 autohtonous and 62 foreign), 11 old native cultivars, 26 biotypes from different populations and 8 wild species. The accessions were phenotypically characterized using 31 different accepted descriptors, variability of characters being observed.

The genetic resources present into this collection are valuable and can be used into the future plum breeding programs for cultivars and rootstocks

#### INTRODUCTION

Identifying and maintaining biodiversity in fruit tree crops has become an important necessity for assuring the survival conditions of future human generations.

Numerous plum collections hold over 5447 accessions of *Prunus domestica*, 802 accessions from *P. salicina* and over 200-300 accessions from *P. cerasifera*, *P. americana*, etc. (Botu, 1999).

Ramming et al. (1992), Mehlenbacher et al. (1992); Botu et al. (2005); Butac at al. (2011), Botu et al. (2012) highlights the value of plum biodiversity, how to conserve genetic resources and their importance in breeding programs and for future generations.

Due to the high genetic variability among the over 40 species and interspecific hybrids (diploid, triploid, tetraploid, pentaploid and hexaploid) of plum used more or less in culture, but also the variability existing within the same species, it is necessary

to save this genetic patrimony from its loss through genetic erosion and genetic vulnerability and to value it.

World plum production amounts to more than 11,282,527 t, which corresponds to only 1.6 kg of plums/year/capita (FAO. 2016). This consumption is considered to be insufficient for a normal diet. Several global organizations are concerned with preserving and making good use of plum genetic resources (European Prunus Database, Biodiversity International, FAO, UPOV, etc.).

The value of plum genetic resources is that they are very well adapted to local environmental conditions, quite rustic and productive and have resistance genes for most dangerous diseases, they are productive and quite rustic.

During 1994-2001 period and through the GENRES - 61 Project of the European Cooperation Program on Genetic Resources (ECP/GR)- Program 1467/94 on *Prunus* species also the *Prunus* genetic resources from Romania were investigated. 292 original accessions and 428 accessions introduced from other countries were recorded from the national *Prunus* collections (Botu et al., 2001).

Later on, a part of the plum genetic resources from national collections were characterized both by phenotypic and genetic descriptors through a national project PN-II-PT-PCCA-2013-4-1399 no. 168 in titled "Conservation of native plum germplasm based on a experimental model of molecular characterization and collection *on farm*" (GERMPLUM).

#### MATERIAL AND METHODS

The plum biological material consists of native genotypes from Romania (original genetic resources) and foreign genotypes existing mainly in the national collection, which needs to be regenerated due to the age of the trees. The plum genetic resources in the national collection include both improved varieties, old varieties, populations (biotypes), species and hybrids.

The biological material was grafted at UCv-SCDP Vâlcea onto selected Myrobalan seedlings ('Mirobolan galben'rootstock). Planting was done in the autumn of 2016 and spring of 2017 on farm land belonging to S.C. Secerişul S.R.L. located in Pleniţa, Dolj county (SW of Romania). Planting distances used were 5.0 x 4.0 m (density of 500 trees/ha).

The phenotypic characterization of the accessions was carried out using the internationally accepted descriptors of Biodiversity International (former IPGRI), F.A.O. and EURISCO. From a total number of 47 descriptors used for plums, 31 descriptors were selected in order to be used to characterize the plum accessions.

In the present paper only the passport and the general descriptors specific to the phenotypic characterization are used, molecular characteristics will be presented later.

The phenotypic characterization of accession will serve for setting up a computerized data bank.

#### **RESULTS AND DISCUSSIONS**

During 2014 - 2017 period the project PN-II-PT-PCCA-2013-4-1399 no. 168 in titled "Conservation of native plum germplasm based on a experimental model of molecular characterization and collection *on farm*" (GERMPLUM) have been carried out. The consortium of the project was composed by University of Agricultural Sciences and Veterinary Medicine (USAMV) from Cluj-Napoca as coordinator and University of Craiova – SCDP Vâlcea and S.C. Secerişul S.R.L. as partners. The project had as main objectives the identification, evaluation, multiplication and establishment of an *on-farm* plum collection.

During this period, 185 genotypes were identified, out of which 115 are original (native) and 70 foreign. Following the multiplication and establishment of the 2.26 ha on-farm collection in Pleniţa 141, accessions are found in this collection, of which 71 are original and 70 are foreign (Table 1). The accessions belong to a number of 13 species, to which are added 5 interspecific hybrids and 2 others.

Table 1

No.	Species	Total no. of accessions identyfied		Accessions introduced into the Pleniţa collection		Out of t	Accessions identyfied but not introduced into the Pleniţa	
		No.	%	No.	%	Autochto- nous	Foreign	collection
1	P. domestica	98	53,0	73	51,8	34	39	25
2	P. insititia	43	23,2	32	22,7	27	5	11
3	P. cerasifera	13	7,0	7	5,0	6	1	6
4	P. spinosa	3	1,6	2	1,4	2	-	1
5	P. salicina	10	5,4	10	7,1	-	10	-
6	P. sibirica	1	0,6	1	0,7	-	1	-
7	P. americana	3	1,6	3	2,1	-	3	-
8	P. microcarpa	1	0,6	1	0,7	-	1	-
9	P. nigra	1	0,6	1	0,7	-	1	-
10	P. besseyi	1	0,6	1	0,7	-	1	-
11	P. maritima	1	0,6	1	0,7	-	1	-
12	P. tomentosa	2	1,1	2	1,4	-	2	-
13	P. subhirtella	1	0,5	1	0,7	-	1	-
14	Interspecific	5	2,7	4	2,8	2	2	1
	hybrids							
15	Others	2	1,1	2	1,4	-	2	-
	Total	185	100	141	100	71	70	44

# The on-farm plum collection from Pleniţa Dolj and the membership of accessions to different species of the *Prunus* genus

Of the 141 accessions in the collection, 73 of them (51.8%) belong to *P. domestica*, 32 (22.7%) to *P. insititia*, 7 (5.0%) to *P. cerasifera*, 2 (1.4%) to *P. spinosa*, 10 (7.1%) to *P. salicina*, etc.

Through the GERMPLUM Project, efforts have been made to save a part of the plum biodiversity, which is greatly affected by erosion and genetic vulnerability. Also, we tried to overcome the negative effects produced in the old national plum collection from UCv-SCDP Vâlcea due to the age of trees, as well as the loss of some of the accessions due to land restitution to previous owners. At the time of counting of plum accessions through the GENRES - 61 Project of the European Cooperation Program on Genetic Resources (ECP/GR) - Program 1467/94 the UCv-SCDP Vâlcea collection was holding 292 original (autohtonous) and 428 introduced (foreign) accessions.

With the help of GERMPLUM project, 141 plum accessions from UCv-SCDP Vâlcea were regenerated and introduced into the on-farm plum collection in Pleniţa. The status of these accessions is presented in Table 2. Original or autochtonous accessions (71 in total) contain 34 improved cultivars, 11 old cultivars and 26 biotypes of different populations. Introduced or foreign accessions from the on-farm collection in Pleniţa consist in 62 improved cultivars and 8 *Prunus* species.

All the 141 plum accessions are presented and recorded based on passport and general descriptors so that they can be registered in the national and foreign data banks. 31 descriptors were used to characterize each of the 141 plum accessions. Along with the recording of the descriptors, 141 phenotypic characterization sheets were written, out of which the limits of variation of the most important accessions characteristics are presented in Table 3.

The phenotypic variability will then be correlated with the molecular data issued as result of SSR analysis carried out at USAMV Cluj - Napoca.

A very high genetic variability was observed among the plum species belonging to *Prunus* genus, but the variability is also present within the same species. This variability will later serve in the breeding program for the obtaining of valuable cultivars and rootstocks based on the genes available in this germplasm fund. Table 3 shows the variability of 10 characters (growth vigor, fruit size, tolerance to Plum-pox virus, etc.) present in these accessions.

Tree growth vigor varies between 1.2 m in tree height (*P. tomentosa*) and 7.0 m (Marianna GF 8-1). The pollination capacity is differentiated from the level of malesterility (Tuleu gras) to that of self fertility (Gogoşele negre, Oteşani 8, etc.), and the flowering time occurs between early March (*P. sibirica*) and early May (Vânăt românesc cl. 4).

The size of the fruit is a very variable character, fruits vary from 4.0 to 6.0 g (Spinigra) up to 120 g or more (Ozark premier).

Fruit yield is very variable in the case of the given plum accessions. The yields start at a level of 0.2 kg/tree at Spinigra (interspecific hybrid) and reaching up to 60.0 kg/tree at Centenar and Stanley cultivars. Some accessions are used as generative or seedling rootstocks (C 163, Oteşani 8, Voineşti B), others as vegetative or clonal rootstocks (Oteşani 11, Miroval).

The range of resistance / tolerance to Plum-pox virus (PPV) attack is very wide. Dobrovica and other accessions proved very susceptible to this attack, but Andreea, Romandreea and Scolduş are tolerant and even exhibiting field resistance to PPV.

Because temperatures in some areas of Romania are sometimes going below -29°C; -31°C, some cultivars from *P. salicina* (like Methley) proved less adapted to the climate.

The *on-farm* plum collection from Pleniţa is in the 2<sup>nd</sup> year (2<sup>nd</sup> leaf) after planting. In the next coming years there is need for financial and scientific support.

Also, the activities of identification and promotion of new plum accessions has to be continuated in order to enlarge the germplasm collections and to use the genetic diversity for breeding new cultivars and rootstocks.

The collection serves for research activities and also for didactical purposes for undergraduate and graduate students but also for research and teaching staff.

#### CONCLUSIONS

During 2014-2017 period, based on the GERMPLUM Project, a on-farm plum collection made up of 141 accessions was established at S.C. Secerişul S.R.L. in Pleniţa, located in Dolj county in the SW of Romania.

The plum accessions in the collection (71 of them original and 70 foreign ones) belong to 13 species of the *Prunus* genus, plus 5 interspecific hybrids and 2 others.

The 141 accessions were phenotypically characterized using Bioversity International (IPGRI), FAO and EURISCO descriptors.

The genetic resources present into this collection are valuable and can be used into the future plum breeding programs for cultivars and rootstocks.
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#### ACKNOWLEDGMENT

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- ✓ Horticulture
- Food produce processing technology
- ✓ Environmental engineering

#### RESEARCH ON THE MULTIPLE WAYS FOR OBTAINING ECOLOGICAL AND QUALITY SEEDS OF LAVANDULA

Broșteanu Elena 1\*

<sup>1</sup> University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture \*Correspondence author. E-mail: e\_brosteanu@yahoo.com

Key words: Angustifolia, Ecological, Lavandula, Vera

#### ABSTRACT

This paper presents the influence of soil and planting density in Lavandula production and seeds quality. The subsidiary objective was to obtain an ecological culture for high quality seeds. The cultivars of Angustifolia and Lavandula ong Silver Mist have been studied at the following planting distances: 0/25 with 71,428 pl ha, 70/30 with 47,613 pl/ha and 70/40 with 35,714 pl/ha. The setting up of the culture starts in the autumn of 2015, when the first row was planted. The seeds production per plant increase direct proportionally with the planting distance. In both varieties, the highest seed yield was obtained at a density of 71,428 plants per hectare. In this case, the average seed yield per hectare was around 222 kg for Lavandula ong Silver Mist and about 165 kg for the Angustifolia variety. The yield obtained is sensible higher, so further quality investigation was performed for proving seeds quality.

#### INTRODUCTION

Lavandula angustifolia Mill which contains a volatile oil with a controlled chemical composition and meets specific quality requirements. Our main goal consisted in type of propagation, both generative, by seedlings and vegetative, by rooted cuttings or by earthling up and separation of old shrubs. Species / subspecies / cultivars of Lavandula in Romania, they were as follows:

- Lavadula Angustifolia Mill
- Lavandula Angustifolia ssp. Angustifolia.

#### MATERIAL AND METHODS

Determination of morpho-anatomical features of Lavandula species / subspecies cultivated in Romania:

• evaluation of the volatile oil content extracted by hydrodistillation and determining its chemical profile.

- production of seedlings from seeds
- by rooted cuttings
- separation of old shrubs

#### **RESULTS AND DISCUSSIONS**

Lavandula is a mountainous, less demanding, sun-loving species. It is a homogeneous, ethomophilous plant and, by generative propagation, it produces many shoots with chemical morphogenetic variability and with diverse economic efficiency.

Lavender seeds are hard, covered by a poorly permeable coat, which leads to long period needed to sprout and spring. After emergence, the lavender plant forms shrubs as a result of growing new branches, increasing the flower production.

Lavender populations predominantly include forms which bloom once a year, but other types bloom gradually or twice during vegetation. During the vegetation period, lavender goes through the following stages:

- leaf-out
- budding
- blooming
- seed ageing

Table 1

Number	Plant	Length of	Length of	Length of	Number of
of	height	flower	flower	flower spike	whirls on
versions	(cm)	(cm)	stems (cm)	(cm)	flower spike
	Х	X±Sx	X±Sx	X±Sx	X±Sx
V1	48.5	27.2±2.4	20.4±1.2	6.8±1.3	6.3±1.1
V2	46.5	26.9±2.3	19.9±2.4	7.8±1.4	6.5±0.1
V3	47.0	25.7±2.2	18.8±1.5	6.9±1.4	6.8±0.8
V4	48.0	26.5±2.4	19.7±2.1	6.8±1.3	6.6±1.8
V5	54.0	31.5±1.5	23.5±2.2	8.0±0.7	7.7±0.5
V6	52.5	29.8±1.8	23.5,±2.3	8.1±1.3	7.4±1.0
VMt	41.5	24.5±1.9	17.4±1.4	6.3±1.1	6.0±0.5



Figure 1. Lavandula angustifolia plantation untreated.

The main factor that limits the spread of various lavender species is temperature. Lavender plants begin vegetation at average daily air temperatures around 10°C. The seed sown on the brink of winter directly in the field or in cold

beddings springs up in spring at 12-13°C provided it has proper humidity. Lavender is a plant specific to dry biotopes and warm climate, with proper morphological features that allow it to be drought tolerant. When cultivated on land with excessive moisture, lavender suffers from rotting roots, which causes ageing and rapid drying of shrubs. Lavender seeds sprout only when the surface soil layer they're in is moist enough.



Figure 2.Lavandula plantation after applying only physical treatment.

Moreover, in case of rooted cuttings, both for lavender and lavandin, moisture is a decisive factor.

Final transplantation of rooted cuttings or seedlings will be done exclusively in wet soil, in case of lack of moisture, plants will necessarily be watered at the planting location.

Lavender and lavandin are light-loving species. Lavender plants which grow under the sun always bloom at a higher rate, developing large shrubs, 3 times more flowers. Light conditions the formation of leaves, as well as assimilation and production of volatile oils. In order to increase the amount of light, lavender plantations are recommended to be located in our country on land facing South or South-West.

The best results are obtained when lavender is grown on deep, limestone-rich, permeable soils, with groundwater at depths of at least 2-2.5m.

Highly sandy soils, heavy, clay, cold and wet soils are not recommended for lavender crops. Nitrogen plays an important role in the rejuvenation of shrubs, as it stimulates growth, which is why young plants will be provided with more phosphorus in the fertilizer mix and more nitrogen will sprayed on old plantations.

Growing seedlings from seeds. Sowing can be done both in late autumn, October -November and in early spring, February-March, but only in specially prepared beddings, which must be 1-1.2 m wide and 8-10 m long and even more. A 1-meter space should be between beddings.

The seeds sprout in early spring when air temperature is constantly maintained at 14-15°C. In case of spring sowing, the seeds germinate and sprout slowly, which requires their 3-4-day moisturizing in water or their layering, which must start 1.5 - 2 months ahead. For this purpose, the seeds are mixed with wet river sand in a ratio of 1 : 5-6 and are kept out until sown, meanwhile being stirred to avoid molding. By the end of the layering period, the seeds germinate and will be immediately sown in warm soil.

After the plants spring out of ground, when they have 2-3 pairs of leaves, thinning is applied, leaving 3-5 cm space between plants. Until the end of vegetation, in autumn, seedlings must be kept clean from weeds, by repeated weeding, must be

watered, fertilized and hoed. Concomitantly, at least twice, all the flower stems will be cut to force the plant to grow branches as close to the ground as possible. The cutting height of floral stems is approx. 8-10 cm above ground level.



Figure 3. Samples of Lavandula angustifolia seeds and yield.

The seedling is ready for transplantation, to the final location, at the end of September and in early October when it is taken out, sorted and stored in layering in sand or wet soil until planting.

Seedlings have the following characteristics:

- length of aerial part 12 cm
- length of main root 15 cm
- minimum 4 mm diameter and 3 branches

Growing seedlings by seeds is a cheap and fast way of propagating lavender.

Propagation by rooted cuttings: Cuttings are harvested autumn, in September-October or in spring, March-April, during the vegetative rest period. Cuttings are harvested from healthy, well-developed plants, aged 3-5 years. Cuttings are cut with a very sharp knife or blade, under buds. Leaves are removed carefully to avoid vegetative buds being destroyed. One wedge-shaped cut is done at the base of the cutting, right after bud. The cuttings thus prepared have a length of approximately 5-6 cm and are ready for planting, which is to be carried out within 1-2 hours. In case of longer storage, but not longer than one day, cuttings are kept in pots with clean water.

The land chosen for the beddings where cuttings will be planted must be weed-free, located near a water source, but without risk of swamping. It will be ploughed to 30 cm deep, leveled, shredded and then gently tamped, then seedbeds will be arranged, allowing 40-50 cm space between them. The seedbeds shall have a well sifted, 8-10 cm thick layer of sand, which, after leveling, is watered abundantly. After watering, to facilitate planting, the next rows will be marked, using markers specially prepared for this work. Planting is done manually, placing them with a 10.5 cm spacing. Immediately or concurrently with planting, the cuttings will be watered abundantly, and the seedbed will be covered with window frames whose glass has been previously varnished and covered with mats until the first roots emerge on cuttings. When the first roots are formed, cuttings are strengthened by uncovering the

seedbed, for a few hours initially, exclusively during the morning or evening. When floral stems appear, they will be cut to half to stimulate branching.



Figure 4. Different Lavadula variants and species, on the experimental plantation

#### CONCLUSIONS

Paper's general conclusions can be structured as follows:

A. The histoanatomical structure of the vegetative organs shows minor differences between the two subspecies (*L. angustifolia* ssp. *angustifolia* / L. *angustifolia* Mill), rather quantitative differences:

- Processed species, subspecies and cultivar
- Moment of the growing period and sampled plant part
- Location of origin 
   Climatic conditions during harvest
- Type of material (fresh / dry) subjected to processing.
- B. The polyphenolic fraction varies qualitatively and quantitatively from:
- Processed species, subspecies and cultivar
- Climatic conditions during harvest
- Type of solvent used for extraction.
- C. The antioxidant activity of the polyphenolic fraction varies with:

Content of polyphenols present in extract, determined by species and subspecies

• Cultivar, samples from Balteni (*L. angustifolia* Mill.) being more active than cultivars collected from varieties cultivated in Dambovita.

#### ACKNOWLEDGMENT

Project's originality firstly derives from the investigation of certain new species and varieties of Lavandula cultivated under pedo-climatic conditions specific to the South-East of Romania. Another element of originality consists in total pharmacobotanical, chemical and biological issues used to explore the species under study. The project contributes to wider phytochemical and correlation existing between pedoclimatic conditions in our country and chemical composition, substantiating the antimicrobial and antioxidant activity of species for Lavandula seed production. Alexan M., Bojor O., Crăciun F., 1989, Medicinal flora of Romania. Vol. I, II. Bucharest: Ceres Publishing House, pp. 118.

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#### THE CONCEPT OF "ROAD OF THE WINE" IN OLTENIA AND THE PLUS OF VALUE FOR A TOURISTIC DESTINATION

Călugăru Iulian Viorel<sup>1</sup>, Giugea Nicolae<sup>1\*,</sup> Mărăcineanu Liviu<sup>1</sup>, Berta Corina<sup>1</sup>, Perţ Carmen<sup>1</sup>

> <sup>1</sup>University of Craiova, Facultaty of Horticulture \* Correspondence author. E-mail: giugeanicolae@gmail.com

Key words: oenotourism, wine, road of the wine, touristic product, touristic potential, touristic destination

#### ABSTRACT

The oenotourism is a recently phenomenon for Romania. In the european countries it has been practiced for a few decades, spontaneous or organized. This expansion is determined by the existence of two reasons fot the wine-growing tourism: on the one hand is the problem of the development of the wine-growing field and on the other hand of a form of alternative tourism to the traditional tourism.

In fact, the road of wine represents a well indicated and delimited touristic route in a zone with wine-growing potential and with enough touristic attractions, natural and anthropical, which can be used by the tourists, individual or in organized groups, in some periods of the year. These roads, depending on their length, but also by the diversity of the objectives, can be seen by the walking tourists or by bike, by car or even by carriage, in a day or in a few days.

The marketing of the touristic products includes the product of ,,touristic destination". If we compare it with other domains of activities, we observe that the tourism reacted with a certain delay in the application of the methods and techniques af marketing. But, in our days, the tourism and specially, the oenotourism in Oltenia is looking to extend and to go thoroughly into these techniques in order to turn to good account the resources and to carry out a better efficiency of the whole activity. The marketing is the process of an organization to be adopted to the market in a creative, productive and profitable manner.

#### INTRODUCTION

The european countries with a long traditon in wine-growing production, understood a long tine ago that the wine is not only a liquid in a bottle, by the contrary the wine is a sory, a history, a legend and a civilization, it is a rural tradition and an urban charm. They understood that is very important for the wine lovers to enjoy the famous wines in the restaurants or at home but in the same time, to discover the universe of the vineyard, the place where it grows – in the wonderful valleys or on the sunny and majestic hills in the mysteriously Oltenia.

The oenotourism and the ecotourism appeared first like an opportunity in affairs, which could be a succes on a long term only if it is a responsiable affair, which respects the market rules and the lasting development in the same time.

Establishing the nair objectives of this study, we proposed to ourselves to identify the factors which can produce the plus of value for a wine-growing touristic destination. In time, the travel reasons of people become very diverse, appearing new

needs and whishes, multiple and more complex, caused by the changes at the level of society.

More than a century ago, nobody thought to pay a visit in a vineyard. The oenotouristic motivation (vocation, inclination) which generates the touristic request is always ery personal, subjective, determined by the psychological impetuses (influenced by the environment).

In the social plan, the tourism (generally) and the oenotourism (specially) have a substantial contribution to the raising of the level of education, culture and civilization of the people. Stimulating the exchange of values, the tourism encourages the enrichment of the cultural horizon for the tourists and for the local population.

#### MATERIAL AND METHODS

The touristic destination represents the place or the geographical space where a visitor or a tourist stay for a night or for a longer perios of time, or the final point in the vacantion of the tourists, no matter if they travel for touristic or for affair reasons (Stăncioiu, A. F., 1999).

The method used in this study was the geographic method, which represents a complex, synthetic approach of the tourism fenomenon in the territory, starting from the existence of potential and built touristic landmarks and from the touristic phenomenon in its complexity, and also from the fact that tourism evolves in the environment, more or less humanized, which is the "raw material" for this and with whom is in close interaction.

Geographic method involves, as a matter of fact, the use of the principles of the distribution in an surface (or area) and geographic integration, specific to geography as a science.

According to this method all information about tourism activities offered by specialists in this field, in relation to the specialization of each, are dispersed, but the overview of the touristic phenomenon results only in the systematization of knowledge and their geographic integration into a territorial and geographis functional system.

It requires a special (expansion, shape, complexity) and positional (geographic) approach of the objectives and of the tourism phenomenon in general, in relation to the areas and forms of the nearness tourism (or at a higher territorial level), and functional and territorial integration of the touristic activity; a causal approach of the touristic phenomenon, but also an ecological and socio-economic approach through the relationship with the environment and human communities (Glavan V., 2005).

#### **RESULTS AND DISCUSSIONS**

The main objective of this research was the identification of some winegrowing trouristic destination in Oltenia, as well as the factors which can produce a plus value of these destination. The wine-growing tourism shortens, in fact, the distances between the wine lovers and the producers, while the tourists who can see live the process of wine producing and the necessary effort of this process will be the first promoters of the consumation of good and authentic wine.

The wine is a real part of the european identity, that's why is very important to maintain this common cultural patrimony in its whole diversity, and to kepp this cultural inheritance for the next generation.

The wine-growing in Romania is now in a period of revival, is in full swing.

Romanians begin to redescover the wines, the producers become more and more experience and wines of a good quality are produced in our country, too.

Although in spite of these trumps, the connection between wine-growing aand tourism is weak represented.

In Oltenia the wine-growing tourism is in an evidently development, being helped by the investments carried out by the wine cellars, as well as by the request from the costumers for this kind of travels, directed to same experiences. The potential of development is great the wine lovers being attracted by the visits in vineyards and wine cellars by the conversations with the experts in wine-growing and not finally, by the discovered in Oltenia. More than these the old mansions near the vineyards whide have been recpnditioned and changed into units for accommodation are points of attraction with their legends for the Romanians and foreign tourists. Until recent time the wine lovers in our country werw forced to travel abroad in order to practice the wine-growing tourism, but in our days they can do this type of tourism just in our coutry. The geographical position, landscape, diversity, history and the native varieties of wines are clear advantages which can be used by the owner of wine cellars in order to attract the Romanian and foreign tourists to the local wine-growing zonees.

The wine cellars in Oltenia can be visited all year round, but the most attractive landscapes are in the period between april and october

Now, we small desribe four wine-growing destinations in Oltenia, which can be included in a concept of "road of wine" in the north part of Oltenia.

1. The wine cellar ,,AVINCIS" is situated in Vâlcea District, on the Hills of Drăgăşani

(Drăgăşani town is situated at a distance of 6 km far fromn wine cellar), at abot 200 km dar from Bucharest (two hours and a half by car).

ANVICIS is the symbol of the rival of the Romanian wines, uder the sign of tradition and modernism. This adventure started by the discovery of one passion in 2007. When Cristina Stoica ame back with his husband, Valeriu, in the brithplace of their family, in the region Drăgăşani. So they started their project to give a new life to the family domain, in order to give back the older charm of the vineyard from the eng of the place for the tourists who like the beautiful and the good wine.

In order to build for a long time the personality of their vineyard, Valeriu and Cristina Stoica tried to use the knowledges of the young and talented French oenolog Ghislain Moritz, graduate of the Dijon University, who had experience in wine-making, working in Bourgone region and in Portugal. This vineyard with 50 hectares (41 hectares active) occupies the whole hill and its slow slopes until the valleys. Out of the old varieties, today are planted "Tămâioasa Românească", "Negru de Drăgăşani" and "Crâmpoşie Selecționată" and besides all these other varieties with a foreign origin which have benn introduced in Drăgăşani wine-growing. So that, varieties as Sauvignon Blane, Pinot Noir, Cabernet Sayvignon or Muscat Ottonel have adapted very well at the climate and soil condition. AVINCIS Wine Cellar from the "Domeniul Vila Dobruşa" offers an unique experience. With a capacity of accommodation for 28 persons in double system or 14 persons in simple system, AVINCIS offers to its visitors 10 modern double rooms complete equipped and 3 flats situated above the wine cellar, with a splendid view about the domain and vineyard.

AVINCIS wine cellar is surrounded by touristic objectives, Dobruşa Villa being an important point of attraction in the region. Dobruşa hermitage, the monasteries of Oltenia (In One-Wood, Horezu, Arnota and the fortified houses from Măldăreşti) are only a part of the beautiful of this zone.

2.BAUER WINE CELLAR – founded in 2012 is situated in Drăgăşani, Vâlcea District, in the middle of the wine-growing Drăgăşani, wine-growing zne Oltenia. This new wine cellar is conceived to permit the process of wine-marking for a small quantity of grapes out of rare or unfound varieties yet by the wine lovers, gathered in the old and well

carefully lots. The philosophy of the wine cellar "Natural Creativity" is the mirror of confidence in diversity, not in the concept of "the best wine". Varieties of grapes: sauvignonasse, sauvignon Blanc, Crâmpoşie Selecţionată, Cabernet Sauvignon, Merlot, Petit Verdot, Fetească Neagră.



Figure 1 AVINCIS wine cellar (http://www.revino.ro)

Drăgăşani wine-growing is rich in traditional varieties of wine, in natural beautiful and diversity, smat people, exceptional experts in wine-making and wines with personality. Bauer wine cellar can be visited every seasin. It is waiting for you to taste its wines in winter, spring, autumn or summer. Preious reservation. Grups between 4 and 18 persons.

3. Ştirbey Wine Cellar – is situated in Drăgăşani, Dealul Olt Street, Vâlcea District. The owners are Ileana and Jakob Kripp and wine-making Oliver Bauer.

Since more than 300 years, the princely family Ştirbey had a wine-growing domain in Drăgăşani. The wines of thw princely family Ştirbey are produced according to some principles developed and kept from generation to generation. Here they cultivate traditional varieties of grapes, adapted and with a good evolution in time at the soil and climate conditions of this vineyard. These varieties of grapes of wine-making, so that are produced only wines from pure varieties in order to emphasize the characteristics of every variety.

Soil, climate, flora and fauna, but also the traditions of people who work in the vineyard influences very much the evolution of wines and grapes, giving to every lot of vineyard another characteristic which can be synthetized as Trroir or, in an universal meaning, as Genius Loci – the spirit of the place.

The owners want to refind this Genius Loci in the wines produced and sold here.

They are sure that the forces of nature give us products healther and more lasting than any teachnical interention. The vines have a good care, with strict manual works, so that the chimical treatments are reduces to the minimum. In the process of wine-making is followed the clock of the nature fot the fermentation and maturising, and it is not forced the process by the technical interventions.

4. Drăgăşani Domain – is situated in Drăgăşani, Dealul Viilor Street, Vâlcea District. Situated on Dealul Viilor, in Drăgaşi Domain and its four points of attraction – the wine-growing, wine cellar, the saloon for wine tasting and the hotel – in an ensemble which proposes to itself to leads the guests to refind the nature and their own aspiration. Thia pilgrimage is influemed by the panoramic view about the wine-growing, afforested hills and flowing Olt, but also by the meeting of the people with their stories

Very interesting are the descriptions of some wines : "Pinot Gris 2015". You Suddenly realise that you are in a flowers filed. You don't scare yourself, such experiences have the tendeny rather to calm you. You raise your head between poppies and can see yellow in your glass. Or is the yellow straw of the field? Yhe fresh aroma make you to feel cool, the air has a semidry aspect, nothing hurry you, everychere there is an unmotion as if you keep your breath. "Sauvignon Blanc 2015". You try to understand words which help you to describe what you drik. And in your mind appear: poems from the seventh from, love letters fom the secondary school, an acacia forest, citric fruit in an castern market, the balance of the ships in the sea, a shell in the sand. Finalu, the fresh taste shakes you and you can seen another imagine-you, the glass, the hat for sun. "Merlot 2015" the cork has a smell as the phrases like all the time in the world. The intense red and violet remins you about a storm from your childhood, when the sky wew full of fire, and the rain were extingnishing it. The aroma of blackberrues, currants, cherries and spices determine you to ask yourself if you are on a verandah or in a forest. The balanced but delicious taste needs some cheeses and closed eyes etc.



Figure 2. Drăgaşi wine cellar (http://www.domeniul-dragasi.ro/)

Limits. In Oltenia there are many wine cellars wines growing, history, touristic objectives and very well roads. But in the same time, we have no enough rooms for accommodation and we new marketing which could promote these wine growing destinations. We haven't structure and coherentness. ,,Three principle condition the succes of the promotion, and these are the three << C >> : continuation, convergence, co-ordination " (Llanguar R., Hollier R., 2001)

#### CONCLUSIONS

The oenotourism in Oltenia must constitute the subject of the project management. The road of wine has to become a touristic route, with wine-growing products, with points for wine-growing products, with points for wine tasting and touristic, cultural and natural objectives which can be developed by well made programmes from one until three days for the people fond of tourism. The multitude of resources / types of resources doesn't facilitate implicit the development of the tourism in a certain region. By the contrary, it needs intense efforts of marketing, The new marketin initiates, negotiates and leads favourable relations for change with the main groups of interest, in order to turn to good account the potential of these relations and to create the fidelity with the brand of the products, because and services not only for their use value to spend his free time, but also for their value of social connection. The geographical position, landscapes, diversity, history and the native varieties of grapes are real advantages and the wine cellars owners can use them in order to attract the Romanian and foreign tourists in the local wine-growing zones.

The oenotourism has a favourable influence about other national economy domains. Consequently it is necessary to be well known the wine-growing destinations in Oltenia, because they can create the concept of "road of wine" in Oltenia and, in the same time they can create a plus of value for a touristic destination with the trumps of the wine cellars in Oltenia.

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## ANNALS OF THE UNIVERSITY OF CRAIOVA Vol. XXII (LVIII) - 2017

Series: ✓ Biology

- ✓ Horticulture
- Food produce processing technology
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#### THE OENO-TURISM A CONSTANT FORM OF TOURISM IN OLTENIA

Călugăru Iulian Viorel<sup>1</sup>, Giugea Nicolae<sup>1\*</sup>, Berta Corina<sup>1</sup>, Carmen Perţ<sup>1</sup>, Mărăcineanu Liviu<sup>1</sup>

> <sup>1</sup>University of Craiova, Faculty of Horticulture \* Correspondence author. E-mail: giugeanicolae@gmail.com

Key words: oenotourism, wine, touristic product, sustainable development, wineries

#### ABSTRACT

The development of the tourism industry has recently been displayed through its returning to nature and original cultural values. The oeno-tourism is one of the most valuable forms of tourism. These forms have as the main purpose the preserving of the environment with a high attention to the tourist' education regarding the protection and the preserving of the environment.

The connection between the tourism and the environment has a high importance because they are the most important conditions to be fulfilled in order to develop the tourism. The touristic development of the population, the variety of the motivations cand lead to a single great contribution designed to stop the damage of the environment caused by different activities, thus, through some rules, which must be taken, to fulfill the protection and the preserving of the environment. Any dangerous action can lead to transformation and, most of the times, is an injury to the environment: the decay and the defeat of its resources also of the economical balance and all these can lead to the damage of the health and of the existence of the next generations. Generally speaking, there are objective factors (when natural phenomena are unfavorable displayed) and subjective factors, which are caused by human activities, that affects and change the environment. Through different activities (controlled and uncontrolled tourism), Man has a great contribution in the natural destruction. Those who are involved in oeno-touristic activities are aware of the effects on the touristic development, of their impact on people's activities.

#### INTRODUCTION

Even though the tourism, at its beginning, represented a growing economical factor for protecting and preerving the environment, nowadays the ecological balance and the natural resources balance is highly affected, mainly through the growth of the tourists flood. This is why it is necessary to reorganise the area, to adapt to the needs of the tourists, to support them, to reorganise the space, and this is a complex duty.

A constant tourism means an industry which tries to have the smallest impact on the environment and on the local culture, but, in the same time, tries to support the incomes, to diminish the unemployment and to support the local eco-systems.

The constant development must become one which fulfills all the needs without affecting the future generations' abilities. An uncontrolled tourism can lead to the development of the environment but also to its damage. According to J.Krippendorf

", if we can lose and then rebuild the economical side of a country, when talking about tourism, where the essence is the Landscape and the Earth, once the balance lost, it stays lost forever."

#### MATERIAL AND METHODS

The touristic destination represents the place or the geographical space where a visitor or a tourist stay for a night or for a longer perios of time, or the final point in the vacantion of the tourists, no matter if they travel for touristic or for affair reasons.

The method used in this study was the geographic method, which represents a complex, synthetic approach of the tourism fenomenon in the territory, starting from the existence of potential and built touristic landmarks and from the touristic phenomenon in its complexity, and also from the fact that tourism evolves in the environment, more or less humanized, which is the "raw material" for this and with whom is in close interaction.

Geographic method involves, as a matter of fact, the use of the principles of the distribution in an surface (or area) and geographic integration, specific to geography as a science.

According to this method all information about tourism activities offered by specialists in this field, in relation to the specialization of each, are dispersed, but the overview of the touristic phenomenon results only in the systematization of knowledge and their geographic integration into a territorial and geographis functional system.

It requires a special (expansion, shape, complexity) and positional (geographic) approach of the objectives and of the tourism phenomenon in general, in relation to the areas and forms of the nearness tourism (or at a higher territorial level), and functional and territorial integration of the touristic activity; a causal approach of the touristic phenomenon, but also an ecological and socio-economic approach through the relationship with the environment and human communities. (Glăvan V., 2005).

#### **RESULTS AND DISCUSSIONS**

The major aim of this research was to identify the traits of the oeno-tourism in order to become a developed one. Also, we wanted to establish some objectives for an everlasting development of the wine-making industry in Oltenia.

A constant development means economic efficiency, social and cultural cohesion, protection of the environment.(Glăvan V., 2005). The development of the oeno-tourism is not automatic because it is connected to image and reputation.

The wine making tourism in Oltenia represents an important opportunity of marketing and a possibility to develop the inside tourism. The oeno-tourism in Oltenia is a method to promote the wine-making industry including a physical, social and cultural dimension of the wine-making scenery.

The oeno-tourism in Oltenia fulfills some conditions and becomes a constant one:

1.Supports the place integrity: the vineries from Oltenia emphasise the local architectural trait of the area by keeping the local architectural motifs and by melting the local culture with its chime, the using of specific materials, the local cuisine, natural attractions and landmarks, aesthetics. The profits from this form of tourism lead to the growth of services.

2.Awakes the quality not the quantity: the vineries owners do not measure the success through their visitors but through the time spent there by the visitors, the expenses and the quality of the experience.

3.Do not abuse the product: The pressure growth is anticipated and applies management limits and techniques. Thus, the groups of visitors are always small. The firms must work to support the natural and cultural resources. Visiting the historical, artistic and architectural landmarks in bad conditions (candles use, lack of technical appliances) led to the damage of important and valuable pieces of art.

4.It is informative: the traveler does not only find information about their destination, but also finds about how to help and support, thus, the experience becomes memorable. By participating at some specific activities, the tourists will learn interesting things. The festive traditions and customs usually represents the end of the wine-making experience which can be in the same time with a religious holiday( The Grapes Dance, The Vineyard Baptism).

5.Supports the local people: the vineries owners try to hire and to train local people, to buy local products and to use local services.

6.Preserves the resources: The ecological aware tourists help the pollution diminish, the use of electric power, the use of water and chemical products.

7.Values the local cultural traditions: the foreign visitors observe and learn the traditions and the local customs. Most vineries include artistic events with folk music and dances. Thus, the firms are likely to practice the wine-making tourism to fulfill the tourists' requirements.

8.Means awesome holidays: the visitors will take with them interesting information and they will send their friends for the same experience, for sure, which will assure a constant flood of tourists.

Regarding the development rules, the building of equipment and the touristic wine-making appliances in Oltenia are fulfilled through: lower buildings with an attractive image, sceneray traits, parking places, natural resouces. (Snak O. and coll., 2001)

The constant oeno-tourism helps the understanding of the touristic activities on the natural, cultural and human environment and assures a touristic development.

The wine-making tourism in Oltenis has the following aims:

-To influence an to motivate the travelling

-To assess the positive results of the past

-To get an open attitude in the relations with the employees

-To practice an active education in order to protect the natural and cultural resources of the touristic industry in order to understand the environmental problems.

Limits. It is necessary to extend a complex research regarding the constant development of the tourism, generally speaking, and of the wine-making tourism. The economic efficacy, the growth of the social cohesion and the protection of the environment must be considered.

#### CONCLUSIONS

Like any other human activity, the oeno-tourism needs touristic resources and participates at the damage and the pollution of the environment.

The oeno-tourism of our area needs a healthy environment with beautiful landscapes as a starting-point for all the other touristic activities. The wine-making tourism of this area represents an opportunity for all the wine-producers and also a way to develop the inside tourism.

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#### Environmental engineering

#### DETERMINATION OF FACTORS THAT REDUCE THE POTENTIAL OF DEGRADATION OF VEGETABLE OILS

Ramona Căpruciu<sup>1\*</sup>

<sup>1</sup>University of Craiova, Str. A.I Cuza, No. 13, Craiova Corresponding author: E-mail address: ramona\_capruciu@yahoo.com

Key words: cold and hot pressed sunflower oil, quality.

#### ABSTRACT

In this study, it is desirable to establish a comparative frame in terms of the degree of oxidation between sun-pressed sunflower oil and sun-pressed sunflower oil obtained from seed lots with a different moisture content. The use of these categories of oil was aimed at obtaining data on their physicochemical stability, organoleptic analysis and the main quality indices (acidity index, peroxide index), determinations with a precise indication of the potential for degradation of the studied oils.

#### INTRODUCTION

In the food industry, the vegetable oil sector occupies an important place by supplying the population with essential products (edible oils, margarines, mayonnaise, etc.) by Gunstone et al, 2011, Popa, V.M et al, 2010.

In order to obtain a good quality oil, the raw material must correspond to the qualitative parameters included in the standards in terms of both the oil content and the moisture content, the parameters being maintained only if optimum conditions are ensured in the storage spaces (M.M. Duda et al. 2007, Sarca Gh., 2004).

Sunflower, as a source of vegetable oil, occupies the first place in our country. High content of unsaturated fatty acids, predominantly linoleic acid (44-75%) and oleic acid (14-43%) and the presence of low linolenic acid (0.2%) give the high nutritional value of the sunflower oil (O'Brien R., 2008). The growing demands of the Romanian market for raw oils obtained by cold pressing are proven by the increasing desire of consumers to know as many technological, nutritional, medical aspects as these oils (D. Mozaffarian et al, 2009, Căpruciu R. 2011). They are also tasteful and contain plenty of protein and liposoluble vitamins.

#### MATERIALS AND METHODS

Determination of humidity content of oilseeds The determination shall be made within 16 hours of receipt of the laboratory sample in closed, labeled packages. It dries in the oven, in a stationary atmosphere. Samples of sunflower seeds are pre-crushed by a laboratory mist and then passed through a 3.15 mm sieve. The screening sample is homogenized and introduced into two troughs, which are fed into the oven together with the lids, for the duration indicated in Stas for each oleaginous feedstockThe time and temperature for sunflower seeds was set at 130 +/-  $30^{\circ}$ C, 1h. After the drying

process ended, the vials were capped and immediately placed in the desiccator for 30 minutes. Once removed from the desiccator, they are weighed again. Calculation is performed according to the formula and is expressed in% umidity.

Determination of organoleptic examination for established oil samples By performing the organoleptic examination according to STAS, the subjective examination of the color, odor, taste and appearance qualities is done. Determination of appearance consists in visual observation in glass container (beaker Berzelius) in natural light. Analyze the clarity, presence of suspensions, flocculation or sediment. Color determination is made by examining the (natural) light of oil samples transported in a colorless glass container, noting whether their color corresponds to the standard and quality. Color change can occur due to certain processes such as wetting, heating, milling or inappropriate drying of the raw material. Examination of smell is done directly, by inspirational evidence. The olfactory test determines possible defects: stale smell (taken from inappropriately stored seed), altered, moldy, rancid, fermented, infestation (eq honey odor to infestation with mites of the raw material from which the oil originates). Determination of taste is made by chewing of 5-10 ml of oil. Before and after each determination rinse the mouth with water. It is appreciated that the taste meets the standard by assessing whether the oil has a chemical taste or other foreign taste. Expression of the results is made by entering in the quality document any reported defects.

Determination of acidity index The acidity index is the amount of KOH, in mg, required to neutralize free fatty acids from one gram of fat. Titrate the dissolved oil in ethanol with 0.1N NaOH solution in the presence of phenolphthalein. Samples of liquid, homogeneous and clear fluid at room temperature are analyzed as such. Samples of liquid oils at room temperature, with insoluble waxes or glycerides, are heated to about 60 ° C, homogenised and filtered through filter paper. Weigh 5 g of the sample (sunflower oil) - (depending on the acidity of the sample) to the nearest 0,01 g and pass it quantitatively into an Erlenmayer glass. Over 100 cm<sup>3</sup> of solvent is placed over the sample. It is then agitaten until complete dissolution of the sample and add 5 drops of indicator. It is then titrated with sodium hydroxide solution to the point of turning of the color (pink in the case of phenolphthalein). Calculation is performed according to the formula and is expressed in % oleic acid.

Determination of peroxide value The peroxide value is used in addition to other quality parameters, for determining the oxidation degree of a product which is composed of fat. This index is a measure unit for the oxygen content linked with peroxide in oils and fats and particularly for the hydroperoxides. The peroxide value was measured by determining the amount of converted iodide to iodine under the action of the active oxygen of the peroxide.

The result was expressed as the number of milligrams of active  $O_2/kg$  of fat. There is only one principle for both methods of the peroxide value determination: the oxidation of iodides to iodine through the active oxygen of peroxide, and measuring the amount of free iodine by titration with 0.01 N sodium thiosulfate solution. An index expressed in milligrams of active  $O_2$  higher than 20, indicates that fats are rancid. If this index is very low, it has no meaning, in this case a simple heating at 130°C is sufficient to destroy the peroxides (Căpruciu R. 2016).

#### **RESULTS AND DISCUSSIONS**

In order to determine which of the two assortments of oil (obtained by hot pressing from seeds with normal humidity (8%) and high-moisture seeds (17%) and obtained by cold pressing from seeds with normal humidity (8% high-grade seeds (17%) retain their high-quality qualitative factors during storage, physico-chemical and

organoleptic analyzes were carried out on both raw material, sunflower seeds and oil samples obtained.

For the steps in which the quality of the oils taken in the assay was established, it began with the organoleptic analysis immediately after production, 14 days after production and 30 days after production. Determination of oil moisture is a quality analysis in the context of obtaining oil samples required for the study (Table 1).

Table 1

#### Determination of humidity on the lots of sunflower seed studied

Component	Sunflower s	seeds
	Lot 1	Lot 2
Humidity (%)	8	17

As a result of this determination, the two seed lots have a different moisture content, making it interesting to note whether this aspect influences the quality of the finished product or not.

After the determination of the humidity, laboratory samples were developed for the analysis of the quality factors. Thus, from each batch of seeds, two samples were formed in order to obtain by pressing (cold or hot) the raw oil. Samples were scored as follows: UR1 - raw oil obtained by cold pressing the seeds of lot 1 (8% humidity); UR2 - raw oil obtained by cold pressing of seed in lot 2 (17% humidity); UC1 - raw oil obtained by hot pressing of seeds in lot 1 (8% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 1 (8% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seeds in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC2 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC3 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC3 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC3 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC3 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC3 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC3 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC3 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity); UC3 - raw oil obtained by hot pressing of see

The organoleptic analysis was carried out under normal laboratory conditions at a constant temperature of 20 °C under natural light conditions, the data obtained being shown in Tables 2 and 3. The overall aspect of the studied samples is oily mass with a high degree of shake suspension in the cold-pressed sun-float oil and with an average stirring suspension in the hot-pressed sunflower oil. Also, when tasting, both cold pressed sunflower oil and hot-pressed sunflower oil showed intense flavor specific to the raw raw material from which it was obtained. The UR2 sample is slightly bitter. For the odor analysis, rapid inhalation from coated pots for a few seconds was performed, the four samples showing a characteristic odor of the raw material from which they originated, more pronouncedly perceptible to sun-dried sunflower oil obtained by hot pressing. The color of the analyzed samples was more intense for the oil obtained by cold pressing. Samples obtained from high moisture seeds showed spots or grains in the oil mass. By comparison organoleptically, the analyzed samples reveal differences in color, consistency, smell and taste, differences in raw material moisture and method of production (cold or hot pressing).

The oil samples taken in the study were analyzed qualitatively by two physicochemical indices recorded in STAS: the acidity index and the peroxide index, the main indices giving clear data regarding the quality of the obtained oil, especially their oxidative potential in storage time. Laboratory analyzes reveal differences between the two categories of analyzed oils (cold and hot pressed) in terms of the value of the physical and chemical indices analyzed, the differences being noted even within the same type of oil (different lots table 4). It is noted that the free acidity expressed in% oleic acid in sunflower oil obtained by hot pressing is lower than that recorded in the cold-pressed sunflower oil. Also, samples of sunflower seed with low moisture content show a lower acidity, maintaining the ratio during the analysis (30 days after production). Table 2

The main organoleptic indexes of sunflower oil after cold pressing

			Organoleptic in	dices	
Oil types		appearance	taste	smell	Colour
		Slightly present sediment	Pleasant, intense	Pleasant,	Brownish brown,
		Suspensions are reported	aroma of sunflower	specific, with	without any other
	UR1	in the oil mass. Semifluid	seeds. It does not	intense	stains or
		consistency	taste rainy or bitter	sunflower seed.	degradations of that
Sunflower cold				No foreign smell	color
pressed		It contains coarse	Pleasant, intense	Pleasant,	Brownish brown,
		sediment. Coarse	aroma of sunflower	specific, dark	slight spots of color
	UR2	suspensions are reported	seeds. It is slightly	brown, brown.	mentioned
		in the oil mass. Semifluid	bitter	No foreign smell	
		consistency			

Table 3

The main organoleptic indexes of sunflower oil after obtaining by hot pressing

# and the second sec ć

	Colour	Yellow	without any	other spot or	color shades		Yellow, with	
	smell	Pleasant, strong,	specific. No	foreign smell	1		Pleasant, specific,	
Organoleput mutes	taste	Intense aroma of	sunflower, persistent at the	opening of glass	It does not taste rainy or	bitter	Medium-flavored aroma	
	appearance	Slightly present	sediment. No oil in	suspension is signaled.	Fluid consistency		Presents medium	
					UC1			
Oil types					Sunflower hot	pressed		

degradation

in oi

slight

no foreign smell

It does not taste racced

sediment. No oil in suspension is signaled. Fluid consistency

<u>2</u>2

#### Table 4

		Oil types						
	Storage time	Sunflo	wer cold	Sunflo	ower hot			
		pre	ssing	pre	ssing			
		UR1	UR2	UC1	UC2			
Acidity index (I.A)	At production	1,1	1,4	0,5	0,9			
	14 days	1,4	1,9	0,7	1,4			
	30 days	1,8	2,3	1,1	1,8			

Determination of the acidity index (I.A) in the studied oil types

In which: I.A. – the acidity index; UR1 - raw oil obtained by cold pressing the seeds of lot 1 (8% humidity); UR2 - raw oil obtained by cold pressing of seed in lot 2 (17% humidity); UC1 - raw oil obtained by hot pressing of seeds in lot 1 (8% humidity); UC2 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity)

Table 4 shows that the acidity index records double values of acidity for sample UR1 versus UC1 immediately after production (1.1% versus 0.5%). Two weeks after production, all samples analyzed showed an increase in acidity, after one month of production and storage under the appropriate conditions (room without light, aerated at 8 °C) only the sample UC1 (raw oil obtained by hot pressing of the seeds in lot 1 (8% humidity) being fit for consumption, according to recorded data. Due to the technological process of production which involves keeping oil samples in contact with the air for a longer period of time (compared to the production of refined oil), both during the actual pressing and during the decanting, there may be oil degradations visible to the eye free (especially color change but also smell and taste).

For this reason, the determination of the peroxide index (Table 5) together with a thoroughly organized organoleptic analysis can lead to the exact determination of the oxidative potential of the analyzed samples.

After determining the peroxide index, it is found that the cold pressed oil samples degrade faster than the hot-pressed oil samples. Also, oil samples obtained from seed with improper humidity show upward values (figure 1) of the peroxide index over a period of 30 days, which means that these oils are not suitable for storage.

Following the physico-chemical analysis of the oil samples analyzed, recommendations can be made regarding consumption and storage period.

Table 5

		Oil types						
		Sunflo	wer cold	Sunflower hot				
The peroxide index	Storage time	pre	ssing	ng pressi				
(lp)		UR1	UR2	UC1	UC2			
(meq O <sub>2</sub> /Kg)	At production	3,41	3,88	3,22	3,64			
	14 days	3,66	4,20	3,65	3,88			
	30 days	4,20	5,80	4,14	4,23			

Determination of peroxide index (lp) in studied oil types

In which: Ip- the peroxide index; UR1 - raw oil obtained by cold pressing the seeds of lot 1 (8% humidity); UR2 - raw oil obtained by cold pressing of seed in lot 2

(17% humidity); UC1 - raw oil obtained by hot pressing of seeds in lot 1 (8% humidity); UC2 - raw oil obtained by hot pressing of seed in lot 2 (17% humidity)

Thus, out of the four analyzed samples, sunflower oil obtained from seeds with normal humidity by hot pressing is recommended for consumption.



Figure 1 Determination of peroxide index (Ip) in studied oil types

It should be noted that the indices obtained for samples from seeds with normal humidity but especially for samples of high humidity seeds indicate that they are not suitable for long-term storage and are recommended to be used within a maximum of two weeks (for sample UR1) and within 1 week (for UR2) as soon as the oil has been in contact with the air (desintegration of the container) and keeping it at 8-10  $^{\circ}$  C.

#### CONCLUSIONS

Following the determination of moisture, laboratory samples were developed for the analysis of the quality factors, from each seed lot being taken two samples to obtain by pressing (cold or hot) the crude oil.

By comparison organoleptically, the analyzed samples reveal differences in color, consistency, smell and taste, differences due to the humidity of the raw material and the method of production (cold or hot pressing).

Laboratory analyzes revealed differences between the two categories of analyzed oils (cold and hot pressed) in terms of the value of the physico-chemical indices analyzed, the differences being noted even within the same type of oil (lots different).

The free acidity expressed in% oleic acid in sun-dried sunflower oil is lower than that recorded in cold-pressed sunflower oil. Samples obtained from sunflower seeds with a low moisture content have a lower acidity, maintaining the ratio during the analysis period (30 days after production). As a result, oil samples obtained from high moisture seeds are not suitable for long-term storage. In order to reduce the oxidative potential of these samples it is recommended that the seeds be subjected to the drying operation with the normalization of moisture before the pressing process. The study concludes that the moisture content of sunflower seeds is a first factor that can increase the potential for degradation of the oils analyzed, this aspect being of interest in keeping the oil in the household after the container has been unsealed and the oil is kept at a temperature room or higher (cooking temperature during cooking) or if the batch was not purchased and is nearing the end of the shelf life.

Air, by the presence of oxygen, is another factor that can lead to the oxidative depreciation of the analyzed oil samples, requiring shortening the contact time between the oil and air mass as a reduction factor of the oxidative potential. Determination of the peroxide index along with a thoroughly organized organoleptic analysis could lead to the exact determination of the oxidative potential of the analyzed samples. Following the physico-chemical analyzes of analyzed oil samples, sunflower oil obtained from seeds with normal moisture by hot pressing is recommended for consumption.

It should be noted that the indices obtained for samples from seeds with normal humidity but especially for samples of high humidity seeds indicate that they are not suitable for long-term storage and are recommended to be used within a maximum of two weeks (for sample UR1) and within 1 week (for UR2) as soon as the oil has been in contact with the air (unsealing of the container) and keeping it at low temperatures.

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#### QUALITATIVE ANALYSIS OF TYPES OF ICE CREAM

Ramona Căpruciu<sup>1\*</sup>

<sup>1</sup>University of Craiova, Str. A.I Cuza, No. 13, Craiova Corresponding author: E-mail address: ramona\_capruciu@yahoo.com

Key words: types of ice cream, quality

#### ABSTRACT

Three types of ice cream, often used by consumers: ice cream with vanilla, cocoa and fruit, ice cream with vanilla, cocoa and fruit and fruit ice cream, wishing -the organoleptic and physico-chemical analysis of these ice-cream varieties with great demand among consumers. The biotechnological assessment of the ice cream assortments under study will be based on the fat content (milk ice cream) and the acidity (fruit ice), following certain physico-chemical changes during storage.

#### INTRODUCTION

Ice cream and ice cream specialties are prepared from a special process based on dairy products, fats, sugars, flavors, dyes, stabilizers, etc. The quality of each ingredient is monitored from a biotechnological, microbiological, physico-chemical and sensory (by Banu et al., 1993, 2009). The quality of fresh milk used as raw material to obtain ice cream influences both the technological process and the quality of the finished product (by Reithofer, 2011). Milk and dairy products have the capacity to provide biologically active components (especially vitamins and trace elements) that are fundamental to the promotion of human health (Scott et al., 1988, Baldi et al., 2005). Fats can provide significant protein intake (Koxholt et al., 2001, Ruger et al., 2002, Shaviklo et al., 2011). Ice cream is a food offering to the liking of consumers, and because it can be obtained by mixing more raw materials, the perceived organoleptic sensations are multiplied by the richness of nutrients found on the label (by Tosaki et al., 2009, Dickinson, 2013).

#### MATERIALS AND METHODS

Determination of fat content by acid-butyric method

The principle of the method consists in separating the fat in the butyrometer by centrifugation after the preliminary dissolution of the protein substances in sulfuric acid in the presence of isoamyl alcohol.

Working mode Add 10 cm<sup>3</sup> of H2SO4 and 5 cm<sup>3</sup> of ice cream thoroughly homogenised in the butyrometer. The frozen pipette is rinsed with 6 cm<sup>3</sup> of distilled water and added to the butyrometer then 1 ml of isoamyl alcohol is added. Remove the butirometer with cotton wool, screw the rubber stopper and mix. After homogenization, the butyrometer is centrifuged for 5 minutes at 1000-1200 rpm, removed from the

centrifuge and placed on the water bath at 65°C. Read the fat content on the butirometer rod and the reading read multiplied by 2.2.

Determination of total acidity of frozen is done by the current titration method, which applies to milk and all dairy products, including ice cream. The acidity of a given volume of sample prepared for analysis is neutralized with a 0,1 n NAOH solution in the presence of phenolphthalein as an indicator. It is expressed in Thörner grade = acidity in 100 cm<sup>3</sup> of the product which is neutralized with 1 cm<sup>3</sup> of NaOH solution 0,1n after calculating and interpreting the results.

Determination of dry substance content consists of using a special balance (Lacta). Introduce 5 g of paraffin and 5 g of ice cream. Heat moderately for evaporation and shake vigorously. When the clock glass is no longer steamed, the evaporation is complete. Keep in desiccator 10 minutes after balancing on the balance with 2 riders. Rider. 1 (2 g) 1 div. = 0.4 g; Rider. 2 (0.5 g) 1 div. = 0.1 g x • 0.4 g + y • 0.1 g = zg water of 5 g frozen.

Determination of sugar from ice cream

Determination of sugar is made by the iodometric method in two variants. The aldehyde group of the reducing sugar is oxidized with iodine in alkaline medium. Excess iodine is titrated with sodium thiosulphate solution in the presence of starch. The titre of the sodium thiosulphate solution (T) expressed in grams of sucrose per ml of solution is calculated according to the formula and expressed in g/cm<sup>3</sup>.

#### **RESULTS AND DISCUSSIONS**

The nutritional impact of ice cream ingredients is often transposed into their biotech assessment to determine how they can influence consumer health. The biotech assessment of ice frost during the hot season is of particular importance and is based on several aspects. It primarily concerns the quality of the raw materials that are part of the ice cream, the strict observance of the technological process and last but not least the keeping and transportation to the beneficiary under appropriate conditions.

In order to determine the nutritional value of the frozen types of ice cream, the chemical analysis of the main compositional elements was performed. The data obtained are listed in Table 1. Greater fat content is found for cream based ice cream than for milk. (8.5% versus 3.5%). In the frozen fruit, the fats are missing. Figure 1 shows the large difference in fat content of the three types of ice cream analyzed. Concerning the sugar content, it can be seen that both types of ice cream (milk-based and cream-based) have a similar content in sugar (14-15%), while fruit ice has recorded values close to twice as high compared to other assortments analyzed (27% versus 14%).

Table 1

Types of ice cream	Fat (%)	Sugar (%)	Dry substance (%)	Acidity (°T)
Of milk:				
<ul> <li>with vanilla, cocoa</li> </ul>	3,5	14	27	20
- with fruit	2,8	15	28	46
By the cream:				
- with vanilla, cocoa	8,5	14	32	21
- with fruit	7,0	15	31	47
Of fruit:				
- with fruit flavor	-	27	27	68

#### Composition analysis of the main types of ice cream



Figure 1 Determination of the fat content of the studied ice cream

And acidity, expressed in degrees Thorner, recorded similar values for milk and cream ice cream (20-21°T). The opposite is the ice cream with fruit, whose acidity was 68 degrees tall. And within the same assortment (eg ice cream), the fruit has higher acidity (46°T to 20°T) - figure 2.



Figure 2 Determination of acidity in the ice cream assortments taken into study

The highest content in dry matter is recorded in cream with three forms (vanilla, 32% cocoa and 31% fruit). The lowest value in dry matter was milk vanilla with vanilla and cocoa and ice cream (27%).

Fats and sugar, together with proteins, represent the basics that give the nutritional value of the types of ice cream studied.

The main biotechnological aspects in evaluating a range of ice cream are mainly transposed into ice cream defects. Ice cream presents a number of defects, the most common being organoleptic: aroma, texture and color.

Ice cream like any food is assessed biotechologically by Stas, following the admissibility conditions (Table 2).

The organoleptic evaluation of the ice cream samples taken in the study did not reveal any major defects. However, it can be mentioned, from the category of flavor defects, the slightly sour, poorly perceptible taste of fruit milk ice cream. An explanation would be the use of strawberries with an average degree of freshness.

Table 2

#### Organoleptic characteristics for ice cream

Characteristics	Admissibility conditions
Taste and aroma	Clear, characteristic, for ice cream of the given type and the raw material used in its manufacture, with no foreign flavors and smells; taste sweet or sweet-sour. To use the food ingredients to taste and smell - must match the taste and flavor of the ingredient introduced. When using flavorings - it must match the flavor and aroma of the flavor enhancer. There is insufficient taste or scent or excessive odor of the flavoring used. When using glaze coating and food ingredients for decoration - it must match the given type of ice cream in combination with the taste, aroma and smell of the coatings used and the decoration ingredients. No smell and taste of fodder, seasons, rînced, fish, burn, metal, mineral, salty, alien. It does not allow insufficient or excessively sweet taste.
Consistency	Dense enough for tanned ice cream. Dish for food ice. Soft, creamy for soft ice cream.
Appearance in section	Homogeneous, without perceptible organoleptic agglomerations of fat and stabilizer (stabilizer-emulsifier). When using the food ingredients with the appearance of the pieces - with the presence of their particles. No perceptible organoleptic ice crystals - for soft and tempered ice cream. It is not allowed the structure and consistency of snow, ice, flakes, flour, sand, rock, hard fusible. The tiled ice cream structure with a fat mass fraction of not more than 5% and a dry mass fraction of dry matter of not more than 30% and ice cream based on sugar syrup are allowed. For glaze ice cream the color of the coating - characteristic of the given type of glaze or chocolate.
Exterior appearance	For tanned ice cream with a good shape, without deformations. Admitted (up to 10 mm) small spills of ice cream and / or glaze over waffles or biscuits. Cracks and other insignificant mechanical damage (not more than 10 mm) of glaze or waffles, crackers, including edges of waffle products are allowed. In glazed ice cream the glaze must cover the ice cream tightly. It is not allowed to shrink and detach ice cream from waffle and biscuit products.

The beginning of the defect is also due to the high acidity found in this ice cream assortment. The taste and smell of the rancid was detected in the case of ice cream with cocoa cream, and was largely due to the use of inappropriate raw materials or the non-observance of the stages of the technological process of production (the basic mixture was maintained too long before to be frozen in the presence of air, resulting in the phenomenon of oxidation of the fat in the composition).

The texture defect such as the sandy texture has also been encountered in the fruit frost, the factors that lead to the formation of a non-uniform mixture are: the poor quality of the raw materials used, the composition of the mixture, the processing method, the freezing and quenching process. Thick texture met with ice cream and vanilla ice cream, persisting for a longer time than fat.

Color defects were encountered in fruit ice (the intense pink color specific to strawberries was not homogeneous in the mass of the product) and in milk cocoa ice cream, where the intense cream color was also uneven in the mass of the product.

#### CONCLUSIONS

Following the determinations, there is a higher fat content in whipped cream based on cream than milk. fruit ice cream. and fats lacking. Fruit ice cream sugar content has almost doubled compared to the other assortments analyzed. Acidity, expressed in degrees Thorner, recorded similar values for milk and cream ice cream and higher for fruit ice, both as a single assortment and in other assortments.

The highest content in dry matter is recorded in whipped cream with the three forms (vanilla, cocoa and fruit), a lower dry amount found in vanilla ice cream and cocoa and fruit ice cream.

The main biotechnological aspects in evaluating a range of ice cream are mainly transposed into ice cream defects.

Some of the ice cream varieties analyzed showed a number of defects, the most common being organoleptic: flavor, texture and color. So:

Fruit milk ice cream: flavor defects (slightly sour, poorly perceived taste);

Coconut ice cream: taste and smell easily rancid;

Fruit ice cream: sandy texture, color defects.

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#### ASSESSMENT OF THE TECHNOLOGICAL QUALITIES OF THE SOME INTRASPECIFIC HYBRID ELITES FOR THE TABLE GRAPES COMPARED WITH THE PARENTAL FORMS BY THE METHOD OF THE DISTRIBUTION ON THE FREQUENCY CLASSES

Ciobotea Cristina-Magdalena<sup>1</sup>, Popa Camelia<sup>1</sup>, Radomir Ana-Maria

<sup>1\*</sup>N. I. R. D. B. H. Stefanesti-Arges, Sreet Bucharest - Pitesti, No.37, Stefanesti, cretcris76@yahoo.com

**Key words**: technological descriptors, distribution on the frequency classes, hybrid elites, parental formes

#### ABSTRACT

The present paper reveals the features and technological characteristics of 21 hybrid elites resulting from the hybridization of four soups for table grapes in three distinct combinations (Muscat lantarni x Canner, Victoria x Black Pearl and Victoria x Victoria) selected from the comparatively field, and also highlights the combined value of the varieties used in the amelioration process by means of distribution by frequency classes.

In order to highlight the technological value of the genotypes obtained from the three combinations mentioned above as well as the combination potential of the varieties involved in the breeding process, the frequency distribution of the average values obtained was used. The multitude of the data obtained was statistically and mathematically processed, based on the descriptive statistics, with general clues on the way of manifestation of the hybrid vines genotypes in the interaction with the biotic and abiotic factors.

#### INTRODUCTION

Based on data obtained, by statistical and mathematical processing, by the descriptive statistics, general conclusions have been formulated concerning the manner of manifestation of the hybrid elites grape-vine in interaction with the biotic and abiotic factors having regard to natural variability of all processes and phenomena involved.

This presents two aspects: - Representation of the entire distribution, by: numerical (frequency analysis) and graphics (bar, histogram, box plot, stem and leaf); central tendency indicators: mode, average, median; dispersion indicators: amplitude, standard deviation; the indicators of the distribution form; index of asymmetry (skewness) and index of vaulting (kurtosis).

**Skewness (skewness)** and *Kurtosis (kurtosis)* în SPSS - Skewness and kurtosis values are normal and equal to 0. In the case of a random sample, extracted from a normal population, it is unlikely to obtain the indices of symmetry and vaulting equal to 0. Must be known if the sample of the analyzed values are derived from a population asymmetric or abnormal vaulte.

The utility of the normality test of the distribution relates to the decision to use parametric tests in the case of the quantitative variables, which are subjected to this condition. Both the K-S test and Shapiro-Wilk are sensitive to the asymmetry and

vaulting. Therefore, when it is used the t test or analysis of variance, vaulting is less important than the asymmetry. For this reason, if the test of normality is significant, it is advisable to check whether this is due to the asymmetry, vaulting or both. For both tests (Kolmogorv-Smirnov and Shapiro-Wilk), is only important the value of p (Si G.). this being understood inversely than the classical interpretations of the p.

#### MATERIAL AND METHODS

In order to highlight the technological value of the hybrid elites resulting from three combinations (*Muscat lantarnii x Canner, Victoria x Black Pearl*, Victoria x *Victoria*) and the combinational value of the varieties involved in the breeding process, the frequency distribution of the obtained mean values was used. Were introduced into the histograms all the values of the three types of hybrid descendants with the parental varieties (26 genotypes), for characters analyzed. For more meaningful representation of the data, it used the class frequency distribution of the average values of parameters determined for elite hybrid and parents.

From here results that the result interpretation of the normality test is determined by the simultaneous correlating of the p-value with the sample size. The Gauss distribution for the normal values, in the form of a bell, is represented by two values: **the average value and standard deviation**.

#### **RESULTS AND DISCUTIONS**

A symmetrical distribution and only slightly platykurtic compared to the average was recorded in the case of yhe average weight of a grape. The coefficient of variation of the values around the mean was average (25,9%), the maximum oscillation limits being between 150 minima and 565 maxims.

With an average grape weight of 565 and 560 g, the selected elites A1 and A19 are at 4 frequency classes in addition to the average. Low values below 250 g show only the *Muscat lantarnîi* variety of the maternal genitor. All the elites had grapes heavier than 250 g (figure 1).



#### Greutatea medie a unui strugure (g)

Figure 1 - Distribution on the frequency classes of average values of the average weight of a grape, to the hybrid elites

Also an empirical distribution with right asymmetry and weak leptokurtic is indicated in the case of the average weight of 100 berries. The maximum oscillation of

the indicator was 1165 g by weight of 100 berries (between 234 and 1400). The average was 524,96 and the higher coefficient of variation (53,3%) the sample being more heterogeneous. Asymmetry is the right, which shows that dominates the elites with the value of the weight grape less than average. The vaulting coefficient had a value of 3,901, which shows the occurrence of excess values only near the mean and far from it, with few representations in the high-value classes (figure 2).



#### Greutatea medie a 100 boabe (g)

Figure 2 - Distribution on the frequency classes of average values of the average weight of the 100 berries, for F1 elites

Selected elites were distributed more around the average, but there were 2 genotypes with double values from the mean: A16 (1400 g) and A19 (1100 g). Lower values recorded the hybrid descendents resulting from *Muscat lantarini* x *Canner* combined, character influenced by the berries of the maternal variety.

The content of the must in sugars ranged between 110 and 210, the maximum variation amplitude was 100 g / l, and the deviation of the values around the average of 160,15. The coefficient of the variation was only 15%. The deviation from normal was reduced. The elites were in a large number around of the average of 160,15 (15 elites), with the values of the sugars from the must ranging from 140-180 g/l. The lowest value for this indicator was the Hi10 elite (110 g / l), and the highest content, the A31 elite (210 g/l) (figure 3).

The histogram of the must acidity for the hybrid descendents has the following characteristics: the mean value was 3,031 g/l of H  $_2$ SO4 and the coefficient of variation of 19,6%.

The excess of the sample was close to the normal distribution. There was a slight right asymmetry, which means that values below the mean prevailed. With acidity below 2,2 g / I of H  $_2$ SO4, the elites from the BP group (BP2, BP9, BP11) and a group A (A17) elite predominated, and with the highest level of acidity, the group Hi (Hi3 and Hi11) (figure 4).

#### Continutul in zahar al mustului (g/l)



Figure 3 - Distribution on the frequency classes of average values of the must content in sugar, to the hybrid genotypes



Aciditatea mustului (g/I H2SO4)

Figure 4 - Distribution on the frequency classes of average value of the must acidity, for F1 hybrids

#### CONCLUSIONS

Conclusions on the study of the **technological characters** of elites resulted by *Muscat lantarnii* x *Canner* hybrid combination (Hi) showed the following points:

• all elites had larger berries than the hybridized variety (*Muscat lantarnii*) and two elites (Hi17 and Hi19) passed the *Canner* variety to this descriptor;

• the sugars content to hybrid descendents from this combination (Hi) ranged from 110 g/l for the Hi13 elite to 180 g/l at the Hi17 elite; the Hi19 elite accumulated 160 g/l, being overtaken by three elites, including Hi9 and Hi11 (175-170 g/l);
• three elites (Hi11, Hi17 and Hi19) inherited from the maternal variety the muscat taste, and an elite (Hi13) acquired from the paternal genitor the specific taste, and another elite presented the frank taste;

• all elites had the greenish-yellowish berry.

Conclusions on the study of the **technological characters** of elites resulted by *Victoria x Black Pearl* (BP) hybrid combination showed the following points:

• because the varieties with large grapes participated in the hybridization process, it is noted that the descendants presented large and very large bunch;

• the *Black Pearl* paternal variety was exceeded, in regards the weight of bunch, by five elites (exception only makes BP2 elite with 310) and *Victoria* variety with weight 470 g was equalized by the elite BP11 (470 g); the rest of the descendants presented have values above 400 g, respectively higher than the average of all analyzed genotypes;

• the *Black Pearl* variety was overtaken by the weight of 100 berries by the all the elites and the *Victoria* variety rated and considered standard for the large berry, was surpassed by the BP 11 elite, with 20 g (650 g, respectively 670 g); four hybrid elites can be ranked in the large berry class because they have values above 500 g, well above the average of 493 g; with regard to the sugar content, BP8 elite is particularly noteworthy, with 206 g/l, the value rarely encountered in table grape varieties, and especially in hybrid elites;

• the acidity of the must showed close values between them, three elites placed above the average and cross-breeds varieties: BP2, BP7, BP8, with concentrations between 3,3-3,8 g/l H <sub>2</sub>SO4;

• it is noted that the BP 2 and BP 7 descendants have a discrete flavor, the rest of the elites beeing whitout flavor;

Conclusions on the study of the **technological characters** of elites resulted by *Victoria x Victoria* hybrid combination (A) showed the following points:

• *Victoria* variety was surpassed in the average weight of 100 berries by the six of the 10 selected hybrid elites (A1, A5, A16, A17, A19, A27);

• sugars content higher than *Victoria* (141 g/l),we meet at the elites: A1, A5, A9, A6, A16, A19, A25, A31;

• it was found that there is a significant positive and linear correlation between the weight of 100 berries (g) and the weight of a grape (g) to the all the studied elites;

• it is interesting and surprising at the same time that the elites A1, A9, A25 have the muscat flavor, elites A5, A6, A16, A17 possess a specific flavor;

• muscat flavor is intense at A1 and A9 and discrete at A6 and A 25.

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#### Environmental engineering

# STUDY ON THE BEHAVIOR OF CAPSICUM GRAFTED ON DIFFERENT ROOTSTOCKS IN SANDY SOIL CONDITIONS

Ciuciuc Elena<sup>1</sup>, Croitoru Mihaela<sup>1</sup>, Vânătoru C.<sup>2</sup>

<sup>1</sup>Research-Development Center for Field Crops on Sandys Soils Dabuleni <sup>2</sup>Development Research Center for Vegetables Buzău *Corresponding author:* ciuciucelena@yahoo.com

Key words: capsicum, grafting, rootstock

# ABSTRACT

Decreasing or eliminating the attack of pests and soil pathogens can be achieved by introduction grafting crop technology. For this purpose Was used as a graft the Işalnita 85 V variety grated and as rootstocks was used 6 lines obtained at SCDL Buzău (L 12, L 60, L63, L66, L70E şi L70P). Determinations were made on the main physiological processes, the height increases of the capsicum plant, the plant health status, the number of fruit / plant, the average weight of a fruit, the total and dynamic production and the biochemical content of the fruit. At the first harvesting by grafting on the L66 rootstock, the highest yields were achieved, ie 13.8 t / ha, with 1 t / ha more than plants without grafting, and the smallest production was made on grafted plants on L12. Total production was 40.0 t / ha for plants without grafting and between 32.9-43.8 t / ha and the grafted on L 70 E by 43.2 t / ha. The plants grafted on the L66 rootstock were distinguished by a well developed root system, with thick, strong roots associated with the lack of nematodes, and contributed to significant production gains.

#### INTRODUCTION

The newly introduced pepper grafting has emerged as a necessity for controlling pathogens and nematodes in the soil and for increasing the tolerance or resistance to stress factors. In This has scope was studied numerous of rootstock for compatibility of the capsicum culture.

Attia M.F. et al., 2003 demonstrated that solanaceae rootstocks were accepted as compatible for both sweet pepper culture and pepper culture. The effect of pepper grafting on Fusarium infested soil was conclusive.

The effect of pepper grafting on Fusarium infested soil was conclusive. Maria Maribel Rodriquez and Paul W. Bosland, 2010, studied the compatibility of tomatoes as rootstocks for Capsicum annuum L. and concluded that grafting peppers on tomatoes provides a mechanism for controlling soil and nematode diseases that negatively affect pepper and that Is still worth investigating. The older paprika plants had a good development, connection to the graft site compared to the young plants. The authors also conclude that rootstock root can be crucial for leaf water content, especially under stress conditions. The fact that grafting reduces stress was also reported by other authors (Lee J.M. et al., 1998, Rivero R.M., 2003, Shibuya T. et al.,

2007, Carmen Martinez-Ballesta, 2010). The authors believe that the physiological and biochemical aspects of the graft-rootstock interaction should be studied.

The nutritional qualities of grafted pepper plants such as total dry substance, soluble dry matter, titratable acidity are comparable of the plants without grafting, while of grafted plants increased vigor and resistance to soil and nematode diseases (Colla G. et al., 2008; Davis AR, 2008a). For the management of nematodes and the replacement of methyl bromide by grafting the pepper plants choose and other authors (Lee J.M. et al., 1998, Attia M. F. et al., 2003, Morra L and Bilotto M., 2006, King S.R., 2008).

#### MATERIAL AND METHOD

At the Research-Development Center for Field Crops on Sandy Soils Dabuleni was studie the behavior of the Isalnita 85 V grape variety was grafted on various rootstocks obtained at SCDL Buzău: L 12, L 60, L 63, L 66, L 70 E, L 70 P, compared to the plants without grafting.

Was determine of the rootstock influence on the health status of the capsicum plants (identification of pathogens of soil and nematodes), determinations of productivity (number of fruit / plant, average weight of fruits) A fruit, total and total sweet pepper production) and the determination of the biochemical content of the fruit. The results were calculated and interpreted statistically.

Seedlings were obtained in the double-shielded solar hood. The grafting method used was that of "sticking with a cotyledon".

Fruit harvesting was done at the consumption maturity, staggered.

#### **RESULTS AND DISCUSSIONS**

The smallest elevations in were made in plants grafted on L 70 E and L 70 P, and the largest waistline was recorded at Isalnita 85 V grafted on L60 (Table 1). The number of fruit/plant was influenced by the rootstock used being of 14.2 fruit/plant on the without grafting plants and ranging from 11.7-15.6 fruit/plant to the grafted plants. The largest number of fruits was recorded in plants grafted on L 66 and L 70 P. The average weight of a fruit oscillated within very narrow limits and ranged from 50-53 g/fruit.

Table 1

Rootstock	Plant height (cm)	Number of fruits / plant	The average weight of a fruit (g/fruit)
Without grafting	35.6	14.2	50
L12	37.4	14.0	51
L60	42.2	14.4	53
L63	35.8	14.8	53
L66	33.4	15.4	51
L70 E	33.0	15.6	50
L70 P	30.6	11.7	51

Biometric determinations made

Five crops were taken as the fruits matured. The first harvest took place on July 22, and the productions made at that date were greatly influenced by the rootstock used (table 2).

The Isalnita 85 V cultivar without grafting achieved on July 22 a production of 12.8 t/ha, which was exceeded by the production obtained from the L66 grafted plants at 13.8 t/ha and to those grafted on L60 that achieved 12.2 t/ha. The lowest yields were obtained at this time on L12 grafted plants (5.5 t / ha), L70P (7.2 t / ha) and L63 (9.4 t / ha).

Table 2

	Production of capsicum obtained on (t / ha):					
Rootstock	22.07	14.08	26.08	9.09	23.09	
Without grafting	12.8	11.6	5.0	5.0	5.6	
L12	5.5	7.2	10.0	11.1	6.1	
L60	12.2	4.4	7.8	5.0	13.3	
L63	9.4	5.0	6.7	8.9	11.1	
L66	13.8	6.7	7.2	10.0	6.1	
L70 E	11.1	6.1	9.4	9.4	7.2	
L70 P	7.2	3.4	5.6	11.1	5.6	

The dynamics of the production of capsicum according to the rootstock used

At the second harvest, on August 14, the production of without grafting plants was maintained at a high level, of 11.6 t / ha, while at grafted plants were low, ranging from 3, 4-7.2 t / ha. At the following harvests, the yields of without grafting plants decreased and were maintained at high levels in grafted plants, the rootstock being resistant to thermal stress conditions.

Total capsicum production was influenced by the rootstock used (table 3). The Isalnita 85 V variety produced a 40 t / ha production in without grafting crops, compared to which, by grafting on the L12 rootstock, a very close production was achieved, the difference being only -0.1 t / ha.

Table 3

Rootstock	Production achieved		Difference	Signifficanc					
	t/ha	%	(t/ha)	е					
Without grafting	40.0	100	Mt.	Mt.					
L12	39.9	100	-0.1						
L60	42.7	107	+2.7						
L63	41.4	104	+1.4						
L66	43.8	110	+3.8						
L70 E	43.2	108	+3.2						
L70 P	32.9	82	-7.1	0					
DL 5% = 6.46 t/ha	DL 1% =	8.79 t/ha	DL 0.1% =	11.77 t/ha					

Influence of rootstock on the production of capsicum

Using the L60 rootstock a production of 42.7 t / ha and a production increase of 2.7 t / ha was achieved, and by grafting on the L63 rootstock there was an increase of 1.4 t / ha. The production of the without grafting variety was also exceeded by that obtained with plants grafted on the L 70 E and L 66 rootstocks, the production yields of which were 3.2 t / ha using the L70 E rootstock and 3.8 t / ha at the L66 rootstock. The smallest production obtained by grafting the Isalniţa 85 V variety was obtained by grafting on the L70 P rootstock, this being 32.9 t / ha, the production difference being 7.1 t / ha.

After the last harvest, the root system was unveiled and the presence / absence of nematodes was determined and the degree of root development based on the rootstock used (table 4).

Table 4

Highlighting the nematode attack on grafted plants on the studied rootstocks

Rootstock	Presence /	Remarks
	absence of	
	nematodes	
Without	Preence	Very few nematodes, well developed root system,
grafting		thick roots
L12	Absence	Rich radicular system, thin roots
L60	Absence	Radical lax system
L63	Absence	Weak root system developed
L66	Absence	Root system developed, thick roots, strong
L70 E	Presence	Nematodes in very small number, lax root system,
		thick roots, few
L70 P	Presence	Much nematodes, root system developed

The rootstock L12 showed a root system rich in roots, but very thin, a poor presence of nematodes, the production obtained by it being very close to the without grafting witness. The rootstock L60 and L63, although presented with a radicular system with few roots, the lack of nematodes positively influenced the produced productions.

Among the studied rootstocks, a well-developed root system with thick, strong roots associated with the lack of nematodes has contributed to the achievement of significant L66 production increases.

The rootstock used has influenced not only quantitative but also qualitative production (table 5).

Table 5

The influence of the rootstock on the quality of the capsicum fruit

Rootstock	Water (%)	Total dry substance (%)	Solubil dry substance (%)	Titratable acidity g citric acid in 100 g f.s.	Carbohy- drates (%)	Vitamin C (mg/100 g f.s)
Without grafting	93.96	6.04	4.2	0.19	3.50	21.12
L12	93.68	6.32	4.6	0.36	3.80	29.92
L60	93.69	6.31	4.4	0.19	3.70	40.48
L63	94.67	5.33	4.4	0.36	3.68	36.08
L66	93.17	6.83	4.6	0.18	3.85	38.72
L70E	94.38	5.62	4.0	0.19	3.35	36.96
L70P	93.54	6.46	4.6	0.23	3.82	36.08

The following determinations were carried out in the laboratory: water content and total dry substance (gravimetric method), soluble dry matter (refractometric method), total sugars (Fehling Soxhlet method), titratable acidity (titrimetric method), vitamin C (iodometric method). Total dry matter content ranged between 5.33% for Isalniţa 85 V grafted on L 63 and 6.83% grafted on L70P. Compared to the without grafting control in most grafted variants, the amount of total dry substance showed higher values. The soluble dry substance content, in all grafted variants, showed values comparable to those of the without grafting control and was between 4-4.6%.

In peppers, the carbohydrate content is in the form of glucose, fructose and sucrose, the amount of glucose and fructose being higher than that of sucrose. Grafting of plants has favorably influenced the accumulation of sugars in peppers, and the best results were obtained at the fruit of the Isalnita 85 V cultivar grafted on L66 (3.85%).

All pepper varieties contain vitamin C in large quantities, but green capsicum has twice as much vitamin C as citrus, and red pepper contains 330% more vitamin C than orange. In 100 grams of pepper are 125 mg of vitamin C, that is, 100% of the recommended daily dose.

In all grafted variants, higher values of vitamin C content were obtained compared to the without grafting control.

If we analyze the influence of the rootstock on the biochemical composition of the fruit, the best results were obtained in the variant where the plants were grafted on the L66.

#### CONCLUSIONS

At the first harvesting by grafting on the L66 rootstock, the Işalniţa 85 V variety produced the highest yields of 13.8 t / ha and the production was low when using the L12 rootstock.

The biggest productions were obtained by grafting on the L66 rootstock. These were 43.8 t / ha providing a production increase of 3.8 t / ha compared to without grafting. All biochemical components of the fruits showed higher values in plants grafted on L 66. The high yields produced by grafting on the L66 rootstock were due to the fact that it was distinguished by a well-developed root system with thick, strong roots associated with the lack of nematodes and contributed to significant production increases.

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# DEVELOPMENT AND EVALUATION OF A REVERSE-PHASE HPLC METHOD FOR THE ANALYSIS OF CAROTENOIDS IN EGG YOLK

Corbu Alexandru Radu<sup>1</sup>, Violeta Nour<sup>2\*</sup>

<sup>1</sup> "Dunarea de Jos" University of Galati, Romania <sup>2</sup> University of Craiova, Romania Corresponding author e-mail: vionor@yahoo.com

Key words: egg yolk, carotenoids, chromatography, method validation

#### ABSTRACT

Carotenoids from the egg yolk are important pigments influencing the consumer acceptance and essential components associated with health benefits either as antioxidants and immunomodulants or as precursor of vitamin A. Recently ther ehas been considerable interest in enhancing the levels of yolk carotenoids by including carotenoids or carotenoid rich forage products in laying hen diets. In order to learn the variety and amount of carotenoids in regular and modified eggs, diiferent chemical analytical methods have been developed. In the current study, a reversed-phase high performance liquid chromatography (RP-HPLC) method with gradient elution and diode array detection (DAD) was developed and validated for the simultaneous determination of seven carotenoids (lutein, zeaxanthin, canthaxanthin, astaxanthin, lycopene,  $\beta$ -carotene and trans- $\beta$ -apo-8'-caroteneal) in egg yolk.

#### INTRODUCTION

Hen eggs are a very popular food of animal origin, being eaten by many people all over the world. They are served in various ways and they are widely used in cookery for leavening, thickening, binding, and emulsifying (Surai & Sparks, 2001). It is well-known their high nutritional value, due primarily to their great content of highly digestible and complete nutritional proteins containing essential amino acids in a profile similar to the ideal balance of amino acids needed by human (Regal et al., 2014). As a result, the protein of the egg is used as a standard for measuring the quality of other food proteins (Sparks, 2006).

Eggs contain high amounts of lipids (aprox. 11%) consisting of triacylglycerols, phospholipids and cholesterol, more than 99% being located in the yolk (Spark, 2005). Eggs are an important and balanced source of essential fatty acids as well as of some minerals and vitamins. They contain significant amount of phosphorus, iodine, selenium and iron as well as vitamins E, A, B12, B2 and folate (Surai & Sparks, 2001).

Egg yolks serve as an important dietary source of highly bioavailable carotenoids that are essential components with positive health effects either as antioxidants or as precursor of vitamin A (Chung et al., 2004; Islam & Schweigert, 2006). Based on their structure, carotenoids are divided into two groups: carotenes and xanthophylls. Carotenes are hydrocarbon containing carotenoids (e.g.,  $\beta$ -carotene and lycopene) whereas xanthophylls are oxygenated derivatives (e.g., lutein and astaxanthin) (Rivera et al., 2011; Gayosso García Sancho et al., 2011). Nutritionists

increasingly recognise the ability of carotenoids to scavenge free radicals and to affect innate, humoral and cell-mediated immune response (Sparks, 2006). The consumption of carotenoids has been associated with a number of health benefits, such as cancer chemoprotection, prevention of heart and vascular disease, and prevention of other chronic and degenerative diseases (e.g., Alzheimer's Disease) (Regal et al., 2014).

Lutein, zeaxanthin,, canthaxanthin,  $\beta$ -cryptoxanthin,  $\beta$ -apocarotenoic ester and  $\beta$ -carotene are the main carotenoids that have been identified in the yolk of hens' egg. Lutein and zeaxanthin are reported to be associated with a reduced incidence of age-related macular degeneration (Goodrow et al., 2006), a major cause of deterioration of sight in elderly people (Alves-Rodrigues and Shao 2004; Stahl, 2005).

Carotenoids are also important pigments in eggs as the colour of the egg's yolk is considered to be an important factor in determining the acceptability for the consumer (Karadas et al., 2006). Some xanthophylls, such as lutein, cause a yellow color while canthaxanthin cause a red color in yolks. The intensity of the yolk color and the shade from yellow to red are dependent mainly on the concentration of carotenoids in the diet (Knoblich et al., 2005).

Recently there has been considerable interest in improving the nutritional quality of eggs by enhancing the levels of yolk carotenoids so as to provide the consumer with a healthier and more balanced diet. Modified or so-called enriched eggs have been obtained by including carotenoids or carotenoid rich forage products in laying hen diets. Therefore, it is imperative to learn the variety and amount of carotenoids in regular and modified eggs (Surai & Sparks, 2001; Singh et al., 2012).

Spectrophotometry (AOAC, 1996; Islam & Schweigert, 2015) and high performance liquid chromatography (Barba et al., 2006) are the analytical methods available for analysis of egg yolk carotenoids. The determination of individual carotenoids such as lutein, zeaxanthin, canthaxanthin,  $\beta$ -carotene and  $\beta$ -apocarotenoic ester in egg yolk has been routinely performed by reversed-phase HPLC (Knoblich et al., 2005; Karadas et al., 2006; Hammershøj et al., 2010). HPLC analysis of carotenoids is usually done with C18 or C30 RP-columns, operated with isocratic or gradient elution using UV–vis or photodiode array or MS detection. A wide variety of mixtures of different organic solvents were employed as mobile phases while, in order to improve carotenoids, AOAC (1993) recommends ethanol/tetrahydrofuran (THF) (50:50 v/v) while other authors use ethyl acetate (100%) or different mixtures of ethanol/hexane, acetone/ethanol/ hexane, ethyl acetate/hexane or acetone/hexane (Barba et al., 2006).

This work aimed at developing and evaluating a suitable, reliable, rapid and simple HPLC method employing a C18 column and UV–Vis photodiode array detection (DAD) for simultaneous determination of carotenoids in egg yolk.

# MATERIALS AND METHOD

# **Reagents and standards**

Standards of lutein, zeaxanthin, canthaxanthin, astaxanthin, lycopene,  $\beta$ -carotene and *trans*- $\beta$ -apo-8'-carotenal were purchased from Sigma-Aldrich (Chemie, Steinheim, Germany). All the solvents used for extraction were of analytical grade and were obtained from Merck (Darmstadt, Germany). All solvents used for HPLC analysis (acetonitrile, ethyl-acetate and methanol) were of HPLC grade and were obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained from a SG-Ultra Clear water purification system (SG Water Company, Barsbüttel, Germany). Standard solutions were obtained by dissolving pure compounds in acetonitrile-methanol-ethyl

acetate (60:20:20, v/v/v) containing butylated hydroxytoluene (BHT) (1% w/v) and the solutions were stored in brown flasks at  $4^{\circ}$ C.

### Samples

The eggs used to develop the method were from hens fed a diet containing waste from tomato processing. Egg yolks were separated manually from their respective whites, then homogenized in a blender and stored at -20 °C until carotenoid analysis. All samples were analysed within three days after extraction. Before analysis, samples were allowed to achieve room temperature.

### Instrumentation

HPLC analyses were performed on a Finningan Surveyor Plus system (Thermo Electron Corporation, San Jose, CA) including a vacuum degasser, a Surveyor Plus LCPMPP pump, a Surveyor Plus ASP autosampler and a PDA5P diode array detector with 5 cm flow cell and with Chrom Quest 4.2 system manager as data processor. Separation was achieved by a reversed-phase Hypersil Gold C18 column (5 mm particle size, 250 × 4.6 mm) provided by Thermo Electron Corporation.

# **Extraction of carotenoids**

Carotenoids were extracted from 0.5 g sample with 10 mL of petroleum ether:methanol:ethyl acetate (1:1:1, v/v/v) containing 0.1% butyl hydroxytoluene (BHT) by homogenizing for 5 min at 2500 rpm using a Vortex homogenizer. The sample was centrifuged for 6 min at 6000 rpm and the supernatant was collected. The residue was extracted following the same procedure until the supernatant was colorless. The combined supernatants were washed by adding 10 mL of 5% NaCl solution, mixing vigorously and incubating for 30 min until two layers were separated. The upper layer was collected, evaporated to dryness under N2 flow and then re-dissolved in 2 mL of acetate (60:20:20, v/v/v) acetonitrile:methanol:ethyl containing butvlated hydroxytoluene (BHT) (1% w/v). The final solution was filtered through 0.45 µm membrane filters for HPLC injection.

# Chromatographic conditions

The mobile phase was filtered through a 0.45  $\mu$ m membrane, and degassed ultrasonically prior to use. The most suitable mobile phase system comprised of acetonitrile:methanol (95:5, v/v) (A), acetonitrile:methanol:ethyl acetate (60:20:20, v/v/v) (B) and water (C) with the following gradient: 96% A and 4% C in the beginning, maintained for 10 min, changed linearly to 100% B in 13 min, maintained 5 min and returned to 96% A and 4% C in 2 min. The C18 column resolved a total of seven carotenoids within 35 min. The mobile phase flow rate was 1.5 mL/min. The column temperature was 20 °C and the absorbance was read at 450 nm.

The standards were diluted appropriately to prepare standard curves and were subjected to HPLC-DAD analysis as described above. Standard curves of each carotenoid were produced by plotting the relative peak areas at 450 nm against the carotenoid concentrations. Identification was done by comparing the retention times with those obtained with a mixed standard solution, by spiking of authentic standards and comparing the spectral data obtained by DAD with reported values. Quantification was performed using Chrom Quest 4.2 software by comparing peak area with standard reference curves.

#### Method validation

Linearity of the detector response was tested by preparing five mixed calibration solutions. The calibration curves were established by plotting peak area versus concentration of the analytes. The correlation coefficients were used as the measure of linearity. The limits of detection (LOD) and quantification (LOQ) were determined by calculating the concentration of the analytes in mg/L at a ratio of signal-to-noise (S/N) of 3 and 10, respectively. The precision of the method was confirmed by repetitive analyses, calculating the average relative standard deviation (RSD) for six replicate determinations. For the recovery test, standard solutions of carotenoids were added to the extracts of egg yolk at two concentration levels and then analysed by the HPLC method for three times. The recoveries for the seven carotenoids were then calcultated as ratio of the measured concentration to the spiked concentration and were expressed as a percentage.

# **RESULTS AND DISCUSSION**

The separation of carotenoids using a C18 column was optimized by testing different solvents and different gradient profiles of co-solvents, temperature and flow rate. In Figure 1 the separation of the eight carotenoid compounds in the standard mixture solution was given. The HPLC method thus developed was evaluated in egg yolk samples by the evaluation of linearity, reproducibility, precision, recovery and detection limit (LOD).

#### Linearity

The linearity of the method was evaluated according to area response. The use of a diode array detector allowed us to confirm the identity of the peak not only by its retention time, but also by the overlay of the UV-VIS spectra with a standard. The calibration graphs for carotenoids were produced by injecting standard solutions in the range 2–20 mg/L. Each point of the calibration graph corresponded to the mean value obtained from three independent area measurements. Retention times, regression equations and correlation coefficients for carotenoid compounds were summarised in Table 1.

As can be deduced from the calibration parameters shown in Table 2, the correlation coefficients of linear regression analysis were always above 0.997. Therefore, the external standard method was used for the quantitative analysis.

# **Reproducibility and detection limit**

Chrom Quest software was used to test peak area and retention time reproducibility. A standard mixture solution of 10 mg/L of each analyte was analysed six times to determine the reproducibility of the peak area and retention time under the optimum conditions in this experiment. The relative standard deviations (RSDs) for retention time were between 0.090 and 0.491% while for peak area the RSDs were between 0.339% and 1.211%. These values indicated the stability of the method in terms of peak area and retention time. The detection limits were between 0.041 and 0.752 mg/L for seven carotenoid compounds.

Table 1

						3,75
Carotonoid	RT	Regres	sion equation	Concentration	Correlation	Detection
caroteriolu	(min)	Intercept	Slope	range	coeffiicient	limit
compounds				(mg/L)	r <sup>2</sup>	(mg/L)
Astaxanthin	3.71	-0.02353	8.19028 e-006	1.2-15	0.9997	0.398
Lutein	4.95	0	7.68226 e-006	0.9-15	0.9996	0.259
Zeaxanthin	5.20	0	6.81732 e-006	0.8-15	0.9994	0.236
Canthaxanthin	6.31	0	8.47711 e-006	2.5-20	0.9990	0.752
Trans-β-apo-	6.51	0	5.34553 e-006	1.6-15	0.9997	0.490
8'-carotenal						
Lycopene	17.29	0	4.46802 e-005	1.8-15	0.9970	0.522
β-Carotene	23.27	0	1.28077 e-005	0.2-20	0.9967	0.041

Calibration results for determining seven carotenoid compounds in egg yolk

### Precision and stability

To test the precision and stability of the HPLC method, a sample solution (extract) was analysed by six repeated injections and the analysis was repeated over three days. For retention time, RSD values were between 0.081 and 0.755%, while for peak areas, RSD values were from 1.692 to 4.815%. These results show that the HPLC method has good precision and stability.



Figure 1. Chromatogram of an external standard mixture at  $\lambda = 450$  nm



Figure 2. Chromatogram at  $\lambda = 450$  nm of an egg yolk extract

#### Sample analysis and recovery

Egg yolk extracts were injected directly and separated under the optimum condition mentioned earlier. A typical chromatogram in the extract of egg yolk is shown in Figure 2. The calculated contents of the eight carotenoids were given in Table 3. The recovery experiments of the seven carotenoids were performed by adding carotenoid standards to the extract of egg yolk, which were treated according to the procedure described in Section 2.4 for six times. The recoveries for the seven carotenoids were between 97.27 and 99.98% (Table 2).

Table 2

11An

Contents and recoveries of seven carotenoids in the extract of egg yolk (n = 6)

Component	Content (mg/L)	RSD (%)	Amount added (mg/L)	Recovery (%)
Astaxanthin	0.00	2.53	1.0	93.60
			2.5	98.56
Lutein	0.92	4.81	1.0	97.19
			2.5	100.08
Zeaxanthin	0.82	1.69	1.0	98.22
			2.5	99.86
Canthaxanthin	0.14	3.26	1.0	93.69
			2.5	101.88
<i>Tran</i> s-β-apo-8'-	0.01	1.94	1.0	92.96
carotenal			2.5	95.04
Lycopene	0.35	2.93	1.0	103.61
			2.5	101.78
β-Carotene	0.02	1.79	1.0	98.87
			2.5	96.89

#### CONCLUSIONS

The RP-HPLC method developed here represented an excellent technique for simultaneous determination of seven carotenoids in the extract of egg yolk, with good sensitivity, precision and reproducibility. The method gives a good resolution among analytes with a relatively short analysis time (35 min). The method can be used as quality control of carotenoids in egg yolk and will play a reference role in the understanding the influence of the diet and environment on the carotenoid content of the egg yolk.

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# THE INFLUENCE OF APPLYING SUMMER PRUNING OPERATIONS ON THE QUANTITY AND QUALITY OF CARDINAL VARIETIES

Costea Dorin Constantin<sup>1\*</sup>, Cichi Daniela Doloris<sup>1</sup>, Căpruciu Ramona<sup>1</sup>

<sup>1</sup> University of Craiova, Faculty of Horticulture A.I.Cuza Street, No. 13, Craiova, Correspondence author. *E-mail*: dinuc3@gmail.com

Key words: table grapes, summer pruning operations, quality.

#### ABSTRACT

Summer pruning works and operations apply during the vegetative cycle of grape vines for the management of growing and fruit bearing seasons, for the improvement of quality, appearance of grapes and earliness of production. They allow a proper ventilation and illumination of stock elements, thus reducing diseases and improving the production quality. The paper shows the results obtained by applying the works and operations to Cardinal table grape varieties grown in Simnicu de Sus viticultural centre and their influence on the quality of grape production.

#### INTRODUCTION

One may say that table grapes must first be appealing to the eye and only then can be tasted. This is why the appearance is very important in case of table grapes, holding a decisive influence on consummers'decision.

Summer pruning works and operation include several plant-growing measures to be performed during the vegetative cycle of grapevines.

Summer pruning holds greater importance in case of table grape varieties, owed to its positive influence over grapes quality. When summer pruning works are carefully and timely applied, their efficiency is higher. Summer pruning must be performed reasonably considering that leaves are useful body parts for plants and that each variety has a specific leaf area / output ratio. For this reason, both nationally and internationally, several research studies have been conducted to determine which summer pruning operations fit the biological features of varieties best for the determination of perfect execution time and work intensity. Amongst the authors who conducted such studies are the following: Acimovici D (2016), Cichi Daniela, Costea D (2008), Costea D.C et al. (2013), Di Lorenzo. et al. (2011), Gatti M. et al (2015), Olteanu I. et al. (2002), Palliotti A, Poni S (2011), etc.

#### MATERIAL AND METHODS

Research studies were conducted on Cardinal table grapes varieties in a vineyard located in Şimnicu de Sus viticultural centre. The experiment was focused on establishing the best choices of summer pruning operations, as well as their best execution timing in order to get high quantity and quality levels of production.

In line with the topic proposed, the observations and determinations were focused on monitoring climate factors for the evaluation of the favourableness of the

study year and the determination of the effect of summer pruning use on the quality and quantity of Cardinal varieties.

The summer pruning applied was evaluated by analyzing biochemical indexes (content of sugars, organic acids, glucoacidimetric index) and productive indexes (grape berry weight, yield per trunk).

The options experimented were several combinations of summer pruning operations, such as shoot thinning and hedging, partial leaf removal, inflorescence thinning and trimming. The research methodology used is the one specific for this field.

#### **RESULTS AND DISCUSSIONS**

Multi-annual average climate data for the Simnicu de Sus viticultural centre are shown in the graphs below:



Precipitation





Graph. 2 – Multi-annual average monthly precipitation and rainfall days (by www.meteovista.co.uk/Europe/Romania/Simnicu-de-Sus/)





An analysis of the multiannual data indicated in the graphs above shows that in most of the years Şimnicu de Sus viticultural centre met the climate conditions required for high quality grape yields.

Table 1

#### Climate Data during the mature ripening – Şimnicu de Sus (by www.accuweather.com/ro/ro/simnicu-de-sus)

Date	Precipitation	High temp.	Low temp.
	(mm)	(° C)	(° C)
16 July	25	27	19
1 August	13	36	18
24 August	16	30	16
19 September	14	32	16

The climate data during the mature ripening process (July-September) shows a lack of precipitation during 2016 year. Table 1 shows that during the two-month period there were only 4 rainfall days (over 10 mm), resulting in the early production of grapes which reached their maturity peak about 2 weeks earlier.

Table 2

The influence of shoot thinning on the production quality of Cardinal variety

	Options	Production (kg/stock)	Finished Production (%)	Sugar content (g/l)	Acidity (g/I H <sub>2</sub> SO <sub>4</sub> )	Glucoacidimetric Index
V <sub>1</sub>	Total extra shoot thinning	1,87	73	133	5,1	26,0
V <sub>2</sub>	50% extra shoot thinning	1,75	79	142	4,9	28,9
V <sub>3</sub>	20% extra shoot thinning	1,72	75	139	5,0	27,8
V <sub>4</sub>	No summer pruning operations (witness)	1,66	74	131	5,2	25,1

The influence of shoot thinning on the quality of production is shown in Table 2. In case of Cardinal varieties the production of V4 (witness) was lower compared to other options. The biggest production was 2.46 kg / stock and resulted from V1 (total extra shoot thinning), where the highest content of sugars was also determined. Out of the shoot thinning options, lower results came out from the 20% extra shoot thinning operation (Table 2).

Partial defoliation consisted in removing old leaves placed in front of grape berries to allow their sun exposure, which is so important for their ripening, the stock ventilation and the easiness of applying plant-growing treatments.

The positive effects of old leaves removal on the quality of table grapes are: the uniform colouring of grape berries in table grape varieties, the increase of sugar content, the decrease of total acidity by degradation of malic acid under the more intense action of light.

The influence of partial leaf removal on the quantity and quality of production of Cardinal variety is shown in Table 3. The best quantity and quality results came out

when the leaf removal operation took place after early ripening and the lowest results were obtained in case of the witness option.

. In terms of quantity, the production of Cardinal variety, as well as sugar content, were higher in case of variants V1 and V2 vs witness.

Table 3

	Options	Production (kg/stock)	Finished Production (%)	Sugar content (g/l)	Acidity (g/I H <sub>2</sub> SO <sub>4</sub> )	Glucoacidimetric Index
V <sub>1</sub>	Leaf removal in early ripening	1,71	73	147	4,5	32,26
V <sub>2</sub>	Leaf removal after early ripening	1,73	79	150	4,1	36,5
V <sub>3</sub>	Leaf removal before early ripening	1,70	75	139	4,8	28,9
V <sub>4</sub>	No summer pruning operations (witness)	1,66	74	131	5,2	21,1

The influence of partial leaf removal on the production of Cardinal variety

Shoot hedging allowed the partial removal of the last 5-7 leaves from the shoot top which were considered to be consummers, due to the fact that they had no more time left to reach maturity. The shoot hedging also had a positive influence on the quantity and quality of production. The biggest production came out when the shoot hedging operation took place in early ripening (1.75 kg/stock), compared to the lowest production obtained in case of the shoot hedging before early ripening (1.66kg/stock)-Table 4

Table 4

The influence of shoot hedging on production of Cardinal variety

		Production	Finished	Sugar	Acidity	Glucoacidimetric
	Options	(kg/stock)	Production	content	$(g/I H_2 SO_4)$	Index
			(%)	(g/l)		
$V_1$	Shoot hedging in	1,75	73	148	4,6	32,71
	early ripening					
$V_2$	Shoot hedging	1,70	79	150	4,3	34,8
	after early ripening					
$V_3$	Shoot hedging	1,63	75	134	5,0	26,8
	before early					
	ripening					
$V_4$	No summer	1,66	74	131	5,2	21,1
	pruning operations					
	(witness)					

In terms of quality, the finished production was high (79%) when the shoot hedging was performed after early ripening. The lowest level of finished production

was V1 (shoot hedging in early ripening), i.e. 73%, while V4 (no shoot hedging) indicated 74%.

	Options	Production (kg/stock)	Finished Production (%)	Sugar content (g/l)	Acidity (g/I H <sub>2</sub> SO <sub>4</sub> )	Glucoacidimetric Index
V <sub>1</sub>	Inflorescence trimming after blooming	1,71	80	152	4.3	46.42
V <sub>2</sub>	Inflorescence thinning after blooming	1,79	82	148	3.5	54.28
V <sub>3</sub>	No summer pruning operations (witness)	1,66	74	131	5,2	21,1

The influence of inflorescence thinning and trimming on production quantity of Cardinal variety

Inflorescence thinning greatly influences the quality of grapes through better commercial appearance, uniformity of maturation, colour and berry size, as well as increase of sugar content. The influence of inflorescence thinning and trimming on the production quantity of Cardinal variety is shown in Table 5. In case of options where summer pruning operations took place, production numbers were higher compared to the witness option.

The lowest production of 1,66 kg / stock came out in case of V3 option (no inflorescence thinning and trimming), while the highest production of 1,79 kg / stock was in V2 (inflorescence thinning after blooming).

# CONCLUSIONS

Summer pruning operations require costly and complex manual work, which means timely performance, specific for each variety, depending on their biological specific characteristics.

When carrying out summer pruning operations, one must bear in mind that leaves are useful body parts for the plant and that every variety has a foliar surface/ production specific ratio.

Research studies indicate that summer pruning operations led to better results. However, given the high costs required, turning them into practice must be performed distinctly:

> the shoot thinning was good results: the ratios were different, depending on their biological features, which in case of Cardinal varieties, recommend the total extra shoot thinning;

 $\succ$  in case of Cardinal varieties the partial leaf removal place after early ripening determined the increase of finished production and sugar content and the decrease of acidity;

> when performed in early ripening, the shoot hedging had a positive impact on the quantity and quality production levels;

> the best results for Cardinal varieties were obtained during the inflorescence thinning and trimming operations.

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# DEVELOPMENT OF VINE-GROWING FARMS IN BULGARIA

Dimitrova Daniela<sup>1\*</sup>, Dimitrov Vladimir<sup>1</sup>

<sup>1</sup>Institute of Viticulture and Enology, 1 Kala Tepe Street, Pleven 5800, Bulgaria \* Correspondence author. E-mail: vachevska\_d@abv.bg

Key words: viticulture, farms, specialization, economic potential

#### ABSTRACT

Overcoming the negative tendencies, emerged over the past few decades, led to a drastic decline in the production of wine and table grapes, required detailed analysis of the factors and conditions determining viticulture development potential. In this context, the study examined the processes of ongoing organizational restructuring in viticulture, as a whole and depending on the legal status of the farms covering the period 2003-2013. The dynamics in the number and average size of farms, the level of specialization in the sector and the farms economic potential were studied. The obtained analysis results showed that the mechanisms of the National and Common Agricultural Policy during the previous programming period did not support sufficiently the restructuring of the sector. The opportunities for stabilizing the production at the current economic conditions should be sought mainly in the framework of the integration between grape-growers horizontally or vertically on the overall organization of the food chain.

#### INTRODUCTION

Regardless of the existing traditions in our country, the socio-economic importance of viticulture continued to lose its position within the framework of the overall development of agriculture and rural areas. (Slavova et al., 2011, Popov et al., 2012, Malamova et al., 2013, Dimitrova et al., 2013, Koteva et al., 2014, Dimitrova and Dimitrov, 2017). The intensive nature of the production of wine and table grapes under the current economic conditions - rising production costs, low purchase prices, insufficient financial support, a limited domestic market and weak competitive positions on the international market had predetermined the dramatic decline of the cultivated vineyard areas, reflecting on the output volume. Wine grapes production was reduced by 41.6% in 2016 compared to 2007 while of the table grapes by 26.2%. The overcoming of the negative trend and the utilization of the existing potential in the country for viticulture development (soils, climate, relief, varietal composition) had been a function of the entrepreneurial interest generated in the sector under the influence of the organizational, economic and management factors of the business environment (Risina, 2007, Borisov, 2007, Kirechev, 2013, Borisov et al., 2014). The objective of the survey was to analyze the situation and to outline the trends in the development of the organizational and economic structure of viticulture under the conditions of the National and the Common Agricultural Policy.

#### MATERIAL AND METHODS

The analyses were based on official statistical data with sources from the Ministry of Agriculture and Food (MAF), Agricultural Statistics Department and

Eurostat. The methods of statistical groupings and index analysis were applied (Petrov et al.). The number of vine-growing farms; the number of the specialized vine-growing farms; the vineyard area in the farm; the average size of the vineyard area per farm; the number of permanent workers on farms specializing in viticulture, the number of family labor force have been discussed in dynamics. The change in the structure of the cultivated vineyard areas was monitored according to the physical size of the farms grouped per size of the utilised agricultural area (UAA) and the economic potential of production structures, expressed in European size units (ESU) in 2003 and in a standard output (SO) in 2013.

The trends in the number of agricultural holdings, the size of the cultivated areas with wine and table grapes varieties and the average area of the vineyards per farm, depending on its legal status – individuals, sole traders, cooperatives, trade companies and associations have been found out.

#### **RESULTS AND DISCUSSIONS**

Vine-growing farms development has been the result of the complex impact of many factors - technological, economic and social, with a view of the complex nature of the production activity and the specificity of the grape and wine market. The data presented in Table 1 showed that for the period 2003 to 2013 the number of vine-growing farms had been drastically reduced by 76.9%. The dynamics was similar with regard to the size of the vineyard areas in the farms where the reported decrease was 33.4%.

Table 1

No	Indicators	2003	2005	2007	2010	2013	2013/ 2003, %
1	Vine-growing farms, number	22358 0	141 330	98 260	87 000	51610	23.1
2	Vineyard area, ha	68490	52 670	51540	52340	45640	66.6
3	Average size, ha	0.306	0.373	0.525	0.602	0.884	288.9
4	Specialized vine- growing farms, number	22650	15380	14420	18120	12080	53.3
5	Utilised agricultural area (UAA), ha	27900	26660	32990	31740	29430	105.5
6	Vineyard area, ha	20900	19660	25880	27780	26640	127.5
7	Average size of the vineyard area, ha	0.923	1.278	1.795	1.533	2.205	238.9
8	Permanently occupied work force in specialized vine- growing farms, number	36140	28050	28750	35010	25070	69.4
9	Family workforce, number	34680	26770	26340	32900	22730	65.5

Changes in the number of farms, the area of vineyards on the farms and the workforce in the period 2003-2013

Source: Eurostat (http://ec.europa.eu/eurostat/data/database) and own calculations

The rate of decrease in vineyards grown on the farms was less pronounced than the reduction in their total number, resulting in an increase in the average area of the vineyards in a single farm (188.9% for the survey period). In spite of the positive effect, it should be outlined that the average size of the vineyards on the farms had

remained significantly lower than the rate of this indicator in some of the leading European grapes and wine producing countries and competitors on the Bulgarian market, such as France (10.3 ha), Spain (6.5 ha) and Italy (2.4 ha). The small size of the cultivated areas on the farms negatively affected the opportunities for efficient organization of the whole complex of activities related to the preparation and marketing of the end product - grapes and wine.

The unfavorable market situation, especially in relation to the purchase prices of wine grapes, as well as the high production and market risk, the limited opportunities for investing in expanding, renovating and modernizing the production activity, due to the lack of sufficient financial resources and difficult access to bank lending, the shortage of qualified workers have been the main part of the macro- and microeconomic conditions that predicted the refusal of a large number of farmers to be involved in viticulture. During the survey period, the number of the specialized vine-growing farms had dropped down by half however their relative share in the total number of farms cultivating vineyards had increased from 10.1% in 2003 to 23.4% in 2013. The area of vineyards in the farms specialized in grapes production had increased – by 27.5%, leading to a higher concentration of the period to 2.2 ha in the last year under review.

A negative phenomenon in the context of depopulated rural areas had been the declining number of permanent workforce in the specialized vine-growing farms (by 30.6% in 2013 compared to 2003), as the decrease in the number of the family labor force, representing almost entirely the permanent employment in the production structures with specialization in viticulture was even higher – by 34.4%. The data clearly revealed that the entrepreneurial interest was not sufficiently promoted with a view of maintaining, expanding, renovating and modernizing the production potential in the sector.



Source: MAF, Agricultural Statistics Department

(http://www.mzh.government.bg/MZH/bg/ShortLinks/SelskaPolitika/Agrostatistics/Structure\_agricultural\_holdi ngs/Results\_copy1.aspx)

Fig. 1: Structure of cultivated vineyard areas per farm group according to UAA for the period 2003-2013, %.

The analysis of the cultivated vineyard area distribution according to the physical size of the farms showed that by 2013 the highest share of the vineyard area had the farms with UAA over 100 ha (38.5% of the total area of the vineyards, decreasing from 40.8% in 2003) (Fig. 1). In absolute terms, there had been a trend towards a decrease in the area of wine and table grapes varieties in this group of farms, amounting to 10 403 ha during the survey period. Similar was the dynamics in

the development of the indicator in the groups of farms with UAA under 2 ha and from 2 to 5 ha where the reduction amounted to 66.4% and 25.1% respectively. At the same time, in all other groups of farms there had been an increase in the size of vineyard area, as it was the highest in the group with UAA from 50 to 100 ha – by 166.4% in 2013 compared to 2003.

In the structure of the cultivated vineyard areas, according to the economic potential of the farms in 2003, the highest share had the market-oriented farms (over 4 ESU), that grew 49.0% of the vineyards in the country (Fig. 2). The farms of mainly natural character (up to 1 ESU) included 35.0% of the area of the vineyards while the share of the vineyard areas in the semi-subsistence farms (with economic size between 1 and 4 ESU) was 16.0%.



Fig. 2: Distribution of the cultivated vineyard areas in the country according to the economic size of the farms in 2003 and 2013.

The analysis of the vineyard areas distribution according to the economic potential of the farms, expressed by the standard output indicator (SO), showed that by 2013 still a significant part of the vineyard area belonged to the small farms (up to EUR 2000 SO) – 7500 ha (16.0%). Almost half of the area of wine and table grapes vineyards was cultivated in farms with high economic potential (SO over EUR 50,000). That fact raised concerns about the viticulture sustainable development in the medium and long term, especially given the need for significant investments in the modernization of the technological process, in the face of a growing shortage of skilled workers, together with the tangible need for upgrading of the output potential in the sector, which was impossible for farms with low economic potential.

The development of the vine-growing farms according to their legal status is illustrated in Table 2 and Table 3. Both in wine and table grapes vine-growing the share of the individuals, owners of vineyards, continued to be prevalent by 2013 – 98.3% of the total number of wine varieties vineyards and 97.5% of the total number of table grapes vineyards.

The number of farms had been decreasing in all organizational forms except for the trade companies. The most severe was the reduction in the farms of the individuals – by 77.6% in 2013 compared to 2003 in the wine-growing farms and by 81.3% in the table grapes farms. However, their share in the cultivated area was still high – 45.7% of the area of the wine grapevines and 60.4% of the area of table grapes vineyards were owned by individuals.

The area of the vineyards had dropped down in the cooperatives (by 76.0% for the wine grapevines and 58.1% for the table grapes), in the associations (65.4% for the wine grapevines and 35.4% for the table grapes) and in the farms of the individuals (45.7% for wine grapevines and 32.5% for the table grapes).

#### Table 2

Change in the number of farms, cultivated areas of wine grapevine vineyards and their average size by legal status

Legal status	Farms, number			Area, ha			Average size, ha	
	2003	2013	2013/	2003	2013	2013/	2003	2013
			2003,			2003,		
			%			%		
Individuals	213438	47709	22.4	35870	19488	54.3	0.17	0.41
Sole traders	727	194	26.7	1676	2603	155.3	2.31	13.42
Cooperatives	285	87	30.5	19024	4565	24.0	66.75	52.47
Trade	205	514	250.7	7376	15864	215.1	36.0	30.86
companies								
Associations	64	16	25.0	442	153	34.6	6.91	9.56

Source: MAF, Agricultural Statistics Department

The size of the cultivated areas had been increasing in the farms with the status of sole traders (by 927 ha for wine vineyards and 129 ha for table grapes vineyards) and in the trade companies (by 8488 ha for wine vineyards and 306 ha for table grapes varieties).

Table 3

Change in the number of farms, cultivated areas of table grapes vineyards and their average size by legal status

Legal status	Farms, number Area, ha				Average size, ha			
	2003	2013	2013/	2003	2013	2013/	2003	2013
			2003,			2003,		
			%			%		
Individuals	36131	6764	18.7	2652	1791	67.5	0.07	0.26
Sole traders	137	56	40.8	87	216	248.2	0.63	3.85
Cooperatives	85	23	27.1	1151	483	41.9	13.54	21.00
Trade	33	82	248.5	104	410	394.2	3.15	5.00
companies								
Associations	16	12	75.0	99	64	64.6	6.18	5.33

Source: MAF, Agricultural Statistics Department

The steady growth of cultivated areas in the farms with the status of sole traders and trade companies showed a positive tendency for the development of these organizational forms in the sector, influenced to a large extent by the existing support mechanisms for the period 2007-2013, mainly The Rural Development Programme, Measure 112 "Setting up farms for young farmers", Measure 121 "Modernization of agricultural holdings" and Measure 141 "Support for semi-subsistence farms undergoing restructuring". The share of these organizational forms in the total size of the cultivated vineyards with wine and table grapes varieties had not been high enough to ensure a long-term effect on the overall development and competitiveness of wine and table grapes production. The existing possibilities for financial support through the CAP 2014-2020 mechanisms, both through the multi-component nature of direct payments and through investment support measures and knowledge transfer and innovation under the RDP, might contribute to strengthening and enhancing the viability of the agricultural holdings in the sector.

#### CONCLUSIONS

The results of the analysis of the organizational and economic structure demonstrated that, the overall restructuring in the viticulture was taking place at a slow pace – still the highest share was of small-sized farms and low economic potential holdings. The need for policy measures to stimulate the process of consolidation of vineyards is tangible. Despite the positive trends related to the increased concentration of the production and the positive development of some of the organizational forms, it was disturbing that in the bigger farms the investment interest in viticulture was decreasing.

The encouragement of the entrepreneurial initiative and its successful implementation for stabilizing the production and supply of table and wine grapes and wine in the country was related not only to the utilization of the provided financial support opportunities for the sector, but also to the realization of the need to integrate the production activity in the overall organization of the food chain and bringing together farmers' efforts in the form of producer organizations, producer groups or other network structures.

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# INFLUENCE OF DIFFERENT GIBBERELLIC ACID (GA 3) CONCENTRATIONS ON THE INTENSITY OF THE MAIN PHYSIOLOGICAL PROCESSES ON VARIOUS GREEN LETTUCE HYBRIDS

Aurelia Dobrescu\*, Gheorghita Hoza, Gabriela Neață, Ana Maria Petre, Monica Luminița Badea, Mohammed Jasim Mohammed

University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Horticulture \*Corespondence author: aurora.dobr@gmail.com

Key words: gibberellic acid, photosynthesis, respiration, transpiration, growth

#### ABSTRACT

The study presents aspects of the intensity of the main physiological processes (photosynthesis, respiration, transpiration) in some salad hybrids under the influence of growth stimulating substance – gibberellic acid. The intensity appraisal of these physiological processes was determined with the LC Pro + instrument directly in the field of experience at the time of harvesting. Green lettuce belongs to the Compositae family, is an annual herbaceous and vegetable plant. It is cultivated in all our country areas, on large surfaces, protected gardens or directly in the field. The administration of gibberellic acid to various green lettuce hybrids had a stimulating role in the development of their metabolic reactions. The obtained results were correlated with the growth process of the targeted plants pursuing the weight of their commercial part. The intensity of physiological processes varies depending on the light intensity, temperature and giberelic acid concentration applied to selected green lettuce hybrids.

#### INTRODUCTION

Green lettuce is an annual herbaceous plant, belonging to Compositae family. It has been cultivated since ancient times, being used in nutrition as well as in medicine due to its therapeutic effects. Green lettuce leaves are short petiolate, spiral arranged, covering the apical meristem. The outer leaves are larger with a welldeveloped middle nervure. Layout of the leaves differs according to assortment (Burzo et al. 2005). The growth of leaves is mainly influenced by light, temperature, nutrition and humidity (Bensink, 1971). The light effect on leaf growth is dependent on both the active photosynthetic radiation intensity and the light radiation interception respectively the foliar surface index. Gaudreau et al. (1994) pointed out that light is the main factor adjusting growth and development in plants. Also the temperature conditions the growth of green lettuce plants, respectively the apical meristem growth located near the soil. The temperature limits for the growth of lettuce plants varies between 7 and 24 ° C, with an optimum of 18 ° C (Lorenz and Mainard, 1980). Among the internal factors that have an influence on growth and ripening in plants, hormones are bioactive substances with a decisive role. The growth hormone concentration changes during the development period (Burzo et al. 2004). The staple objective of this study is to highlight the gibberellic acid concentration influence on the intensity of the main physiological processes (photosynthesis, respiration, transpiration) occurring in various green lettuce hybrids.

#### MATERIAL AND METHODS

The researches took place in the first decade of April, following the main physiological processes: photosynthesis, respiration, transpiration of the mature leaves of inland origin green lettuce hybrids under the influence of the growth stimulating substance gibberellic acid (GA 3). The intensity of photosynthesis, respiration and transpiration processes was determined with the LC Pro + analyzer directly in the experience field. Photosynthesis and respiration values were expressed in  $\mu$ mol/CO2/m<sup>2</sup>/s and the transpiration values in  $\mu$ mol/H<sub>2</sub>O/m<sup>2</sup>/s. Also, the light intensity was situated beetwen 180 and 926 µmol/m<sup>2</sup>/s and the temperature beetwen 10.8 and 19.5° C. The obtained results were correlated with the growth process achieved by the investigated plants regarding the weight of their commercial part. The analyzed green lettuce hybrids were: Touareg, Shangore, Alanis, Centore, Analena and Sotalis. The examined variants outline for each hybrid included the V1 variant representing the witness hybrid, V2 referring to the plants that were treated with GA 25 mg/L concentration and V3 representing GA 50 mg/L treated plants. Physiological researches were performed at the time of harvesting and the data obtained was recorded in the graphics.

#### **RESULTS AND DISCUSSIONS**

According to the data presented in Graphics as far as *Touareg* hybrid goes plants belonging to V2 recorded an intensity of 1.65 times greater than V1 and 1.15 higher than V3. Regarding transpiration a remarkable thing is highlighted: the process unfolds directy dependent upon temperature, according to the scientific literature data. The highest transpiration intensity is registered at V3, being 1.22 higher compared to V1 and 1.11 compared to V2. Note that the determinations were performed at different temperatures, the highest being at V3. As respects the respiration process, a high intensity was obtained at V2 plants, which demonstrates that gibberellic acid in the concentration used (GA 25 mg/L) had a stimulating role in the metabolic reactions carried out in the plants. Therefore, the metabolic reactions enhancement led to the growth process stimulation, so the growth expressed in plant weight was the most obvious at V2 plants, being 1.17 times higher than V1 and 1.10 times higher than V3. Thus, for this hybrid, it is possible to appreciate the efficient application of the GA 25 mg/L concentration (figure 1).



Figure 1. The Intensity of Physiological Processes of *Touareg* 

The Shangore hybrid, analyzed under the same fertilization conditions has the highest photosynthesis intensity at V2, being 1.20 higher compared to V1 and 1.12 higher compared to V3. The photosynthesis process is also stimulated in this hybrid by GA 25 mg/L, but we appreciate that photosynthesis is 1.62 times lower than *Touareg*. The transpiration process unfolds with a similar intensity, the differences being

insignificant, which makes it possible to assume that in this case gibberellic acid does not change the conduct of transpiration process. The respiration process determined in this hybrid variants indicates increasing values from V1 to V3, but with reduced differences. Regarding *Shangore* hybrid, although there are relatively few differences in physiological processes, it is observed the influence of GA 25 mg/L on the growth processes stimulation (figure 2).



Figure 2. The Intensity of Physiological Processes of Shangore

The Alanis hybrid variants indicate the highest photosynthesis intensity at V1, being 2.1 times higher than V2 and 1.21 higher compared to V3. It was found that GA 50 mg/L concentration applied to V3, accelerated the photosynthesis process by 1.73 times compared to V2. As far as the transpiration process, it is very intense at V3 plants, being 1.36 higher than V1 and 1.16 higher than V2, which shows us that the water absorption process is stimulated under the action of GA 50 mg/L. The respiration process records the highest intensity at V1, being 1.76 times higher than V2 and 1.42 times higher than V3. It is noticed that in this hybrid case, V1 plants have the intensity of photosynthesis and respiration much more intense than those of the plants under the influence of growth stimulators, yet the commercial weight of the latter is highly appreciated (figure 3).



Figure 3. The Intensity of Physiological Processes of Alanis

Centore hybrid records various intensities of the photosynthesis process, but with insignificant differences, which can not give us relevant information about the gibberellic acid treatment at V2 and V3 plants. As far as the transpiration process goes, there is registered a high intensity on V1 plants, being 2.51 times higher than V2 and 1.49 times higher compared to V3. This aspect suggests a balanced water regime for V1 plants. The respiration process records a high intensity at V3 plants, followed by V2 plants and the lowest at V1 plants. The physiological indicators values are reflected

in the increase process, considering that the V2 plants have a clear growth over the V1 (figure 4).



Figure 4. The Intensity of Physiological Processes of Centore

Analena hybrid under the influence of growth stimulating substance GA realizes the main physiological processes with the highest intensities in V1 plants. This hybrid shows a commercial weight increase of the fertilized variants, although the physiological processes are carried out with similar intensities (figure 5).



Figure 5. The Intensity of Physiological Processes of Analena

Researches on *Sotalis* hybrid revealed that the physiological processes show insignificant differences beetwen variants. Thus, the photosynthesis process is carried out with a similar intensity at V2 and V3 plants and 1,006 times higher at V1. As far as the transpiration goes, we notice the highest intensity in V1 hybrids and the lowest in V3 plants, which explains the negative influence of GA 50 mg/L stimulator on water absorption, The respiration process is carried out with a higher intensity at V2, being 1.14 times higher than V1 and 1.65 times higher than V3. From a physiological point of view, the variants V1, V2, V3 did not record significant differences, however, regarding the plant growth of the fertilized variants there was a slight increase compared to unfertilized variants (figure 6).



Figure 6. The Intensity of Physiological Processes of Sotalis

Biometric synthesis data of Green Lettuce, 2017

Table 1

Hybrid	Variant	AVG. nob weight at	nob weight at AVG. weight of		
		harvest time	salable nob	weight	
	unfertilized	252	243	9	
SOTALIS	GA 25 mg/L	294	285	9	
	GA 50 mg/L	297 278		19	
ANALENA	unfertilized	298	283	15	
	GA 25 mg/L	333	323	10	
	GA 50 mg/L	379	354	25	
CENTORE	unfertilized	301	274	27	
	GA 25 mg/L	401	376	25	
	GA 50 mg/L	350	312	18	
TOUAREG	unfertilized	338	304	34	
	GA 25 mg/L	398	352	46	
	GA 50 mg/L	361	321	40	
ALANIS	unfertilized	328	289	39	
	GA 25 mg/L	377	349	28	
	GA 50 mg/L	388	340	48	
SHANGORE	unfertilized	336	300	36	
	GA 25 mg/L	398	373	25	
	GA 50 mg/L	350	314	36	

#### CONCLUSIONS

Regarding the *Touareg* hybrid, the efficient application of the gibberellic acid must be in a concentration of 25 mg/L. As respects *Shangore* hybrid, although there are relatively few differences in physiological processes, it is noticed GA 25 mg/L to have a stimulating influence over the growth processes. In *Alanis* hybrid case, although V1 plants have a higher intensity of photosynthesis and respiration than the fertilized plants, the latter have a more obvious growth. In the matter of *Centore*, gibberellic acid applied in a concentration of 25 mg/L is thought to achieve a metabolism that stimulates the growth process. In relation to *Analena* hybrid there is a obvious growth of the fertilized variants, although physiological processes are carried out with similar intensities at all variants. Regarding *Sotalis* hybrid, there were no significant physiological differences in all three variants, however, fertilized plant are observed to have a slightly increase in plant growth compared to unfertilized ones. As respects *Analena* and *Sotalis* hybrids, GA doesn't influence the physiological processes and the 50 mg/L use is not justified as the plant growth processes show reduced differences.

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# INFLUENCE OF FERTILIZATION MODE ON PRODUCTION OF SOME TOMATO CULTIVARS GROWN IN AN ECOLOGICAL SYSTEM

Domocoş Daniela<sup>1</sup>, Apahidean Alexandru Silviu<sup>1</sup>, Cărbunar Mihai<sup>2</sup>, Mariana Bei<sup>2</sup>, Apahidean Alexandru Ioan<sup>1\*</sup>, Ştef Adrian Valeriu<sup>1</sup>

> <sup>1</sup>University of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, 3-5 Mănăştur Street, Cluj-Napoca, 400372, Romania, <sup>2</sup>University of Oradea, Faculty of Environmental Protection, B-dul Gen.Magheru 26,.Oradea, Romania Corresponding author. E-mail: \*alexandru.apahidean@usamvcluj.ro

Key words: tomatoes, cultivar, fertilization, production, ecological system

#### ABSTRACT

Tomatoes are used in human diet, fresh or in cooked food (soups, sauces, pots, stuffed tomatoes, etc.). They are recommended as food in asthenia, inadequacy, chronic poisoning, congestive conditions, atherosclerosis, vascular diseases, arthritis, gout, rheumatism, azotemia, biliary and urinary lithiasis, constipation, enteritis. They are industrially used in the production of tomato paste, broth, canned, simple or spicy juices. In order to achieve economically profitable productions, organic farming system requires increased attention to crop fertilization. The experience was carried out during the year 2016, in an ecologically certified vegetable microplant, in Husasau de Tinca, Bihor County. The experimental factors were cultivar and fertilization. Experimental culture was set up in a polyethylene tunnel in April.

#### INTRODUCTION

Tomatoes are an important source of many antioxidant properties: carotenoids (lycopene,  $\beta$ -carotene), flavonoids, phenolic acids (chlorogenic acid, gallic acid) and ascorbic acid (Hallmann, 2012). They provide valuable protection for the human body. To prevent oxidative stress a diet based on the consumption of antioxidants is needed (Horotan, 2014).

Dry matter content of tomato fruits ranges between 5.5-7.5%, 3-4% glucose and glucose glucose, 1-1.3% antide and 0.5-0, 7% minerals from the fresh substance.

Fresh fruits contain vitamin C (15-30 mg/100g), carotenoids (0.8mg/100g), vitamins in group B (B1 0.06mg, B2.0.04mg, B6 0.1mg) and P, vitamin K (24 mg/100 g). Tomato fruit has a high content of mineral substances and organic acids (0.3-0.5%), mainly represented by citric and malic acid (Dinu et al., 2016).

Favorable ratio of carbohydrates to acids gives fruit and tomato juice a pleasant taste and a refreshing effect.

Mineral nutrition of tomatoes has some particularities that must be taken into account when fertilizing. Thus, specific consumption varies depending on the system of cultivation, variety, production, degree of supply of soil with mineral elements (Indrea et al., 2012).

Tomato assortment is very varied and dynamic, and varieties and hybrids with superior agroproductive qualities are grown every year and can be grown in different systems. In the assortment there are different fruit cultivars (most of them are red, but they are also green, brown, black, yellow, pink or even tigrated). The chemical composition of fruits differs significantly from one color to another (Lagunovschi-Luchian & Vînătoru, 2016). The lycopene content varies depending on cultivar and place of culture. For field cultures, content of lycopene varies between 5.2-23.6 mg/100 g, whereas for tomatoes cultivated in greenhouses the content is between 0.1-10.8 mg/100 g (Abushita et al., 2000).

Nitrogen nutrition should be guided so as to avoid excess because, especially under unfavorable conditions (insufficient light, poor supply of K and Mg), causes excessive foliar growth and delays the formation and maturation of the fruit (Ciofu et al. 2003). If in the conventional agricultural system the possibility of nutrition management is ensured by the diversity of fertilizers that have complex composition with macro and microelements, organic fertilization is mainly used for fertilization with organic fertilizers. Experiments with Cropmax 0.25% provided 12.5% production increases and by application of Humus the production increase was 17.7% (Dinu et al., 2009). In accordance with the requirements of Reg.EU 834/2007, the maximum amount of N from organic and mineral fertilizers must not exceed 170 kg per hectare per hectare (Stoleru, 2013).

Cultures of tomatoes in protected areas are carried out between 25 March and 15 September, with the aim of producing early productions, beginning with the end of May, at a lower cost price than the greenhouse production (Stan et al., 2003).

#### MATERIALS AND METHODS

The experience was carried out in 2016, being located in an ecologically certified vegetable micro-farm from Husasau de Tinca in Bihor County, in the western part of Romania.

The purpose of the experience was to determine how some tomatoes cultivars behave in different fertilization systems, cultivated in a polyethylene tunnel, under the conditions specific to organic farming. Objectives were to determine the dynamics of production and total fruit production.

Pineapple, Potiron Ecarlate, Double Rich, Brandywine Pink, Merveille des Marchés, Caroten de Plovdiv, Estiva F1 and Blue Beautz have been used in the experience, the seed being produced for organic crops.

Ananas is highly appreciated by consumers for the qualities it has: it has few seeds, the pulp is firm, dense, sweet, juicy, very fragrant and with an incomparable flavor. Color of the pulp resembles that of the pineapple. The fruits are large (200-400 g, sometimes they can reach 1 kg) yellow marked in red. It is a late variety, with indeterminate growth, with leaves similar to potatoes.

*Potiron Ecarlate* grows indefinitely, with large fruits of 250-600g, red in mixture with a little yellow, dense, acidic, tasty, very fragrant pulp, ideal for filling.

Double Rich is an cultivar with indeterminate growth, adapted to short summers or cooler summers. The fruits of 300-500g, are red, firm pulp, with few seeds and sweet sour taste. They have double the content of vitamin C than the average of other tomato varieties.

*Brandywine Pink* is a very old variety considered to be one of the best varieties, with undetermined growth, with potato-like leaves. The fruits are large (500-900g), slightly flattened, dark pink, with a very fine flavor. Fruits are sensitive to cracking.

*Merveille des Marchés* is a very old variety with undetermined growth, vigorous and compact growth, with large fruit production. The fruits are red, with pink pulp, medium-sized (180-200g) with few seeds.
*Caroten de Plovdiv* is a variety obtained in Bulgaria by researchers from the Maritza Institute of Vegetable Culture in Plovdiv.

*Estiva F1* is an undetermined growth hybrid, producing medium fruits (180-220g), red in colour, tasty fruits.

Blue Beautz is a variety with undetermined growth that produces fruit weighing 200-250g with dense pulp. Color is a mixture of red and indigo. The fruits are resistant to cracking and sunburn. Fruits are rich in anthocyan with antioxidant capabilities.

For differentiated fertilization, Agriful (applied to the ground) and Tekamin Brix (foliar application) were compared to the nonfertilized variant. Combining the two experimental factors resulted in 24 experimental variants that were placed in three repetitions.

The experience was placed in a polyethylene tunnel plant (fertlized in autumn with with 40 t/ha of half-decomposed manure), crop being set up with seedlings produced in biofuel-heated seedlings shelter. Sowing was done on 6.02.2016. Seedlings were transplanted in pots of 9x9x9.5 cm and planted in the polyethylene tunnel on 16.04.2016.

During the production of seedlings, specific works and two treatments with nettle macerate (to fortify plants and prevent disease attack) have been applied. First treatment was performed immediately after rising and the second after 10 days of transplanting. During the vegetation period, the usual maintenance work was carried out. To prevent disease attack, nettle treatments were performed and in June, two treatments were done to combat aphids using a mixture of fern macerate and black soap (vegetable soap made from olive oil). Plant growth was stopped after 8 inflorescences. Fruit picking started in June and lasted until September.

Observations have been made on plant growth, quantitative and qualitative production.

## **RESULTS AND DISCUSSIONS**

Harvesting of fruits began with some cultivars in June and with others from July. First ripened fruits were harvested from Double Rich, Brandywine Pink, Merveille des Marches. By the fertilization on the ground with Agriful, a more favorable harvest dynamic was ensured for all cultivars. Foliar fertilization with Tekamin Brix ensured a better dynamics of harvested production compared to unfertilized variants but lower compared to variants fertilized with Agriful.

Tomato production ranged from 8.48 kg/m<sup>2</sup> at Brandywine Pink and 10.86 kg/m<sup>2</sup> at Estiva F1 hybrid (Table 1). As compared to the average of the experience, the cultivars Ananas, Brandywine Pink and Caroten de Plovdiv produced lower yields, less than 9.24 kg m<sup>2</sup>, differences in production compared to average being significantly negative.

Potiron Ecarlate cultivar achieved a production of 9.74 kg/m<sup>2</sup>, the difference from the average of the experience being significant and the Estiva F1s cultivar had a production of 10.86 kg/m<sup>2</sup>, the production increase compared to the average of 17, 53% and the production gap, very significant (Table 1).

Unilateral influence of the fertilization system applied in the experimental culture highlights the favorable effect on the production of the two supplementary fertilization variants with Agriful and Tekamin Brix respectively, accepted in the ecological culture system. Tomato production was 8.61 kg/m<sup>2</sup> for the additional nonfertilized variant and 10.08 kg/m<sup>2</sup> for the Agriful variant (Table 2).

Table 1

Unilateral influence of the	cultivar on tomato	production
-----------------------------	--------------------	------------

Cultivar	Prod	uction	Difference to	Significance
	kg/m <sup>2</sup>	%	culture	of the
	-		average (t/ha)	difference
Ananas	8.63	93.39	-0.61	0
Potiron	9.74	105.41	0.50	*
Ecarlate				
Double	9.11	98.59	-0.13	-
Rich				
Brandywine	8.48	91.77	-0.76	0
Pink				
Merveille	9.19	99.45	-0.05	-
des				
Marchés				
Caroten de	8.49	91.88	-0.75	0
Plovdiv				
Estiva F1	10.86	117.53	1.62	***
Blue	9.44	102.16	0.20	-
Beauty				
Average	9.24	100.00	-	-
DL	P 5%			0.45
DL	P 1%			0.83
DL	P 0.1%			1.14

Table 2

Unilateral influence of the fertilization system on production of tomatoes

Ferilization	Prod	uction	Difference	Significance	
	kg/m <sup>2</sup>	%	to culture	of the	
	-		average	difference	
			(t/ha)		
No fertilization	8.61	100,00	-	-	
Soil fertilization	10.08	117,07	1.47	***	
with Agriful					
Foliar	9.03	104,87	0.42	*	
fertilization with					
Tekamin Brix					
DL P 59	%			0.38	
DL P 19	%		0.94		
DL P 0.	1%			1.23	

The total production of tomatoes was influenced by the cultivar used and fertilization mode (Table 3).

The additional fertilization on the ground with Agriful provided production bonuses ranging between 12.43% and 24.42% depending on the cultivar, the production differences being very significant in the Ananas, Double Rich Brandywine Pink, Merveille des Marchés, Merveille des Marchés Estiva F1 and Blue Beauty. Additional fertilization with Tekamin Brix provided lower production yields, below 10%, with the exception of the Blue Beauty cultivar at which the production increase was 11.59% and the production difference compared to the control variant was distinctly significant.

Va	ariant	Prod	uction	Difference	Significance
Cultivar	Fertilization	kg/m <sup>2</sup>	%	to culture	of the
	system			average	difference
				(t/ha)	
Ananas	No fertilization	7.82	100.00	-	-
	Agriful	9.73	124.42	1.91	***
	Tekamin Brix	8.34	106.64	0.52	*
Potiron	No fertilization	9.25	100.00	-	-
Ecarlate	Agriful	10.40	112.43	1.15	**
	Tekamin Brix	9.59	103.67	0.34	-
Double	No fertilization	8.33	100.00	-	-
Rich	Agriful	9.87	118.48	1.54	***
	Tekamin Brix	9.15	109.84	0.82	*
Brandywine	No fertilization	7.75	100.00	-	-
Pink	Agriful	9.26	119.48	1.51	***
	Tekamin Brix	8.45	109.03	0.70	*
Merveille	No fertilization	8.60	100.00	-	-
des	Agriful	10.10	117.44	1.50	***
Marchés	Tekamin Brix	8.87	103.13	0.27	-
Caroten de	No fertilization	8.03	100.00	-	-
Plovdiv	Agriful	9.13	113.69	1.10	**
	Tekamin Brix	8.32	103.61	0.29	-
Estiva F1	No	10.01	100.00	-	-
	fertilization				
	Agriful	11.96	119.48	1.95	***
	Tekamin Brix	10.63	106.19	0.62	*
Blue	No fertilization	8.54	100.00	-	-
Beauty	Agriful	10.26	120.14	1.72	***
-	Tekamin Brix	3.77	106.49	0.23	*
DL P 5	%				0.51
DL P 1	%				0.83
	1%				1.16
	170				1.10

Combined influence of cultivar and fertilization system on the production of organic tomatoes

Table 3

# CONCLUSIONS

On the basis of the results obtained from the research carried out on the tomatoes grown in polyethyelene tunnel, in the specific conditions in the West of Romania, using the cultivars Ananas, Potiron Ecarlate, Double Rich, Brandywine Pink, Merveille des Marchés, Caroten de Plovdiv, Estiva F1 and Blue Beautz, fertilized with Agriful and Tekamin Brix respectively, the following conclusions were drawn:

- fruit harvest began in June at the Double Rich, Brandywine Pink, Merveille des Marches;

- Agriful fertilization on the ground has ensured a more favorable harvesting dynamics for all cultivars;

- unilateral influence of the cultivar revealed tomato production ranging from 8.48 kg / m2 to the Brandywine Pink cultivar and 10.86 kg / m2 in the Estiva F1 hybrid;

- additional soil fertilization, with Agriful assuring production increases ranging between 12.43% and 24.42% depending on the cultivar;

- Estiva hybrid achieved the maximum production of 11.96 kg / m2 in the fertilized variant with Agriful.

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# TRENDS IN THE EVOLUTION OF CLIMATIC FACTORS IN THE DEALUL BUJORUI VINEYARD UNDER THE CURRENT CLIMATE CHANGES CONDITIONS

Enache Viorica<sup>1\*</sup>, Donici Alina<sup>1</sup>, Tabaranu Gabriel<sup>1</sup>

<sup>1</sup>Research and Development Station for Viticultural and Winemaking Bujoru Correspondence author E-mail enacheviorica57@gmail.com

Key words: climate risk, vine, air temperature

#### ABSTRACT

In the last period of time, the action of climatic factors on vine ecosystems is becoming increasingly prominent. In addition to the damage caused by extreme temperatures, there is an increased intensity and increasing frequency of extreme weather phenomena. The knowledge generated by previous research on the implications of global climate change on the viticultural ecosystem has been used to move to a superior level of sustainable use of vineyards under the current climate change conditions. The evolution of air temperature, relative humidity and sunstroke in the Dealul Bujorului vineyard 1980-2016 during was studied. Climatically comparatively the periods 1980-2006 and 2007-2016 were analyzed. Observations and determinations made allow us to assess the evolution of climatic factors under the current climate changes conditions. It was observed a trend for increased frequency of extreme values of air temperature and increasing duration of sunshine during in the vegetative period.

## INTRODUCTION

Recently, we witness the variation of global climate factors as a result of global warming caused by greenhouse effect enhancing. Climate change has been highlighted frequently in recent decades by increasing air temperature, considerable atmospheric precipitation (rainfall and snowfall) and extreme weather phenomena (Enache Viorica et al, 2007). The long-term analysis has marked a climate change trend. Simulations with complex global climatic models indicated that the phenomenon is determined by both natural and anthropogenic factors (IPCC, 2007). According to the researches, it is concluded that air temperature may rise by 0,1°C / decade in the coming decades. A number of international studies have concluded that the average temperature has risen over the past century. An increase of 0,6°C of the average air temperature was reported. Obvious trends have also been manifested in the evolution of the daily minimum temperature that has increased more than the maximum daily temperature (Easterling et al., 1997).

Based on these considerations, the paper aims to present the tendency of evolution of some climatic factors in the Dealul Bujorului vineyard, analyzing a series of data from 1980-2006 taking as reference the decade 2007-2016.

#### MATERIAL AND METHOD

The research was carried out within the Bujoru Research and Development for Viticulture and Wine Growing Station. Data on average daily, monthly, and annual

average temperatures were processed and analyzed. For the purpose of assessing the trend of temperature evolution data were collected from the Tg Bujor weather station (AGROEXPERT system). We analyzed:

- Annual average and vegetation period;

- Monthly average temperature;

- Minimum and maximum annual temperature;

- Temperature of temperatures above 30°C and below -18,0 °C;

- Deviation of average monthly temperatures from 2007-2016 compared to 1980-2006;

- Actual insolation (hours);

- Relative humidity (%).

A comparative analysis was carried out between 1980-2006 and the reference period 2007-2016.

# **RESULTS AND DISCUSSIONS**

The air temperature determines the area of the vine culture, the system of cultivation, the triggering and the passage of the vegetation phenophases, the quantity and the quality of the production. The vine is relatively pretentious for heat, cultivating in areas with an annual average temperature of more than 9°C. If the average annual temperature is higher than 10 ° C, quality wine-wine products can be obtained. In figure 1 shows the average annual temperatures over the two time periods: 1980-2006 and 2007-2016. Annual average temperatures were compared to the mobile average on 5 year and multiannual average. Different average annual temperatures and average temperatures during the vegetation period were presented. The 5-year mobile average indicates a trend of average temperature increases between 1980 -2006 and a downward trend over the period 2007-2016. Between 1980 and 2016, out of the total of 27 years, 10 years are below the multiannual average and 12 years below the multiannual average of the vegetation period. Of the 10 years of the reference period 2007-2016, 5 years are below the multiannual average and 7 years below the multiannual average of the vegetation period.

From the point of view of the monthly evolution of the temperatures, it is observed that during the months of March, July, August, November and December, the average monthly temperature is higher than the average of the reference period 2007-2016 (figure 2).



Figure 1 The average annual temperature and the vegetation period in the Dealul Bujorului vineyard during 1980-2006 and 2007-2016



Figure 2 The average monthly temperature in the Dealul Bujorului vineyard between 1980-2006 and 2007-2016

The monthly average temperature during the vegetation period from the reference period 2007-2016 shows a growth trend in April, May, June and September with a maximum negative deviation of 0,8°C / October and a positive maximum deviation of 0,8 / August compared to the 1980-2006 period (figure 3).





Notice a downward trend for the average temperature to drop in August 2007-2016 as compared to the period 1980-2006. If from the 27 years of 1980-2006 during the vegetation period were registered 21 years with maximum air temperatures> 35°C, representing 77,8%, in the period 2007-2016, 9 out of 10 years recorded maximum temperatures of air> 35 ° C, representing 90%.

The frequency of days with maximum air temperatures> 30°C in relation to the total number of days for the period 1980-2006 is the maximum in July (37,6%). Comparing the two intervals, the frequency of days with air temperatures> 30° is higher



in April, May, June and July for the period 1980-2006 and lower in August and September (figure 4).

Figure 4 Frequency of days with air temperatures> 30 °C in relation to the total number of days

In the figure 5 shows the evolution of the minimum and maximum annual temperatures in the Dealul Bujorului vineyard between 1980-2006 and 2007-2016 periods. In the conditions of temperate continental climate, the minimal temperatures are important for the vine culture. During vegetative rest, the buds of the vine freeze at temperatures between -16,0° C and -18,0° C. Between 1980 and 2006, in 12 years out of 27, minimum air temperatures were <-18,0° C, representing 44,5%, compared to the baseline when it was 6 years, accounting for 60%. The frequency of minimum temperatures <-18° C in the reference range is increasing compared to the 1980-2006 period.



Figure 5 Evolution of the minimum and maximum annual temperatures in the Dealul Bujorului vineyard between 1980-2006 and 2007-2016

The light sources of the vineyard are appreciated after the sum of the hours of effective sunshine during the vegetation period (real sunshine -  $\Sigma$ rs). High values of this indicator favor the accumulation of anthocyanins in red wine grape. From the data presented in figure 6, light sources in the 2007-2016 reference range are increasing over the 1980-2006 period, with a surplus of 212.4 hours, especially in July, August and September.



Fig. 6 The evolution of real sunstroke in the Dealul Bujorului vineyard between 1980-2006 and 2007-2016

The growth and fructification processes of the vine are carried out in optimum conditions with air humidity of 60-80%. For the whole analyzed period, the relative air unity is within normal limits, with the exception of July and August 2007-2016, when the monthly average is lower due to high temperatures and to low rainfall (figure 7).



Figure 7 The evolution of real sunstroke in the Dealul Bujorului vineyard between 1980-2006 and 2007-2016

# CONCLUSIONS

Comparing the 1980-2006 period with the reference period 2007-2016, it was found that:

1. The 5-year moving average indicates a tendency to increase average air temperatures between 1980-2006 and a downward trend over the period 2007-2016, both on an annual basis and during the growing season.

2. There is a downward trend in the average temperature in August 2007-2016 compared to the period 1980-2006.

3. The frequency of days with air temperatures> 30,0°C is higher in April, May, June and July for the period 1980-2006 and lower in August and September compared to 2007-2016.

4. The frequency of the years with maximum temperatures> 30,0°C increased in the reference range to 90% compared to the period 1980-2006 (77,8%).

5. From the 27 years of 1980-2006 during the vegetation period were registered 21 years with maximum air temperatures>  $35,0^{\circ}$ C, representing 77,8%, in the period 2007-2016, 9 out of 10 years recorded maximum temperatures of air >  $35,0^{\circ}$ C, representing 90%.

6. The frequency of minimum temperatures <-18,0°C in the reference range is increasing compared to the 1980-2006 period.

7. Light sources are rising in the 2007-2016 reference range.

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# USE OF EXTRACTS FROM RED GRAPE POMACE IN VINE PROTECTION

Fîciu Lidia<sup>1</sup>\*, Bădulescu Adriana<sup>2</sup>

<sup>1</sup>\*Research and Development Institute for Viticulture and Enology, Valea Călugărească, Romania <sup>2</sup> Research and Development National Institute for Biotechnologies in Horticulture Stefanesti Arges \* *Correspondence author. E-mail*: ficiulidia@yahoo.com

Key words: grape pomace, polyphenolic compounds, grapevine downy mildew

#### ABSTRACT

The aim of this study was to investigate the efficiency of the treatment with Cu<sup>2+</sup> (chelatic) complexes and extract of grape pomace in the protection of the vine. Grape pomace was obtained by extraction from three black varieties. Cu<sup>2+</sup> complexes were obtained based on polyphenol compounds separated from red grape pomace. The causal agent pathogen was the downy mildew produced by the Plasmopara viticola fungus and the treatments were performed in the field conditions. Three control variants have been tested: classical treatment against downy mildew with synthesis and contact products, treatment with copper chelate in aqueous solution 0.1% and treatment with 0,2% aqueous solution of grape pomace. As a result of the observations made in the case of chemical treatment, the degree of attack was only 9%, followed by the solution treated with Cu<sup>2+</sup> (chelatic) complexes, respectively 20% and the treatment with extract of grape pomace, 27%.

#### INTRODUCTION

The downy mildew is produced by the *Plasmopara viticola* fungus and is the most dangerous disease because it attacks all the green organs in formation, causing very great damage to the culture (Tomoiagă Liliana 2006, Dagostin et al. 2011, La Torre et al. 2014). Worldwide there are few studies on the use of extracts from grape pomace to naturally stimulate the resistance of plants to the attack of cryptogamic diseases. Extracts from grape pomace alter the morphogenesis of parasitic fungi such as *Plasmopara viticola, Uncinula necator, Botrytis cinerea*, preventing spore germination, granulating conidia cytoplasm, destroying plasma membranes, etc. (Woods et al. 1995). The research by Sackenheim et al. (1994) under controlled conditions highlighted the positive effect of the extract from grape pomace in reducing the attack of the *Plasmopara viticola* fungus on the leaves. The possibility of using the extract from grape pomace in the control of cryptogamic diseases in vines is of interest for organic viticulture since 2006, the use of copper products in the control of manure in the vine was restricted at European Union level living, these products being accepted in organic viticulture.

#### MATERIAL AND METHODS

The study was conducted at the Research and Development Institute for Viticulture and Enology, Valea Călugărească in the 2015-2016 period. The experimental device was located within a plot of cultivated Merlot / SO4 variety, with

planting distances of 2.0 x 1.0 m, located on wide terraces, under the conditions of a brown-red mollusc soil. The way of soil maintenance was that of a black field. Three variants of control have been tested: V1-classical treatment against downy mildew with synthesis and contact products (Dithane M-45, Acrobat Mz 69 WG and Kocide 2000), V2-treatment with copper chelate in aqueous solution 0.1% and V<sub>3</sub>-treatment with 0.2% aqueous solution of grape pomace. The control variant was untreated (V1). Cu2+ (chelatic) complexes were obtained by Polytechnic University of Bucharest, based on extracts rich in polyphenolic compounds obtained from red grape pomace. The application of the products was carried out during the vegetation period in the phenophases BBCH11 (first leafs), BBCH16 (six leafs), BBCH55 (before flowering), BBCH71 (and of flowering) and BBCH73 (the grapes reached 30% of the size), with the vermorrel. After each treatment, observations and determinations were made to record the degree of attack on leaves and grapes and to determine the efficacy of the products used to combat the Plasmopara viticola fungus. The rating of attack is expressed by intensity and frequency (GA% = FxI / 100), and these values were the basis for interpreting the obtained results.

# **RESULTS AND DISCUSSIONS**

Grape pomace was obtained by extraction from three black varieties: Burgund mare, Feteasca neagra and Merlot.

Physico-chemical characteristics of fresh grape pomace were performed (table 1). Fresh grape pomace presented a humidity of 6.70-7.73 g/100 g and an acid pH. Sugars are present in quantity between 0.6-1.19 g/l, while the amount of sugars in grapes had values ranging between 211 and 230 g/l.

Table 1

Parameters	The raw m	The raw material-grapes for red wines								
	Burgund mare	Feteasca neagra	Merlot							
Moisture (g/100g)	7.73 ± 0.07	7.37 ± 0.17	6.70 ± 0.07							
pН	3.68 ± 0.04	$3.90 \pm 0.03$	3.67 ± 0.03							
Sugar (g/l)	0.46 ± 0.06	0.66 ± 0.09	1.19 ± 0.18							
Total acidity (g/l tartaric acid)	3.9 ± 0.30	4.6 ± 0.25	4.9 ± 0.35							
The ashes	7.99 ± 1.05	7.53 ± 1.03	6.56 ± 0.64							

Physico-chemical characterization of fresh grape pomace

The obtaining of aqueous extract from grape pomace has been carried out in the following manner: 100 g marc pinched and mountainous, has been named in 1000 ml ethyl alcohol 70% and mixed using a shaker for 2 hours at 100 rpm. The resulting extract was centrifuged for 30' at 3600 rpm, then passed the rotoevaporator at 80°C, separating the alcohol extract. The extract was brought to 100 ml with distilled water (I). In order to correct the acidity extraction (which must have a neutral ph) was carried out and a buffer solution which consisted in: 11.9 g disodium phosphate, Na2HPO4 solution • 2H2O dissolved in 1000 ml of distilled water and 0.9 g potassium dihydrogen phosphate KH2PO4 solution dissolved in 100 ml of potassium dihydrogen phosphate solution, resulting the buffer solution (II). The final extract was obtained through a mixture of 100 ml of extract with distilled water (I) and with the buffer solution (II).

With regard to the climatic conditions, the level of rainfall was very low during the winter months, when it grew at 0.2 mm in December compared to 44.5 mm

multiannual average, but has become surplus in the spring (87.6 mm versus 35.3 mm multiannual average recorded in March) and in July, favoring the development of diseases, most notably downy mildew. In the warmest months (June, July and August) have registered 81.8 mm, 70.2 mm and 78.2 mm. Evolution of average values of rainfall in the period January-September 2016 is represented in Figure 1. The amount of the average temperature °C in January-February period was between - 4.3 ° C in January and 169.3 ° C in July (Figure 2).



Figure 1. The average rainfall January-September





Analyzing the data collected in conjunction and with the climatic conditions in the period of vegetation of vineyards that were particularly favourable to the attack of downy mildew on leaves, inflorescences and grapes, the first symptoms of leaf downy mildew attack have been observed from 15.06.2016. Due to the hot and humid climate, the evolution of the disease was fast so that the degree of attack in untreated (control) was quite high, 43%. In the case of chemical treatments the degree of attack was only 9%, followed by the solution treated with  $Cu^{2+}$  (chelatic) complexes, respectively 20% and the treatement with extract of marc, 27%.

Due to the climatic conditions in the period of vegetation of vineyards, which were very favourable to the attack of downy mildew, the treatment with copper chelates, but especially with the pomace extract were not effective enough to halt the onslaught of downy mildew, the reason for that especially young grapes were partially affected, which has led to a reduction in the production of grapes.



Figure 3. The evolution of the attack of downy mildew (GA%)

#### CONCLUSIONS

Compared to the treatment with chemicals, the degree of attack was double in case of treatment with Cu<sup>2+</sup> (chelatic) complexes and triple in case of treatment with extract from grape pomace, but much less than in untreated variant, when the loss of production was 100%. In the case of these variants, when the weather conditions are very favourable to the attack of downy mildew (warm and wet weather) are recommended complementary 1-2 treatments with systemic products (especially in the phenophases a flowering and the increase of grape berries).

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# CHARACTERIZATION OF SPONTANEUS FLORA AND INSECTS IN VALEA CĂLUGAREASCĂ VITICULTURAL ECOSYSTEM

Fîciu Lidia<sup>1</sup>\*, Bădulescu Adriana<sup>2</sup>, Brîndușe Elena<sup>1</sup>

<sup>1\*</sup>Research and Development Institute for Viticulture and Enology, Valea Călugărească, Romania <sup>2</sup> Research and Development National Institute for Biotechnologies in Horticulture Stefanesti Arges \* Correspondence author. E-mail: ficiulidia@yahoo.com

Key words: flora, harmful insects, useful insects, ecosystem.

# ABSTRACT

The mail goal of this paper was to invent the spontaneus flora and insects (useful and harmful) in Valea Calugareasca viticultural ecosystem. Flora and fauna represent a natural heritage, and it allows us to appreciate the situation of future threats to the vineyard culture. In the viticultural ecosystem taken into study the following areas of interest were delimited: the main crop (vine) in 85.3%, the hedges (5.4%), the canals (4.6%), the rows of trees (0.004%) and isolated trees (0.002%).

28 floral species were identified following inventory. Of these, the most abundant were: Agropyron repens and Capsella bursa pastoris. The rare species were: Ranunculus ficaria, Crocus, Hypericum perforatum. As regards fauna, 61 species of harmful fauna and 138 species of useful fauna were identified.

# INTRODUCTION

The conservation of biodiversity is, nowadays, one of the major issue at the international level due to intensified human impact on the biosphere. In this context, the maintenance of biodiversity is necessary not only to ensure life in the present, but also for future generations, because it preserves the regional and global ecological balance, guarantee the regeneration of biological resources and maintaining environmental quality required.

Wild flora and fauna represent a natural heritage of aesthetic, cultural and scientific value (Ellenberg et al. 1992).

Biodiversity is essential for "services of ecosystems", the services being offered by nature: adjusting the climate, water and air, soil fertility and the production of food, fuel, fibre and medicines.

# MATERIAL AND METHODS

In a vineyard ecosystem located at The Research and Development Institute for Viticulture and Enology, Valea Calugareasca, the spontaneous flora, useful and harmful fauna were inventoried.

Inventory of flora was achieved through collecting and mapping of species with the help of frames of  $1 \text{ m}^2$ . Journeys were carried out at regular intervals so as to be captured on all stages of vegetation and as many species as possible. In order to identify the plants specialized determinant were used (determinator for flora: "Flora ilustrată a României" – V. Ciocîrlan, 2009, "Flora R. P. R. " - Vol. I-XIII,1952-1976).

Following the field visits was drawn up the inventory of flora and, also data on plant fenologia were recorded. The following field observations were made: systematic database of species, abundance, dominance, phenology. Abundance was calculated as the total number of species in a given ecosystem area respectively. This indicator is expressed in absolute value. Depending on abundance, the species may be relatively rare, common, abundant, very abundant. Dominance was calculated starting from the abundance as the ratio of livestock species and the amount of other herds in the studied area. The notion of dominance is an indicator of productivity, showing what percentage of each species is present in the biocoenosis.

Inventory of harmful and useful entomofauna were made using the Barber traps, of leaves samples and the fracture technique. Barber traps are the classic method for collecting ground-moving arthropods (epigee in the case of nocturnal or diurnal grasshoppers, but also other invertebrates with high activity on the soil, such as aranas, miriapodes, etc. Six traps per variant were installed in each experimental plot.

Leaf samples were an efficient method of estimating populations at the leaf level. This method consists in the preleavtion of 50 leaves per variant, collected randomly once a week (on the same day of the week). They were put in bags with the batch and date labels, transported to the laboratory, and examined under a microscope.

The fracture technique was used to evaluate fauna (harmful and useful) in the experimental variants, completing the other techniques (visual control, buried traps or Barber). The basic principle of the method is to shake a number of vegetative organs above a conical or square device (an entomological net).

# **RESULTS AND DISCUSSIONS**

It was necessary to identify and delimitation of habitats being a paramount relevance for the realization of the programs of monitoring and management of biodiversity. The microhabitat for vine has been identified as an analysis of its structure and morphology (Figure 1). The total studied area was 0.129 ha, from which 0.11 ha the area occupied with vine (main crop).



Figure 1. Analysis of structure and morphology of vineyard microhabitat

Soil type of ecosystem is preluvosol reddish molic (Br MB), the structure was destroyed towards the poliedric large developed subangular dark brown and wet-dry dark brown, porous, fine effervescence, moderately friable, with earthworm channels. The grain size fractions of soil in the ecosystem to study are shown in table 1.

Table 1

Soil type	Depth (cm)	Coarse	Fine sand	Dust	Clay	Textural
	(CIII)	Sanu			< 0.002 mm	CIdSS
Preluvosol	0-20	1.76	34.72	20.92	42.60	TT
reddish molic	20-40	12.91	6.41	29.38	51.30	TT
	40-60	1.26	21.55	23.69	53.30	AL
	I La amazur A					

Enter the vineyard soil composition from experimental plots (%)

TT = clay loamy; AL = clay.

As a result of valuation have been identified a number of 28 species of spontaneous flora which grow and develop in the wine-growing ecosystem got in this study. The plants are listed in table 2 after the scientific name and family.

Table 2

Spontaneous flora species reported in	vinevard ecosystem
-t	- , , , ,

Species	Taxon	Abundance	Dominant
Fam. Malvaceae	Althaea officinalis	17	0.839
Fam. Poaceae/	Agropyron repens	258	12.734
Gramineae			
Fam. Amaranthaceae	Amaranthus retroflexus	97	4.787
Fam. Apiaceae	Aethusa cynapium	76	3.751
	Matricaria inodora	31	1.530
	Polygonum convolvulus	98	4.837
Fam. Asteraceae	Cirsium arvense	113	5.577
	Chrysantheum vulgare	96	4.738
	Taraxacum officinale	82	4.047
Fam. Rubiaceae	Galium aparine	215	10.612
	Galium verum	36	1.776
Fam. Caryophyllaceae	Stellaria media	49	2.418
Fam. Lamiaceae	Lamium purpureum	130	6.416
	Salvia pratensis	10	0.493
Fam. Plantaginaceae	Plantago lanceolata	158	7.798
Fam. Urticacea	Urtica dioica	50	2.467
Fam. Umbelliferae	Aegopodium podagraria	28	1.382
Fam. Primulaceae	Primula officinalis	38	1.875
Fam. Papaveraceae	Chelidonium majus L.	42	2.073
	Papaver rhoeas	25	1.233
Fam. Ranunculaceae	Ranunculus ficaria	5	0.246

Fam. Violaceae	Viola odorata	20	0.987
Fam. Fabaceae	Lathyrus vernus	32	1.579
Fam. Campanulaceae	Campanula patula	25	1.233
Fam. Iridaceae	Crocus	5	0.246
Fam. Hypericaceae	Hypericum perforatum	8	0.394
Fam. Brassicaceae	Sinapis arvensis	22	1.085
	Capsella bursa pastoris	260	12.833

As regards fauna, there were identified 61 species of harmful fauna and 138 species of useful fauna (Table 3).

Table 3

Usefu	and harmf	ul fauna col	lected		
Specify	06-24.06	11-25.07	08-25.08	Total	The relative
	2016	2016	2016		abundance
					%
	HARMFU	L FAUNA			
Gryllus campestris L.	-	2	3	5	8.20
Cicada viridis L.	2	2	-	4	6.56
Ceresa bubalus L.	-	1	1	2	3.28
Pulvinaria vitis L.	1	1	1	3	4.92
Alopecosa pulverulenta C.	-	1	1	2	3.28
Epicometis hirta Poda	-	2	1	3	4.92
Apis sp.	1	2	-	3	4.92
Carabus violaceus L.	2	1	-	3	4.92
<i>Eurigaster maura</i> Pul.	1	2	1	4	6.56
Melolontha melolontha L.	2	2	-	4	6.56
Haltica ampelophaga Guer.	-	3	-	3	4.92
Lebia humeralis D.	-	2	3	5	8.20
Vespa germeanica L.	-	-	3	3	4.92
<i>Tetranychus urticae</i> Koch	3	-	-	3	4.92
Eriophyes vitis Pgst.	2	-	-	2	3.28
Anomala solida Erw.	3	-	-	3	4.92
Tanymecus palliathus F.	2	1	-	3	4.92
Hyphantria cuneae L.	-	-	3	3	4.92
Lobesia botrana Den.et Schiff.	-	-	3	3	4.92
Total harmful fauna				61	100.00
	USEFUL	FAUNA			
Forficula auricularia L.	10	16	8	34	24.65
<i>Blitophaga undata</i> Mull.	1	1	1	3	2.17
Calosoma auropunctata L.	1	1	2	4	2.90
Harpalus pubescens L.	-	2	3	5	3.62
Harpalus distiguendus Duft.	-	-	2	2	1.45

Coccinella 7-punctata L.	8	2	2	12	8.70
Propylea 14-punctata L.	5	1	-	6	4.35
Coccinula 14-punctata L.	8	1	1	10	7.25
Thea 22-punctata L	4	2	1	7	5.07
Coccinella septempunctata L.	5	2	1	8	5.80
Scymnus sp.	4	5	2	11	7.97
Philaenus spp.	-	7	-	7	5.08
Allobophora caliginosa L.	-	-	2	2	1.45
Allobophora rosea L.	-	3	1	4	2.90
Fridericia bulbosa Rosa	-	2	2	4	2.90
Lithobus fornicatus L.	1	1	1	3	2.17
Geophillus sp.	-	2	1	3	2.17
Nabis ferus L.	2	2	2	6	4.35
Eurydema ornata L.	1	1	2	4	2.90
Aphodius Iuridus F.	-	1	2	3	2.17
Total useful fauna				138	100.00
Total general				199	-
The relative abundance of harmful fauna (%)				61	30.66
The relative abundance of useful fauna (%)				138	69.34

In the first category highlighted the following species: *Cicada viridis* L., *Gryllus campestris* L., *Melolontha melolontha* L., *Hyphantria cunea* L., *Vespa germeanica* L., *Eurigaster maura* Pul. The category of useful species is represented by *Forficula auricularia* L, *Blitophaga undata* Mull., *Calosoma auropunctata* L., *Harpalus pubescens* L., *Sciaridae*, *Chalcidoidea*, *Ichneumonoidea*, *Formicoidea*.

Copies of each report category and the number of species showed a net favourable situation for useful fauna. The report overall FU (useful fauna)/FD (harmful fauna) was 69.34/30.66.

## CONCLUSIONS

From the 28 floral species the most abundant were: Agropyron repens, Capsella bursa pastoris, Galium aparine, Plantago lanceolata and Cirsium arvense. Rare species were Ranunculus ficaria, Crocus, Hypericum perforatum, Salvia pratensis and Althaea officinalis.

The most represented families were: *Asteraceae* - 420 species, *Brassicaceae* - 282 species, *Poaceae/Gramineae* - 258 species, *Rubiaceae* - 10 species. Genres that include most species are: *Capsella* - 260 species, *Agropyron* - 258 species, *Galium* - 215 species, Genul *Plantago* - 158 species, *Lamium* -130 species.

The useful and damaging fauna of the Valea Calugarească vineyard ecosystem was very varied.

The species that have registered a numerical abundance, important from the point of view of the protection of the culture were those belonging to the *Homoptera* order.

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# SOME FRUIT CHARACTERISTICS OF BLACKTHORN (PRUNUS SPINOSA L.)

Gavrilă Călușaru Florentina Claudia<sup>1,\*</sup>, Ionica Mira Elena, Cosmulescu Sina Niculina<sup>2</sup>

<sup>1,\*</sup>University of Craiova, Horticulture Faculty, Doctoral School of Plant and Animal Resources Engineering, Romania, e-mail: calusaru\_flory@yahoo.com

<sup>2</sup>University of Craiova, Horticulture Faculty, Department of Horticulture & Food Science, A.I.Cuza Street, 13, Craiova, Romania; e-mail: sinacosmulescu@hotmail.com

Key words: Prunus spinosa L., fruits, physical-chemical characteristics, diversity.

#### ABSTRACT

This study has been conducted upon 15 genotypes of Prunus spinosa L. growing wildly in spontaneous flora in South Oltenia Region (Dolj county). Ripen fruits of these genotypes have been harvested and analysed. Some physical-chemical characteristics have been observed. Average fruit weight ranged between 1.10 g and 4.20 g and the average pulp mass ranged between 61.53 and 93.75%. Dried substance content ranged between 17.50% and 25.50% and the values of titrable acidity were established as 1.94; 2.08; 2.78; 2.28; 1.91; 1.27; 1.47; 1.88; 1.51; 1.21; 1.71; 1.74; 1.54; 2.11; 1.61, respectively (g malic acid content/100g fresh fruit). This study aims at showing that there is a great diversity of Prunus spinosa L. genotypes, especially regarding the characteristics of fruit evaluated in the same ecological conditions. The last but not least, these genotypes of Prunus spinosa L. could be very useful not only as a tree-growing point of view, but also as food additive.

#### INTRODUCTION

At present, Prunus spinosa L. is the only species of this genre which can be found in spontaneous form in Romania, especially in steppe areas where it grows in small bushes (0.3-0.8 m) stretching up to the hills areas. It plays an essential role in root-sucking in both valley and low hills areas where it grows wildly especially in village common areas (Cociu et al. 1999). Prunus spinosa L. (blackthorn or sloe) is a species of flowering plant in the rose family of Rosaceae, subfamily Prunoidae, Prunus genre. The fruit is 1-1.5 cm globular drupe (Cosmulescu 2014) blueish black, little, short pedunculate bloomy, ripening in the autumn, covered of prune (lliescu 2002). The pulp is green, dense and very astringent to taste. They can be eaten both raw and overripe (Ghena et al. 1997). These fruits can be a valuable source of natural antioxidants (Cosmulescu et al. 2017). Even though they are edible and they can be used for medicinal purposes, it is hard to find products of blackthorn fruits on the market or even with their additives (Sikora et al. 2013). Blackthorn (Prunus spinosa L.) is used in phyto-therapy for the treatment of many diseases related to various forms of cough, it is mild laxative, diuretic, spasmolytic and anti-inflammatory agent. It has anti-septic effect (due to the presence of tannins) and shows activity against inflammatory of mucosal layer of the digestive system (Velickovic et al. 2014). Medicinal characteristics of blackthorn were shown by fruit flowers, bark and root of the plant. Apart from phytotherapy, blackthorns are also used in food industry for production of jams and various

beverages: liquor, wine, juice, compote and tea (Velickovic et al. 2014). According to Olszewska and Wolbis (2001) extracts from the flowers have been used as diuretic. anti-inflammatory, spasmolytic and mild laxative and "blood cleansing" agents, and their active substances are flavonoids. The fruits are also dietetic products rich in tannin which have anti-inflammatory and anti-bacterial effects. Apart from, flavonoids, kaempferol, quercetin and their heterosides can be mentioned among their constituents. The blackthorn (Prunus spinosa L.) is a thorny deciduous shrub whose fruits are astringent and they can be eaten fully ripen but it is more common to be prepared using sugar, honey or alcoholic drinks in order to obtain a digestive liquor used for its laxative, astringents, diuretic and purgative effects. According to Ruiz-Rodrigues et al. (2014) blackthorn could be a valuable and cheap source of antioxidants and she highlights their role in modern and traditional diet. Over a vast expanse of north-eastern Iberian Peninsula, sloes from blackthorn are macerated to make a schnapps called "pacharan". It is a traditional drink of Navarra, Spain flavoured with the essential oils of anises and sugar. In their study Fraternale et al. (2009) assessed the antioxidant activity of Prunus spinosa L. growing wildly in Urbino area, central Italy. Their study revealed that three most representative anthocyanins contained by Prunus spinosa L. fruit juice (cyanidin-3-rutinoside, peonidin-3-rutinoside and cyanidin-3-glucoside) are likely to play an important role in its antioxidant properties. Due to its antioxidant properties, different concentration of smashed blackthorn can be used for preparing ice-cream. Furthermore, this led to acceptable effects upon physical, chemical, thermic and sensorial properties of ice-cream. The use of blackthorn at different concentrations for ice-cream production caused an acceptable effect on some physical-chemical, thermal and sensory properties of icecream (Kavaz Yuksel 2015). According to Barros et al. (2010) blackthorn contains very useful bioactive phytochemicals such as phenolics, vitamins (ascorbic acid and tocopherol) and carotenoids. The combination of bioactive compounds and rich nutritional composition (high contents in carbohydrates, low contents in fat with precious contribution of polyunsaturated fatty acids as well as omega 3 and omega 6 fatty acids make them a special food. Marakoglu et al. (2005) showed not only the chemical and physical properties of blackthorn but also the fact that all materials contained high amounts of K (18706,98 mg/ kg), Ca (1524.22 mg/ kg), Mg (968.15 mg/ kg), P (1514.1 mg/ kg), S (500025.97 mg/ kg), Na (530.11 mg/ kg), B (26.99 mg/ kg), Al (26.33 mg/ kg) and Fe (16.18 mg/ kg). Analytical values revealed nutritional properties and mineral contents of blackthorn fruit. Moisture content, crude protein, crude oil, crude fibre, crude energy, water soluble extract and alcohol-soluble extract values obtained by Marakoglu et al. (2005) was 69.37%, 3.4%, 2.06%, 4.6%, 249 kcal/g, 78.28% and 14.74%, respectively. This paper aims at studying physicalchemical characteristics and properties of blackthorn fruits growing wildly in spontaneous flora in Oltenia region in order to identify important genotypes not only as tree-growing but also as food additive point of view.

## MATERIAL AND METHOD

There have been identified and labelled 15 genotypes of *Prunus spinosa* L. growing wildly in southern part of Oltenia region, Dolj county, namely Gura Vaii village, and Calopar village, Dolj county. Each label contained some codes such as the initial of the village (fruit where harvested from) and the genotype number according to their identification. Moreover, geographical coordinates were established for each type using Altimeter programme. There were harvested 100 fruits from each genotype, namely 7 genotypes from Gura Vaii and 8 genotypes from Calopar village, and then were taken to the laboratory in cooled plastic bags. Next, they were labelled and

measured (length and diameter of the fruit, unit mass, fruit volume, fruit density, kernel and pulp mass). After that they were prepared for chemical analyses (total dry matter, soluble dry matter content, acidity). The length and diameter of fruit were measured by digital micrometre. The fruit mass was measured by electronic balance. Water and total dry matter were determined by using water evaporation method from the finegrained analytical average sample by maintaining the sample in a thermoregulatory oven reaching temperatures no higher than 85-105°C (Nour 1998). The results are expressed using millimetre for length and diameter, grams for fruit mass, water percentage and total dry matter. Total dry matter was established using refractometric method (Ionica 2014) using digital refractometer and the results were expressed as percentages. The titrable acidity was determined by titration of a known amount of fruit water extract with 0.1N NaOH using phenolphthalein as an indicator. The results are expressed as g acid/100g fresh matter.

Statistical analysis. The data obtained after the above evaluation have been processed so as mean values and standard deviation or standard errors. This treatment was carried out using Data Analysis option in Microsoft Office Excel.

## **RESULTS AND DISCUSSION**

In this study the chemical and physical properties of 15 genotypes of blackthorn fruits growing wild in the spontaneous flora in Oltenia region were assessed. After the investigations carried out in the laboratory, the highest average fruit weight was registered in genotypes in Gura Vaii village 3.07± 0.47 g (GV3) whereas the lowest amount was established in genotypes in Calopar village (2.01 ± 0.16 g la C5). The highest fruit unit mass was observed in GV3 genotype in Gura Vaii village (4.20g) whereas C6 genotype in Calopar was lower (3.10 g.). The minimum mass of fruits was equal for both genotypes 1.10 g for both GV1 and C2. As regards the pulp mass, the highest level was observed in GV1 genotype (93.75%) whereas the lowest one was observed in C1 genotype (61.53%) (Table 2). For 4 of 7 genotypes in Gura Vaii village the pulp mass was higher than 90% whereas only one genotype in Calopar village reached this point. The medium length fruits for 15 genotypes was reported at the level of 14.25 mm for genotypes in Gura Vaii village and 12.96 mm in Calopar village. The highest rates for medium length fruits were observed in 2 genotypes from Gura Vaii (GV8 and GV3) ranging from 17.74 mm to 17.11 mm, respectively, compared to the values obtained for the same characteristic in two genotypes of Calopar population, namely 14.04 mm (C6) and 13.89 (C5) (Table 1). The maximum height of fruits was established as 22 mm for GV8 genotype in Gura Vaii village whereas C1 genotype in Calopar reached only 18.80 mm. The minimum height of fruits was established as 9.50 mm for GV 1 genotype and 9.90 mm for C3 (Table 3). The large diameter of the fruit had a maximum value at GV8 (20.70 mm) and a minimum value for the genotype GV1 (9.40 mm), and in the Calopar population, the maximum and minimum value for the same characteristic was 18.50 mm and 9.30 mm, encountered in the same genotype (C1). The highest small diameter was established as 20.50 mm for GV8 genotype from Gura Vaii area and the lowest one was established as 8.80 mm for GV1 genotype. As regards the genotypes in Calopar area the highest and the lowest degree was established as 18.10 mm and 9.20 mm, respectively, for C1 genotype.

The average results of this study were established as being higher than those of Marakoglu et al. (2005) (Table 1 and Table 2) for fruits growing wild in Konya province in Turkey, except for diameter of fruit and sphericity which were almost similar to those observed for genotypes from Calopar village namely: length (12.12 mm), unit mass (1.28 mm), fruit diameter (12.59 mm), geometric mean diameter (12.43), sphericity (1.03) and volume (1288.5 mm<sup>3</sup>). Coefficient of variation for each value is given in table 1 and 2. For

C1 genotype from Calopar area and for GV1, GV2 and GV8, from Gura Vaii, the variation coefficient for kernel mass shows greater percentages which were established as follows: 20.00%, 27.78%, 20.83% and 25.71%, respectively, which show a great non-uniformity for this feature. The same non-uniformity was also observed in genotypes C1, C6, GV1, GV8; in case of kernel percentage the coefficient of variation was 20.57%, 21.49%, 24.82% and 24.57%, respectively. In terms of fruit weight, the variation coefficient indicates a small/medium variation.

As regards physical characteristics of blackthorn fruits we can mention that the values of mass, geometric mean diameter and sphericity were established. The average weight of fruits that belonged to the genotypes studied here was lower, within the range of 1.46  $\pm$  0.19 and 3.07  $\pm$  0.47, compared to that found by Erturk et al. (2012) for Turkish genotypes, 5.86 g for red fruits and 7.83 g for dark-purple fruits.



120 120,00% 100 100.00% 80 60 40 80.00% 60,00% 40.00% 20 20,00% 0.00% 2.00 2.10 , AO 2.20 2.30 2.10 2.60 20 2,00 290 200 Frequency Bin Cumulative %

Fig.1 Percentage distribution for fruit weight harvested in Gura Văii population

Fig.2 Percentage distribution for fruit weight harvested in Calopar population

Also, the technological properties such as geometric mean diameter of fruit were established as 20.08 for GV8 and 18.46 for C1 genotype (the highest level). Minimal values were 9.30 for GV1 genotype and 9.83 for C2 genotype, whereas the highest average values were established as 16.84 and 16.33 for GV8 genotype and GV3 genotype, respectively. Sphericity (shape index/form index) had average values ranging between 1.02 and 1.07 for genotypes in Gura Vaii area and from 0.98 to 1.06 for genotypes in Calopar area.

Concerning the fruit weight, of the total fruit harvested from the 7 genotypes belonging to Gura Vaii population, only one fruit had the minimum weight of 1.10 g, representing 0.17% of total fruit, and one single fruit had the maximum weight of 4.20 g. In the range of weight between 1.20 and 2.00 g were 43.96%, between 2.00 g and 3.00 g were 47.58%, and between 3.00 and 4.00 grams were 8.10% of the fruit (Figure 1). Of total fruit harvested from the eight genotypes belonging to Calopar population, 75.73% of fruits weighed between 1.10 and 2.00 g, 24.12% of the fruit weighed between 2.00 and 3.00 g, and a single fruit had 3.10 g, representing 0.15% of total fruit (Figure 2). To determine the fruit quality, chemical analyses were performed and the results are presented in Table 3. The highest value for titratable acidity was found in GV3 genotype (2.78 g malic acid / 100 g fresh product), and the lowest in C3 genotype (1.21 g malic acid / 100 g fresh product). A high percentage of total dry substance showed fruits from genotype GV8 (37.66%) and the lowest content was noted for genotype C2 (27.71%). Considering soluble dried substance, the values ranged from 17.50% (GV5 genotype) to 25.50% (C5 genotype). Our results on titratable acidity (Table 3) are similar to those found by Erturk et al. (2012) in Prunus spinosa L. genotypes with dark purple, red and vellow purple fruits in Turkey; the highest acidity was found in dark red fruits (4.99%) and the lowest in dark purple fruits (3.87%).

Table 1

Average values of some physical characteristics of Prunus spinosa in Gura Văii and Calopăr areas

	cv%	3.92	4.90	2.83	5.90	1.96	1.96	4.67	5.71	3.77	6.12	3.92	3.77	1.96	2.91	2.91
icity IF	Max/ Min.	1.13/ 0.93	1.12/ 0.90	1.14/ 1.01	1.15/ 1.02	1.09/ 1.00	1.00/	1.22/ 0.98	1.27/ 0.89	1.17/ 1.00	1.15/ 0.83	1.12/ 0.92	1.21/ 1.00	1.12/ 0.99	1.11/ 1.00	1.14/ 0.95
Spher	Average ± s	1.02±0.04	1.02±0.05	1.06±0.03	1.07±0.03	1.02±0.02	1.02±0.02	1.07±0.05	1.05±0.06	1.06±0.04	0.98±0.06	1.02±0.04	1.06±1.06	1.02±0.02	1.03±0.03	1.03±0.03
eter IM	cv%	5.55	6.45	7.16	5.90	6.04	8.35	16.15	16.33	6.96	7.50	8.71	5.82	7.89	7.05	7.36
ean diam	Max./ Min.	12.26/ 9.30	14.30/ 10.46	18.86/ 13.73	15.93/ 12.50	14.96/ 11.36	15.93/ 9.83	20.80/ 12.43	18.46/ 9.93	13.66/ 9.83	14.33/ 10.13	15.30/ 10.30	14.76/ 10.96	17.13/ 11.00	14.40/ 10.70	14.30/ 9.96
Geometric m	Average ± s	10.82±0.60	12.09±0.78	16.33±1.17	14.24±0.84	13.42±0.81	12.57±1.05	16.84±2.72	12.43±2.03	11.64±0.81	12.54±0.94	12.40±1.08	13.24±0.77	13.82±1.09	12.48±0.88	12.22±0.90
Ê	cv%	6.00	6.58	7.52	6.42	6.45	8.02	17.02	16.87	7.52	77.7	9.05	6.45	7.84	7.45	7.87
meter(m	Max./ Min.	11.90/ 8.80	14.10/ 10.20	18.70/ 13.20	15.60/ 12.00	14.80/ 11.10	14.30/ 9.50	20.50/ 11.30	18.10/ 9.20	13.30/ 9.60	14.20/ 10.00	14.90/ 10.00	14.50/ 10.60	16.60/ 10.90	14.30/ 10.30	14.30/ 9.60
Small dia	Average ± s	10.50±0.63	11.86±0.78	15.82±1.19	13.71±0.88	13.17±0.85	12.35±0.99	11.80±2.76	12.03±2.03	11.30±0.85	12.35±0.96	12.15±1.10	12.87±0.83	13.64±1.07	12.21±0.91	11.94±0.94
æ	cv%	5.71	6.79	7.46	6.57	6.27	7.92	16.45	16.56	7.39	7.94	9.02	6.29	7.62	7.25	7.48
eter D (m	Max./ Min.	12.40/ 9.40	14.30/ 10.40	18.70/ 13.30	15.90/ 12.10	15.00/ 11.10	14.50/ 9.80	20.70/ 11.80	18.50/ 9.30	13.50/ 9.80	15.00/ 10.30	15.30/ 10.20	14.60/ 10.90	16.70/ 11.00	14.40/ 10.50	14.30/ 9.90
Big Diam	Average ± s	10.85±0.62	12.07±0.82	16.08±1.20	14.00±0.92	13.40±0.84	12.50±0.99	16.60±2.73	12.26±2.03	11.50±0.85	12.72±1.01	12.41±1.12	13.03±0.82	13.78±1.05	12.41±0.90	12.16±0.91
	cv%	6.31	7.44	7.13	5.26	5.99	7.84	15.45	16.41	7.21	8.36	8.78	5.47	8.33	6.86	7.31
t H(mm)	Max./ Min	12.70/ 9.50	14.50/ 10.20	20.00/ 14.10	16.50/ 13.40	15.30/ 11.80	14.70/ 10.20	22.00/ 13.40	18.80/ 10.40	14.50/ 10.00	14.60/ 9.90	15.70/ 10.40	15.20/ 11.70	18.10/ 11.10	14.80/ 11.00	14.50/ 10.20
Heigh	Average ± s	11.11±0.7	12.36±0.92	17.11±1.22	15.02±0.79	13.68±0.82	12.75±1.00	17.74±2.74	12.92±2.12	12.20±0.88	12.56±1.05	12.64±1.11	13.89±0.76	14.04±1.17	12.83±0.88	12.58±0.92
Genotype		GV1	GV2	GV3	GV4	GV5	GV7	GV8	5	8	ឌ	2	S	8	C7	ö

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		%v3	24.82	19.79	17.28	13.99	16.46	8.36	24.57	20.57	17.41	16.13	15.44	9.32	21.49	14.22	16.95
	kernel mass	Max./Min.	18.75/6.25	20.00/9.52	32.00/13.79	31.57/13.79	23.80/9.52	19.04/13.63	23.80/6.45	38.46/15.38	30.76/10.00	30.76/12.50	28.57/11.76	20.00/15.78	35.29/11.11	28.57/13.04	30.76/15.00
	%	Average±s	12.25±3.04	13.64±2.70	20.95±3.62	22.59±3.16	17.74±2.92	15.90±1.33	13.92±3.42	21.92±4.51	19.64±3.42	20.46±3.30	20.08±3.10	17.92±1.67	17.31±3.72	21.16±3.01	20.53±3.48
		cv%	3.46	3.12	4.58	4.10	3.55	1.63	3.98	5.78	4.20	4.15	3.88	2.03	4.50	3.88	4.48
	ulp mass	Max./Min.	93.75/81.25	90.47/80.00	86.20/68.00	86.20/68.42	90.47/76.19	86.36/80.95	93.54/76.19	84.61/61.53	90.00/69.23	87.50/69.23	88.23/71.42	84.21/80.00	88.88/64.70	86.95/71.42	85.00/69.23
	%	Average ± s	87.75±3.04	86.35±2.70	79.05±3.62	77.47±3.18	82.25±2.92	84.03±1.37	86.08±3.43	78.07±4.51	80.33±3.37	79.53±3.30	79.92±3.10	82.08±1.67	82.68±3.72	78.59±3.05	79.23±3.55
		cv%	27.78	20.83	14.29	12.50	17.14	9.68	25.71	20.00	17.86	15.15	15.79	13.89	17.65	12.82	16.67
	l weight G(g)	Max./Min.	0.30/0.10	0.30/0.20	0.90/0.30	0.60/0.30	0.50/0.20	0.40/0.30	0.50/0.20	0.50/0.20	0.40/0.20	0.40/0.20	0.50/0.20	0.40/0.30	0.60/0.20	0.50/0.30	0.50/0.30
	Kerne	Average ± s	0.18±0.05	0.24±0.05	0.63±0.09	0.48±0.06	0.35±0.06	0.31±0.03	0.35±0.09	0.35±0.07	0.28±0.05	0.33±0.05	0.38±0.06	0.36±0.05	0.34±0.05	0.39±0.05	0.36±0.06
		cv%	11.49	11.43	15.31	11.57	8.59	8.16	13.44	11.18	13.01	10.43	10.58	7.96	13.57	11.48	10.40
	weight G(g)	Max./Min.	2.00/1.10	2.30/1.30	4.20/2.00	2.90/1.60	2.30/1.50	2.30/1.60	3.10/1.80	2.20/1.20	2.00/1.10	2.00/1.30	2.40/1.40	2.40/1.70	3.10/1.50	2.40/1.40	2.20/1.30
	Fruit	Average ± s	1.48±0.17	1.75±0.20	3.07±0.47	2.16±0.25	1.98±0.17	1.96±0.16	2.53±0.34	1.61±0.18	1.46±0.19	1.63±0.17	1.89±0.20	2.01±0.16	1.99±0.27	1.83±0.21	1.73±0.18
	Genotype		GV1	GV2	GV3	GV4	GV5	GV7	GV8	5	C2	ő	<mark>6</mark>	C5	90	C7	ő

Itable 2. Average values of fruit mass, kernel mass, percentage of pulp and kernel in the fruits analyzed in the two populations of *Prunus* spinosa

Table nr. 3

Values and percentages of total dried substance, acidity, soluble dried substance of *Prunus spinosa* L. from Gura Vaii (GV) and Calopar areas, Dolj county

N.a.	Constant of a	Tetel day	Tituatable esidit.	
INO	Genotype	l otal dry	l itratable acidity	Soluble dry matter
		matter %	(100 g malic acid	%
			content/fresh fruit)	
1	GV1	33.94±0.02	1.94±0.05	20.50±0.05
2	GV2	32.50±0.01	2.08±0.03	23.50±0.06
3	GV3	31.89±0.03	2.78±0.02	23.50±0.15
4	GV4	31.11±0.10	2.28±0.01	19.50±0.02
5	GV5	27.83±0.01	1.91±0.06	17.50±0.25
6	GV7	28.51±0.02	1.27±0.15	18.00±0.04
7	GV8	37.66±0.01	1.47±0.25	24.50±0.06
8	C1	36.74±0.01	1.88±0.03	23.50±0.03
9	C2	27.71±0.01	1.51±0.04	19.50±0.02
10	C3	28.12±0.10	1.21±0.02	18.50±0.35
11	C4	30.03±0.15	1.71±0.01	19.00±0.04
12	C5	35.73±0.02	1.74±0.02	25.50±0.05
13	C6	29.37±0.04	1.54±0.31	20.50±0.04
14	C7	31.21±0.06	2.11±0.25	20.50±0.05
15	C8	31.20±0.05	1.61±0.04	20.50±0.06

Marakoglu et al. (2005) report an acidity of 1.97% and a pH of 3.53% for fruit in Konya, Turkey. The fruits of *Prunus spinosa* L. in Madrid, Spain, studied by Ruiz-Rodríguez et al. (2014) showed a high content of anthocyanins (1431.75 mg pelargonidine 3-glucoside Eq/100 g FW) and phenolic acids (728.81 mg E q gallic acid/100 g FW), low vitamin C (11.27 mg ascorbic acid/100 g FW) and a high level of total phenolic compounds (2294.57 mg/100 g FW). Wolbis et al. (2001) isolated from the flowers of *Prunus spinosa* L. a mixture of alpha and beta amyrine, a mixture of ursolic and oleanic acids, ursolic acid, a mixture of beta-sitosterol, gamma sitosterol 3-0 beta-D-glucopyranoside were isolated.

## CONCLUSIONS

Determining the physical and chemical characteristics of the fruits is a step that precedes the establishment of their edible mass and an important step for the qualitative and quantitative assessment of production and the drawing of the fruit utilization directions. Fruit weight ranged between 1.10 g and 4.20 g and the percentage of pulp mass ranged between 61.53 and 93.75 %. The soluble dry matter content ranged from 17.50% to 25.50% and the titratable acidity was expressed as g of malic acid/100 g of fresh product ranging from 1.27 to 2.78. Through this study it was shown that there is a great variability between the genotypes of blackthorn, in terms of characteristics of fruit under the same ecological conditions, and these genotypes could be important as tree-growing point of view and also as food additive.

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# RESEARCH CONCERNING THE BEHAVIOUR OF WALNUT GENOTYPES WITH DIFFERENT GEOGRAPHIC ORIGIN UNDER THE ECOLOGICAL CONDITIONS OF NORTH OF VÂLCEA COUNTY

Giura Simona<sup>1</sup>, Scutelnicu Anca<sup>1</sup>, Preda Silvia<sup>1</sup>, Botu Mihai<sup>1,2</sup>, Manthos Ioannis<sup>3</sup>, Mitrea Rodi<sup>2</sup>

<sup>1</sup> University of Craiova – SCDP Vâlcea, Rm. Vâlcea

<sup>2</sup>University of Craiova, Faculty of Horticulture, Department of Horticulture and Food Science, Craiova <sup>2</sup> University of Craiova – SCDP Vâlcea, Rm. Vâlcea

<sup>3</sup>Institute of Plant Breeding & Genetic Resources, Dept. of Nut Trees, HAO-DEMETER , Lamia, Greece \* Correspondence author. E-mail: simona\_vam01@yahoo.com

Key words: Juglans regia L., cultivars, ecological conditions, frost

## ABSTRACT

The common walnut nut finds favorable conditions for cultivation in the Oltenia region located into the SW of Romania, where the climate is temperate, with some Mediterranean influences. Present study is evaluating during 2016 and 2017 years a total number of 26 accessions of walnuts of different geographic origins (14 from Romania, 9 from the USA, 1 from France, 1 from Russia and 1 from Japan) from the UCv-SCDP collection regarding yield, phenology, fruit maturation and resistance to late spring frosts.

# INTRODUCTION

Common walnut (*Juglans regia* L.), known also as Carpathian, Persian or English walnut is a very valuable nut crop. Although walnut is adapted to various ecological conditions from almost 50 countries from both hemispheres, it is quite susceptible to low temperatures during winter, but also to early and late frosts.

In Romania, the walnut culture dates back to the ancient Dacians, when the Latin poet Ovid wrote about walnut that "he is not pretentious, he grows by the side of the road, and he is not afraid neither of the wind, nor of rain, nor of heat or of cold" (Cociu et al., 2008).

Walnuts can be found almost in all suitable regions of Romania from the plains till high hills. Walnut cultivation has become very important due to the advantages of its products (fruits, wood, leaves) (Botu et al., 2010).

Favorable ecological conditions for walnut cultivation are found in the Oltenia region, a region located in southwestern Romania, where the climate is temperate, with Mediterranean influences, with an average annual temperature of 10-11,5°C. (Cosmulescu et al., 2012).

Survival and obtaining good fruit production in temperate continental areas depends on winter temperatures (Olsen, 2010), and especially late spring frosts (Arora and Rowland, 2011).

*Juglans regia* L. is a species of high plasticity and, to a certain extent, tolerates low winter temperatures in different geographic areas and does not suffer winter damage (Améglio T. et al., 2001).

Even inside *Juglans regia* species there are differences regarding resistance to low temperatures during winter, respectively to early or late frosts. Walnut cultivars with lateral bearing proved to be more susceptible from this point of vue then the cultivars with terminal bearing (so called Carpathian walnuts).

Resistance to winter temperatures is not just a characteristic of varieties, according to Ameglio et al. (2001), low temperature resistance is due to the accumulation of carbohydrates during the previous year and its storage in the trunk.

Terziev (2002) states that lesions are more severe when trees have been exposed to low temperatures for a longer period of time.

Gandev (2013) mention that the French cultivar Lara is less winter hardy then another French cultivar, Fernor and Bulgarian cultivar Izvor 10 regarding reproductive organs. There are also differences between types of reproductive organs, catkins being usually more susceptible to frost then female buds.

Germain et al. (1999) mentioned that the Californian varieties (lateral bearing cultivars) were inappropriate for cultivation in the colder climate of France.

According to Nedev et al. (1983), low temperature damages in walnut cultures was reported only when temperatures dropped below -20°C. The Carpathian walnut is considered to be the most resistant, at temperatures that can reach up to -32°C; -35°C (Domoto, 2002).

Botu et al. (2010) report that in the Vâlcea area the winter temperature of -22.7°C did not affect the growth and fruiting of the Californian and French lateral bearing cultivars.

Nedev et al. (1983) and Terziev (2002), cited by Gandev (2013), mention that the temperature of -24.4°C is crucial for walnut cultivars.

# MATERIAL AND METHODS

The study was conducted between 2016 and 2017 in the walnut collection belonging to the University of Craiova - SCDP Vâlcea. The collection is located at 260 m elevation, at 45°8'45" N and 24°22'01" E, under non-irrigated conditions. Average annual rainfall in the area is 715 mm.

The biological material used in this study consists of 26 accessions of walnut with different geographical origins: 14 genotypes from Romania, 9 from the United States, 1 from France, 1 from Russia and 1 from Japan. All genotypes are grafted on *Juglans regia* seedling. The planting distance from the collection is 9.0 x 8.0 (139 trees/ha). The age of trees in the collection varies between 18 to 22 years old.

Research methods are related to the aims of the paper and include comments

on:

- geographic origin of studied cultivars;
- vigor of the trees;
- fruit bearing type;
- blooming time of female and male flowers;
- dichogamy;
- influence of low temperatures on vegetative buds, mixed buds and catkins;
- resistance to low temperatures during spring (March and April).

For observations on the influence of temperatures on buds, twigs were collected from trees at a height of 2.5-3 m above the ground. 3 trees of each cultivar were sampled, from each tree 10 twigs were harvested. The different types buds were sectioned with a scalpel, and the percentage of frost damage was reported using a binocular microscope.

# **RESULTS AND DISCUSSIONS**

The walnut genotypes with different geographical origins, studied in the Northern area of Oltenia (Vâlcea county), showed differences regarding growth, fruit bearing and fruit maturation.

In case of genotypes with geographical origin in Romania, the plant vigor ranges from medium (Velniţa, HC O2 and HC O3) to the high (Germisara, Jupâneşti, Sibişel 44, Valcor, Valrex, Valmit, Valcris, Unival, Valstar, Sarmis and VL 102 H).

Accessions originating from France, the USA, Japan and Russia exhibited from high vigor (all of the genotypes from USA, except *Juglans nigra*, and Franquette from France), genotypes from Japan (*J. sieboldiana*), Russia (*J. mandshurica*) and *J. nigra* presented very high growth vigor (Tables 1 and 2).

The walnut accessions, which were evaluated, have three types of bearing fruits:

- terminal: 11 genotypes from Romania (Table 3) and 7 foreign ones;
- lateral: 2 genotypes in Romania (HC O2 and HC O3) (Table 1) and 4 foreign genotypes (Table 2).
- Intermediate: Germisara cultivar from Romania and American cultivar Hartley.

During the study period phenological data was recorded. Pollination is very important to achieve yield. Walnut pollination is done by wind.

Walnuts are monoecious (Polito, 1996; cited by Krueger), bearing staminate and pistillate flowers, separately located on the same tree. The walnut cultivars are generally cross compatible. The period of pollen shedding does not completely overlap the period of female pistillate flowers receptivity, which is known as dichogamy (Forde and Griggs, 1975; cited by Krueger).

Dichogamy can be influenced by environmental conditions; same cultivar under different ecological conditions can have slightly modified behaviour regarding blooming time and dichogamy. In some exceptional climatical years, dichogamy can be modified, a known protandrous cultivar can switch to homogamous type or a protogynous one can pass to homogamous. Passing from protandrous to protogynous type of dichogamy or from protogynous to protandrous type in case of same genotype under exceptional variable climatic conditions of same location are extremely rare.

Phenology under normal climatic conditions was recorded in case of walnut genotypes during spring of 2016.

Out of the Romanian walnut genotypes, 10 proved to behave during the study period as protogynous and 4 as protandrous (Jupâneşti, Valcor, Valrex and Sibişel 44). 6 of the foreign accessions behaved as protogynous and 6 as protandrous (Tables 1 and 2).

In 2017, the cold wave of air of -2.1°C recorded on April 22<sup>nd</sup> has affected drastically the flowering of walnut genotypes (Figure 4). The beginning of blooming for Romanian genotypes occurred at the end of April, and the end of the blooming took place beginning of May. Earlier accessions from this point of vue were HC O2, HC O3 and Valmit, and the latest one was Velniţa.

In the case of the foreign genotypes, Hartley and Serr cultivars exhibited early blooming. *J. sieboldiana*, Franquette and *J. nigra* accessions proved to be the most late. The blooming time began in the first 10 days of May.

Table 4 represents the absolute minimum temperatures for the January 1<sup>st</sup> to June 1<sup>st</sup> 2017 period. The cold air reached -1.7°C on March 27<sup>th</sup>, 2017, when bud break of walnut genotypes occurred for most genotypes (Table 3). Bud break took place in spring of 2017 almost 3 weeks earlier than the multi-annual average period.

14.04. - 02.05.2016 12.04. - 26.04.2016 18.04. - 08.05.2016 27.04. - 02.05.2016 29.04. - 12.05.2016 10.04. - 02.05.2016 14.04. - 30.04.2016 26.04. - 08.05.2016 02.05. - 14.05.2016 26.04. - 08.05.2016 16.04. - 30.04.2016 16.04. - 30.04.2016 18.04. - 30.04.2016 23.04. - 29.04.2016 Male flowers Blooming time 15.04. - 28.04.2016 12.04. - 18.04.2016 16.04. - 24.04.2016 18.04. - 28.04.2016 15.04. - 22.04.2016 29.04. - 09.05.2016 18.04. - 27.04.2016 30.04. - 08.25.2016 18.04. - 28.04.2016 13.04. - 20.04.2016 12.04. - 18.04.2016 03.04. - 12.04.2016 14.04. - 22.04.2016 19.04. - 30.04.2016 Female flowers protogynous protogynous protogynous protogynous protandrous protogynous protandrous protandrous protandrous protogynous protogynous protogynous protogynous protogynous Dichogamy intermediate Bearing erminal erminal terminal terminal erminal terminal erminal erminal erminal terminal terminal lateral atera type medium medium medium medium vigor high high high Tree high high high high high high high Geographic Geoagiu Geoagiu Geoagiu Craiova Craiova Välcea Välcea Välcea Välcea Välcea origin Pitești Välcea Välcea aşi Accession Germisara Sibişel 44 Jupänești VL102 H name Velnița Sarmis HC 03 Valcris Valcor Unival Valstar HC 02 Valrex Valmit Ň. 0 2 ŝ 4 ŋ ω თ N m 4 0 ÷

Main agro-biological characteristics of the autochtonous walnut accessions studied at UCv-SCDP Vålcea (2016)

Table 1

ng time	Female flowers	13.04 23.04.2016	16.04 26.04.2016	16.04 21.04.2016	19.04 28.04.2016	17.04 30.04.2016	12.04 28.04.2016	29.04 13.05.2016	22.04 30.04.2016	06.04 14.04.2016	01.05 08.05.2016	01.05 14.05.2016	03.05 13.05.2016
Bloomir	Female flowers	30.04 08.05.2016	01.05 10.05.2016	24.04 30.04.2016	18.04 01.05.2016	30.04 08.05.2016	16.04 24.04.2016	15.04 27.04.2016	14.04 20.04.2016	10.04 17.04.2016	27.04 11.05.2016	30.04 11.05.2016	26.04 07.05.2016
Dichogamy		protandrous	protandrous	protandrous	protogynous	protandrous	protandrous	protogynous	protogynous	protandrous	protogynous	protogynous	protogynous
Bearing	type	terminal	intermediate	terminal	lateral	lateral	terminal	lateral	terminal	lateral	terminal	terminal	terminal
Tree	vigor	high	very high	very high	very high								
Geographic	origin	France	U.S.A.	Japan	U.S.A.	Russia							
Accession	name	Franquette	Hartley	Adams 10	Serr	Vina	Manregian	Pedro	Idaho	Payne	J. sieboldiana	J. nigra	J. mandshurica
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Table 2

Table 3

Bud break of the walnut cultivars studied in 2017 at UCv-SCDP Vâlcea

No.	Accession	Start of	End of						
	name	bud break	bud break						
1	Germisara	07.04.2017	15.04.2017						
2	Jupâneşti	03.04.2017	14.04.2017						
3	Franquette	27.04.2017	20.05.2017						
4	Hartley	03.04.2017	13.04.2017						
5	Velniţa	31.03.2017	13.04.2017						
6	Adams 10	05.04.2017	13.04.2017						
7	Serr	21.03.2017	31.03.2017						
8	Vina	24.03.2017	07.04.2017						
9	Sibişel 44	10.04.2017	17.04.2017						
10	Manregian	24.03.2017	03.04.2017						
11	Pedro	05.04.2017	13.04.2017						
12	Valcor	24.03.2017	11.04.2017						
13	Valrex	24.03.2017	04.04.2017						

No.	Accession	Start of	End of
	name	bud break	bud break
14	Valmit	22.03.2017	31.03.2017
15	J. mandshurica	04.04.2017	13.04.2017
16	J. sieboldiana	16.03.2017	31.03.2017
17	J. nigra	05.05.2017	17.05.2017
18	Valcris	24.03.2017	03.04.2017
19	Unival	24.03.2017	03.04.2017
20	Valstar	24.03.2017	07.04.2017
21	HC O2	22.03.2017	31.03.2017
22	HC O3	20.03.2017	24.03.2017
23	Sarmis	21.03.2017	04.04.2017
24	Idaho	24.03.2017	08.04.2017
25	VL 102 H	05.04.2017	16.04.2017
26	Payne	21.03.2017	03.04.2017

Table 4

Minimum daily temperatures recorded at Rm. Vâlcea Weather Station (2017)

Climatic	Year	Monthly temperature values (°C)								
element		January	February	March	April	May	June			
Daily low temperature (°C)	2017	-18.2 °C (Jan.10 <sup>th</sup> )	-9.3 °C (Feb. 11 <sup>th</sup> )	-1.7 °C (Mar. 27 <sup>th</sup> )	-2.1 °C (Apr. 22 <sup>nd</sup> )	6.8°C (May 11 <sup>th</sup> )	10.1°C (June 10 <sup>th</sup> )			

After frost, twigs were harvested from each accession and samples of buds were analyzed.

The data presented in Table 5 show that frost caused a different degree of deterioration of the vegetative, mixed buds and catkins to the studied genotypes.

In case of Romanian genotypes the vegetative buds were affected from 0% Germisara, Velniţa and VL 102 H) to 70.8% (Sarmis). Values over 50% also were recorded for Valcor and HC O2 genotypes. Lower values of damage due to frost were observed for Valrex, Valmit, Valcris and Unival cultivars.

Mixed buds were affected differently from vegetative ones. Valmit, Unival and Jupâneşti cultivars did not show any damage, while the HC O2, HC O3 and Sarmis genotypes emphasized 60-70% damage (Figure 1).

The male flowers disposed in drooping catkins very affected by frost. Catkins from Velniţa, Sibişel 44, Valrex and Valcris cultivars and those from HC O2 and HC3 selections were affected in proportion of 70-100% (Fig. 2). Only 20% of the catkins from Germisara cultivar were affected (Table 5).

The degree of damage caused to the group of American accessions varied from 0% (Hartley, Manregian and Pedro) to 100% (Payne) in case of vegetative buds. Also, vegetative buds of Serr, Idaho, Vina and *J. nigra* were affected in high percentages (42.5 - 63.1%). Mixed buds were damaged in different proportions (100% in case of Serr and Payne; 66.6% for Adams 10; 70% for Idaho; 60.0% for Manregian). Catkins kept the same trend of damages (100% for Serr; 85.7% for Manregian; 71.8% for Pedro; 69.5% for Hartley) (Figure 3).

In case of the French cultivar Franquette the vegetative and mixed buds were only slightly affected by frost in the spring of 2017 due to their late bud break. This is in

accordance with Gandev and Dzuhvinov (2014) to confirm various frost susceptibility of walnut cultivars attributed to different phenological development. Table 5

No.	Origin Accession		Mixed buds	Vegetative	Catkins
	-	name	affected	buds affected	affected
			(%)	(%)	(%)
1		Germisara	20	0	17.9
2		Jupâneşti	0	33.3	34.3
3		Velniţa	20	0	100
4		Sibişel 44	30	44.1	70.2
5		Valcor	30	61.2	30.0
6	România	Valrex	30	10.5	95.5
7		Valmit	0	20	25.6
8		Valcris	45.4	18.1	79.4
9		Unival	0	24	37.8
10		Valstar	33.3	44	26.9
11		HC O2	70	57.1	100
12		HC O3	60	45	86.9
13		Sarmis	70	70.8	66.6
14		VL 102 H	41.6	0	22.9
15		Hartley	0	0	69.5
16		Adams 10	66.6	29.1	51.3
17		Serr	100	63.1	100
18		Vina	30	42.5	29
19	U.S.A.	Manregian	60	0	85.7
20		Pedro	0	0	71.8
21		Idaho	70	45.4	60
22		Payne	100	100	63.2
23		J. nigra	0	70.2	67.4
24	France	Franquette	0	0	46.6
25	Japon	J. sieboldiana	70	61.7	69.7
\$26	Russia	J. mandshurica	0	21.2	55.2

Influence of frost on walnut accessions in the North of Vâlcea County



Fig. 1. Walnut mixed bud affected by frost in 2017.



Fig. 2. Walnut catkin affected by frost in 2017.



Fig. 3. Walnut vegetative bud affected by frost in 2017



Fig. 4. Walnut field collection from UCv-SCDP Vâlcea affected by frost in 2017

*J. sieboldiana* accession originating from Japan was affected by frost with damages ranging from 61.7% in case of vegetative buds to 70.0% for mixed buds.

The mixed buds of *J. mandshurica* accession, originated in Russia, were not affected by frost while 55.2% of the male flowers were destroyed.

Observation will have to be continued the next spring in order to fully evaluate the consequences of 2017 frost on the next year yield.

Also, taking into account the visible variability of the climatic conditions, long term phenological data collection is necessary.
## CONCLUSIONS

During the study period (2016 – 2017) the genotypes originating from U.S.A., grown under the environmental conditions from North of the Vâlcea county, were most affected by frost occurred in April 22<sup>nd</sup> 2017.

The Franquette cultivar originating from France was the least affected by the low temperatures in the spring of 2017 due to the later bud break and phenological development.

Further observation should continue in the next years taking into account the visible climatic variability.

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# COMPARATIVE STUDY ON POLYPHENOLS CONTENT AND ANTIOXIDANT EFFECT OF OLIVE CULTIVARS FROM THE THESSALY REGION, GREECE

Gougoulias Nikolaos<sup>1</sup>\*, Kamvoukou Christina- Anna<sup>2</sup>, Ntalla Maria-Nektaria<sup>3</sup>, Papachatzis Alexandros<sup>1</sup>

<sup>1</sup> Department of Agricultural Technology, Technological Educational Institute of Thessaly, 41110 Larissa, Greece <sup>2</sup>School of Pharmacy, Aristotle University of Thessaloniki, Greece <sup>3</sup>Department of Computer Science and Engineering, Data Analysis, Technological Educational Institute of Thessaly, 41110 Larissa, Greece \*Correspondence author. email: ngougoulias@teilar.gr

Key words: Antioxidant activity FRAP; Antiradical activity DPPH; Olive fruits; Total phenols

#### ABSTRACT

Seven different varieties olive fruits (Amfisis, Chalkidikis, Kalamon, Megaritiki, Koroneiki, Arbequine and Arbosana) cultivated on the region of Larissa, Greece were studied for total polyphenol content, phenolic fractions and for antioxidant properties. It has been established that total phenols content, move in broad ranges depending on the variety, chemical soil properties, and with the ecological conditions. The content of TP in the olives varieties varied from 9.48 to 19.63 mg (GAE)/g FW, (NFP) from 1.43 to 5.11 mg (GAE)/g FW and (FP) from 5.89 to 14.52 mg (GAE)/g FW. The antioxidantactivity FRAP of the seven varieties of fresh fruits ranges from 37.2 to 77.7  $\mu$ mol (AAE)/g FW. The antiradical activity (DPPH•) ranges from 158.41 to 281.31  $\mu$ mol (Trolox)/g FW. The olives grown on the region of Larisa, Greece offer a high intake of antioxidants for the prevention of many diseases.

#### INTRODUCTION

The fruits are natural reservoir of phenolic compounds and of natural antioxidants (Gougoulias 2014). Olive fruits (*Olea europaea* L.) are one traditional Greek product, component of the Mediterranean diet, and natural reservoir of phenolic compounds and of natural antioxidants (Boskou & Visioli 2003). Olive fruits are rich to flavonoids, secoiridoids, phenolic acids and phenolic alcohols, which exert high antioxidant activity (Dağdelen et al. 2013). Phenolic compounds influence the sensory properties of the olive fruits (Brenes et al. 1995).

The concentration and composition of phenolic compounds in the olive fruits depends from the cultivar, the irrigation and the climatic conditions (Romani et al. 1999, Tovar et al. 2002, Vinha et al. 2005).

The aim of the present study is to determine the content of phenolic compounds and the antioxidant activity, of olive fruits from different varieties, grown on the Thessaly region, Greece.

# MATERIAL AND METHODS

Experimental: The study was carried in the farms Alatas, during the 2015 -2016 seasons, to two olive orchards. The one olive orchard (A) is located on the region Gonnoi of Larissa (latitude 39°51'N, longitude 22°29'E, 200m altitude), where are grown the olive varieties Amfisis, Kalamon, Chalkidikis and Megaritiki. The olive trees are over a hundred years old, with distance between two consecutive trees 6m and between two consecutive lines 7m. 2000 m<sup>3</sup> irrigation water per hectare were added. Also, 50 kg sheep manure corresponding to 1.20 kg Nitrogen, 1.10 kg Potassium, 0.4 kg Phosphorus and 13.8 kg Organic matter per tree were added. The other olive orchard (B) is located on the region Kranona of Larissa (latitude 39°26'6"N, longitude 22°17′55″E, 100m altitude), where are grown the olive varieties Koroneiki, Arbequine Arbosana. The olive trees are fifteen years old, with distance between two and consecutive trees 3m and between two consecutive lines 4m. 4500 m<sup>3</sup> irrigation water per hectare were added. Also, 30 kg sheep manure corresponding to 0.65 kg Nitrogen, 0.60 kg Potassium, 0.2 kg Phosphorus and 7.8 kg Organic matter per tree were added. The climate in the area is continental, with cold rainy winters, average temperatures 6°C, hot summers, average temperatures 25°C and average annual precipitation 420 mm.

The basic characteristics of olive fruits are shown in Table 1. The collection of fruits took place at November of 2016, at the stage of complete maturation. Two trees by each olive variety were chosen, were sampled of olive fruits one kilogram with four replicates from each tree, from all the orientations and without type of disease. These samples were stored at -18°C and were further subjected to analyses.

Table 1

Varieties	Size	Color	Use	Origin
Amfisis	Big	Black	Dual-purpose	Greece
Chalkidikis	Big	Yellowish-green	Table olives	Greece
Kalamon	Big	Black	Table olives	Greece
Megaritiki	Medium	Black	Dual-purpose	Greece
Koroneiki	Small	Black	Oil production	Greece
Arbequine	Small	Black	Oil production	Spain
Arbosana	Small	Black	Oil production	Spain

#### Basic characteristics of olives

**Preparation of the methanol extracts:** The flesh was separated from the kernel and 100 g of flesh for each treatment were subjected to freeze drying for further extraction and determination of humidity. The dry mass was crushed and stored in clean bottles in refrigeration (Boskou et al. 2006). Two g of dry sample was extracted two times with 50 mL of 80% aqueous methanol for 24 h at 150 rpm, the methanolic extracts were combined and washed two times with 25 mL n-hexane in order to eliminate the oil of the methanolic extract (Rigane et al. 2011). The separation of the phases was performed with separating funnels. Subsequently the methanolic extract was evaporated under nitrogen, and the residue was dissolved in 50 mL of 80% aqueous methanol, stored in clean bottles in refrigeration in the dark until its use.

**Methods of analyses:** Total polyphenolic (TP) content was determined with the Folin-Ciocalteu (F.-C.) reagent according to the method by (Singleton & Rossi 1965) and the results were expressed as gallic acid equivalent (GAE) in mg/g fresh weight. The content of Nonflavonoid phenols (NFP) was determined with the F.-C.

reagent after removing the flavonoid phenols (FP) with formaldehyde according to the method by (Kramling & Singleton 1969) and was expressed as gallic acid equivalent (GAE) in mg/g fresh weight. Flavanoid phenols (FP) were determined as a difference between the content of total phenols (TP) and nonflavonoid phenols (NFP). Their amount was evaluated as gallic acid equivalent in mg/g fresh weight.

The antioxidant activity of ferric reducing antioxidant power (FRAP) of the methanol extracts was determined on the basis of the method by (Benzie & Strain 1999) and was expressed as ascorbic acid equivalent (AAE) in µmol/g fresh weight. The antiradical activity (DPPH') of the methanol extracts was determined according to the method by (Brand-Williams et al. 1995) using the stable free radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH•). The activity was evaluated as Trolox equivalent (TEAC) in µmol/g fresh weight.

Soil was analyzed using the following methods which are referred by Page (1982).

Organic matter was analyzed by chemical oxidation with 1 mol/L  $K_2Cr_2O_7$  and titration of the remaining reagent with 0.5 mol/L FeSO4.

Inorganic nitrogen was extracted with 0.5 mol/L CaCl<sub>2</sub> and estimated by distillation in the presence of MgO and Devarda's alloy, respectively.

Available P forms (Olsen P) was extracted with 0.5 mol/L NaHCO<sub>3</sub> and measured by spectroscopy. Exchangeable form of potassium was extracted with 1 mol/L CH<sub>3</sub>COONH<sub>4</sub> and measured by flame Photometer (Essex, UK). pH and (EC), Electrical conductivity measured in the extract (1 part soil : 5 parts H<sub>2</sub>O).

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid  $0.005 \text{ mol/L} + \text{CaCl}_2 0.01 \text{ mol/L} + \text{triethanolamine } 0.1 \text{ mol/L})$  and measured by atomic absorption. The samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture (Varian 1989).

**Statistical analysis:** Data were analyzed using the MINITAB (Ryan et al. 2005) statistical package. The experiment had four replications. Analysis of variance was used to assess treatment effects. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

#### **RESULTS AND DISCUSSIONS**

Soil samples collected from olive orchards were analyzed for nutrient element and organic matter content (Table 2). In the olive orchard (A) the soil was Sandy Clay Loam (SCL) and in the olive orchard (B) Sandy Loam (SL).

In the olive fruits studied, the Kalamon variety characterised by the highest TP content, with 19.63 mg (GAE)/g FW, followed by those of the Koroneiki and Chalkidikis with 15.79 and 15.50 mg (GAE)/g FW respectively, and the lowest by the Megaritiki and Amfisis with 9.64 and 9.48 mg(GAE)/g FW respectively (Table 3). Our results are in agreement with the data obtained by other authors who have established differences in phenols content in the different olive fruits varieties (Boskou et al. 2006). The established trend in the variation of phenols within one and the same variety under the action of climatic factors is observed also by other authors (Petridis et al. 2012).

The content of flavonoid phenols (FP) in the studied fruits ranges from 5.89 to 14.52 mg (GAE)/g FW (Table 3). The FP content in the fruits Amfisis, Arbosana Arbequine, Kalamon and Koroneiki constitute 84.9%, 80.1%, 77.3%, 73.9 and 72.3 respectively of TP amount, whereas for fruits Chalkidikis and Megaritiki reached 70.3 % and 61.2%, respectively. The content of non-flavonoid phenols (NFP) ranges from 1.43 to 5.11 mg (GAE)/g FW (Table 3). The NFP content in the fruits Megaritiki, Chalkidikis, Koroneiki, Kalamon and Arbequine constitute 38.9%, 29.7%, 27.7%,

26.0% and 22.7% respectively of TP amount, whereas for fruits Arbosana and Amfisis reached 19.9% and 15.1%, respectively.

Soil properties	Olive orchard (A)		Olive orchard (A)		
		Soil d	depth		
	(0-30) cm	(30-60) cm	(0-30) cm	(30-60) cm	
Texture	SCL	SCL	SL	SL	
pH (1:5)	6.70 ± 0.34	6.97 ± 0.35	6.30 ± 0.30	6.72 ± 0.32	
EC (1:5), dS/m	0.19 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	
CaCO <sub>3</sub> (%)	2.20 ± 0.11	4.89 ± 0.25	1.50 ± 0.08	1.82 ± 0.09	
Organic matter (%)	2.62 ± 0.15	1.90 ± 0.10	2.00 ± 0.12	1.24 ± 0.05	
N-inorganic (mg/kg)	192.2 ± 9.5	144.6 ± 8.3	161.4 ± 9.3	112.2 ± 7.7	
K-exchangeable (mg/kg)	189.4 ± 9.1	98.6 ± 4.93	222.4 ± 9.9	164.8 ± 7.6	
P -Olsen (mg/kg)	25.6 ± 4.56	13.4 ± 0.74	16.4 ± 0.97	9.3 ± 0.56	
Cu-DTPA (mg/kg)	4.4 ± 0.23	2.1 ± 0.11	6.2 ± 0.28	4.2 ± 0.20	
Zn-DTPA (mg/kg)	2.0 ± 0.08	0.7 ± 0.04	1.1 ± 0.06	0.4 ± 0.02	
Mn-DTPA(mg/kg)	4.8 ± 0.23	2.6 ± 0.16	7.3 ± 0.45	6.2 ± 0.37	

#### Soil chemical properties of olive orchards studied

Data represent average and SE deviation, (n) = 4.

Table 3

Table 2

Total phenols content, flavonoid and non-flavonoid phenols in the olives studied

Olive fruits cultivars	Total phenols (TP)	Flavonoid phenols (FP)	Non-flavonoid phenols (NFP)					
	mg (GAE)/g FW							
Amfisis	9.48e	8.05c	1.43e					
Chalkidikis	15.50b	10.90b	4.60b					
Kalamon	19.63a	14.52a	5.11a					
Megaritiki	9.64e	5.89d	3.75c					
Koroneiki	15.79b	11.42b	4.37b					
Arbequine	10.97d	8.48c	2.49d					
Arbosana	13.09c	10.49b	2.60d					

Columns with the same letter do not differ significantly according to the Tukey's test (P=0.05).

The results of the FRAP antioxidative assay, of the seven varieties of fresh fruits reveal that the Amfisis and Arbosana varieties exert the highest activity (as ascorbic acid equivalent) with 77.7 and 74.8  $\mu$ mol (AAE)/g FW respectively and those of the Megaritiki variety the lowest with 37.2  $\mu$ mol (AAE)/g FW (Table 4). These values are higher than those reported by other authors for the same varieties, but from different regions of Greece (Petridis et al. 2012). The correlation between the antioxidant activity (FRAP) of fruits and the contents of TP was low, with correlation coefficient (r<sup>2</sup>) equal to: 0.006.

The antiradical activity (DPPH•) of fruits from the olives varieties studied ranges from 158.41 to 281.31  $\mu$ mol (Trolox)/g FW (Table 4). The Amfisis and Arbosana varieties exert the highest activity with 281.32 and 253.12  $\mu$ mol (Trolox)/g FW

respectively, and the Megaritiki variety exert the lowest with 158.41  $\mu$ mol (Trolox)/g FW. These values are higher than those reported by other authors for some Turkey varieties (Gurel et al. 2014). The correlation between the antiradical activity (DPPH•) of fruits and the contents of TP was low, with correlation coefficient (r<sup>2</sup>) equal to: 0.08.

Table 4

Antioxidant activity FRAP and antiradical activity DPPH• in the olives studied

	Antioxidant activity FRAP	Antiradical activity DPPH
	µmol (AAE)/g FW	µmol (Trolox)/g FW
Amfisis	77.7a	281.32a
Chalkidikis	50.2b	222.14b
Kalamon	61.8b	261.32a
Megaritiki	37.2c	158.41c
Koroneiki	51.1b	229.21b
Arbequine	55.7b	227.38b
Arbosana	74.8a	253.12a

Columns with the same letter do not differ significantly according to the Tukey's test (P=0.05).

## CONCLUSIONS

Phenolic compounds, antioxidant and antiradical activity of olives were studied depends strongly on the variety and by the climatic conditions of the each region. Olives which are cultivated on the region of Larissa, Greece are a source of bioactive components that could be included in functional foods composition.

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# THE EFFECT OF *POSIDONIA OCEANICA* ON SOIL ORGANIC MATTER BIODEGRADATION AND OTHER SOIL CHEMICAL PROPERTIES

Gougoulias Nikolaos<sup>1</sup>\*, Goula Ioanna-Maria<sup>1</sup>, Papachatzis Achilleas<sup>3</sup>, Ntalla Maria-Nektaria<sup>2</sup>, Efthimiou Spyridoula<sup>1</sup>

<sup>1</sup> Department of Agronomy Technology, Technological Educational Institute of Thessaly, 41110 Larissa, Greece <sup>2</sup>Department of Computer Science and Engineering, Data Analysis, Technological Educational Institute of Thessaly, 41110 Larissa, Greece

<sup>3</sup>School of Agriculture, Aristotle University of Thessaloniki, Greece

\*Correspondence author. email: ngougoulias@teilar.gr

Key words: posidonia oceanica; soil chemical properties; soil organic matter biodegradation

## ABSTRACT

The effects of posidonia oceanica on soil organic matter biodegradation and on soil chemical properties an incubation experiment were studied. The air dried posidonia plant tissues incorporated at five different rates (0, 0.3, 0.6, 0.9 and 1.2 g per 50 g of soil) resulted in increases in organic carbon mineralization, mineral nitrogen forms, available potassium in organic and available phosphorus. The level of available forms of Fe and Zn was increased at all the rates of added posidonia, whereas Mn was increased at the three upper rates. The level of available Cu did not show statistically significant differences in comparison with the control. Also, the addition of posidonia resulted in a increase in total forms of Na, K, P and Zn. The results of this study indicated that posidonia could be applied to the soil without any extremely negative effect on the soil chemical and biological properties.

#### INTRODUCTION

The addition of organic materials in the soil, affects the composition and biological activity of microflora. When applying of organic fertilizers in the soil, dominate microbial nutrition (Riber et al. 2014). In biological agriculture is mandatory to replace chemicals with natural additives. Between them, the plant debris for their role in the soil fertility, the plant essential oils for their role as pesticides against on the soil pathogens (Gravanis et al. 2004).

*Posidonia oceanica* is a Mediterranean seagrass that produces onshore deposits. Many authors have studied the use of *Posidonia oceanica* as substitutes to peat and as a growing medium for the soilless cultivation of Tomato (Mininni et al. 2015, Montesano et al. 2013). Studies have shown that the *Posidonia oceanica*, can be used as composting material with poultry droppings for improving soil fertility for horticulture (Tangour et al. 2015). Also, posidonia residues can be used as organic mulch and soil amendment (Grassi et al. 2015).

Our previous studies have shown, the effect of oregano and basil on degradation of organic fertilizers, and it was found that the presence of foliar tissues of oregano, act a slowing effect on degradation of organic fertilizer, while the presence of basil, accelerate it (Chouliaras et al. 2007, Gougoulias et al. 2010).

The objectives of this research were to study, in the laboratory, if the application of *Posidonia oceanica* dry matter into soil has an effect on soil organic matter and on soil chemical properties.

#### MATERIAL AND METHODS

In coastal areas of the island Skopelos accumulate large quantities of seaweed-sea *Posidonia oceanica*, and because of the environmental problems created transported to landfills. The October 2015 held the collection of *Posidonia oceanica* from the coastal areas of island Skopelos, Greece. Because of the high salinity, *Posidonia oceanica* (EC =16.85 dS/m) stirred the material for one hour with distilled water at ratio (1: 5), filtered, air dried, well milled and were used.

Incubation experiment: In this study, 12.3 g of a sheep manure containing 5 g of organic matter (Table 1), obtained from the farming establishments of TEI of Thessaly, and was added to 50 g of air-dried soil that was poor in organic matter, derived from the same region (Table 1). Into 50 g of this soil plus 12.3 g of the sheep manure, 0, 0.3, 0.6, 0.9 and 1.2 g of air-dried and well milled of leaf tissues Posidonia oceanica were added. Thus an experimental unit is constituted by 50 g of soil, 12.3 g of manure and a variable amount of Posidonia oceanica. In the incubator, the treatments were prepared in four replicates and kept at 28 °C for a period of 15 weeks. During the first three weeks of the incubation period, the moisture was maintained at two-thirds of field capacity, but for the next three weeks the soils were left to dry. This process was repeated until the end of the incubation period according to (Wu and Brookes 2005) they reported that the alternation of drying and rewetting soil samples enhances mineralization of both soil biomass organic matter and non-biomass organic matter. Soil aeration and regulation of moisture enhance the growth and metabolic activity of aerobic soil microorganism (Gordon et al. 2008). At the end of the incubation period, soil samples were analyzed.

**Methods of analyses:** Samples were analyzed using the following methods which are referred by (Page et al. 1982).

Organic matter was analyzed by chemical oxidation with 1 mol/l  $K_2Cr_2O_7$  and titration of the remaining reagent with 0.5 mol/l FeSO<sub>4</sub>.

Both ammonium and nitrate nitrogen were extracted with 0.5 mol/l CaCl<sub>2</sub> and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) was extracted with 0.5 mol/l NaHCO<sub>3</sub> and measured by spectroscopy. Exchangeable forms of potassium and sodium ware extracted with 1 mol/l CH<sub>3</sub>COONH<sub>4</sub> and measured by flame Photometer. Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol/l + CaCl<sub>2</sub> 0.01 mol/l + triethanolamine 0.1 mol/l) and measured by atomic absorption.

Organic phosphorus was measured after mineralization by combustion of the sample and subtraction of the mineral phosphorus amounts, which had previously been estimated in the laboratory. The mineral amounts were extracted with 1 mol/l H2SO4 and all forms were measured by spectroscopy.

For the determination of total metals Mn, Cu and Zn , 1 g of material, digestion at 350  $^{\circ}$ C + 10 ml HNO<sub>3</sub> + 5 ml HCLO<sub>4</sub>. According to the method described by (Varian 1989), the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus), with the use of flame and air-acetylene mixture.

**Statistical analysis**: Data analysis was made using the MINITAB (Ryan et al. 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

Table 1

Chemical properties of soil samples, manure and *Posidonia oceanica* foliar tissues used in the experiment

Property	Soil	*Manure	Posidonia oceanica
Teature	Construction		Air dried material
Texture	Sandy Loam	-	-
рН	7.74 ± 0.3	8.55 ± 0.4	7.84 ± 0.3
EC, dS/m	$0.42 \pm 0.02$	2.93 ± 0.2	2.56 ± 0.3
Organic matter (%)	0.85 ± 0.04	40.54 ± 2.4	-
CaCO <sub>3</sub> (%)	8.48 ± 0.6	-	-
N -Total (g/kg)	1.55 ± 0.13	11.57 ± 0.8	4.55 ± 0.4
N-NH4 <sup>+</sup> (mg/kg)	28.6 ± 4.3	-	-
N-NO <sub>3</sub> <sup>-</sup> (mg/kg <sup>-1</sup> )	94.1 ± 10.4	-	-
K-exchangeable (mg/kg)	264.8 ± 9.2	-	-
K-Total (g/kg)	5.20 ± 0.2	19.88 ± 1.3	0.71 ± 0.05
Na-exchangeable (mg/kg)	211.1 ±6.5	-	-
Na-Total (g/kg)	0.61 ± 0.02	8.40 ± 0.5	10.67 ± 0.5
CEC (cmol/kg)	18.5 ± 0.9	-	-
P -Olsen (mg/kg)	11.1 ± 4.2	-	-
P -Total (g/kg)	0.36 ± 0.02	6.85 ± 0.2	0.48 ± 0.04
Cu –DTPA (mg/kg)	1.13 ± 0.07	-	-
Zn -DTPA (mg/kg)	1.01 ± 0.06	-	-
Mn -DTPA (mg/kg)	2.74 ± 0.17	-	-
Cu –Total (mg/kg)	-	39.6 ± 9.7	44.8 ± 3.3
Zn -Total (mg/kg)	-	212.5 ± 9.8	61.6 ± 3.7
Mn -Total (mg/kg)	-	77.8 ± 4.8	66.1 ± 7.1
Fe-Total (g/kg)	-	5.37± 0.9	0.33 ± 0.01

\*Digested sheep manure four months; Electrical conductivity, (EC) and soil pH is determined in (1:5) soil/water extract; Data represent average means and SE deviation. (n)=4.

# **RESULTS AND DISCUSSIONS**

The results of the laboratory experiment showed that decomposition of soil organic matter added as manure or preexisted organic matter in soil, was increased with the increase of posidonia concentration in soil, as compared with the control (soil plus manure), (Figure 1). In particular, the data showed an increased mineralization of soil organic carbon by 23.8 to 42.6 % at all rates addition of posidonia compared to the control.

The addition of dry plant tissues posidonia in the mixture (soil plus manure), in all treatments, in comparison with the control, at the end of the incubation period increased available forms of P, Zn, Fe, ammonium and nitrate nitrogen (Table 2). In contrast, available form of Cu by the addition of posidonia in the mixture, in all treatments, at the end of the incubation period did not show statistically significant differences in comparison with the control (Table 2).





Table 2

Effect of posidonia dry matter concentration on soil available forms of elements

Treatments	Available forms			DTPA			
	P-Olsen	$N-NH_4^+$	N-NO <sub>3</sub> <sup>-</sup>	Cu	Zn	Mn	Fe
		(mg/kg soil)					
С	276.9b	24.5b	161b	1.02a	2.38c	2.02b	12.22b
CP1	419.7a	178.8a	322a	0.92a	5.34b	2.22b	16.74a
CP2	423.5a	149.8a	357a	0.92a	5.88b	3.59a	16.42a
CP3	406.3a	145.5a	322a	0.73a	7.67a	3.17a	17.67a
CP4	422.2a	164.2a	301a	0.89a	6.74a	3.05a	17.21a

For each chemical property of soil mixtures, columns of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). C, control (soil plus manure); CP1, CP2, CP3 or CP4, control and posidonia 0.3, 0.6, 0.9 or 1.2g, respectively.

The analysis of variance performed for the organic phosphorus and exchangeable forms of potassium data indicated that the organic phosphorus and exchangeable potassium was significantly increased by the added posidonia in all treatments (Table 3).

Table 3

Effect of posidonia dry matter concentration on soil chemical properties

Treatments	Exchangeable forms		Organic	EC	рН
	Na	K	Р	Extract (1: 5) in water	
	(mg/kg)			(dS/m)	
С	1110.9c	1110.9c 2130.6b		1.66e	7.62a
CP1	2196.5b	5927.9a	517.9b	2.18d	7.55a
CP2	2334.5a	2334.5a 6180.1a		2.68c	7.78a
CP3	2472.5a	2472.5a 5675.6a		3.30b	7.69a
CP4	2677.2a	5675.6a	474.5b	4.12a	7.71a

For each chemical property of soil mixtures, columns of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). C, control (soil and manure); CP1, CP2, CP3 or CP4, control and posidonia 0.3, 0.6, 0.9 or 1.2 g, respectively.

Also, the exchangeable form of sodium was increased by the added posidonia dry matter, in all treatments. In particular, the exchangeable form of sodium was increased with increasing posidonia dry matter concentration. Electrical conductivity also increased in all treatments by the added posidonia. However, the greatest increase was caused by the highest rate of dry plant tissues posidonia. The soil pH did not show statistically significant differences compared to control, in all cases by the added posidonia (Table 3).

The addition of dry plant tissues posidonia in the mixture (soil plus manure), at the end of the incubation period in comparison with the control, increased total forms of Na, K, P, Cu and Zn, in all treatments (Table 4). In contrast, total forms of Mn and Fe by the addition of plant tissues posidonia did not show statistically significant differences in comparison with the control, in all treatments (Table 4).

Table 4

Treatments	Total forms							
	Cu	Zn	Mn	Na	K	Р	Fe	
	(mg/kg soil)			(g/kg soil)				
С	48.2a	57.6b	504.9a	1.315c	8.098c	1.454b	3.286a	
CP1	26.83b	71.0a	556.9a	3.136b	10.479a	2.076a	3.347a	
CP2	23.56b	85.9a	540.4a	3.136b	10.479a	2.276a	3.322a	
CP3	22.91b	82.1a	524.0a	3.135b	9.431b	2.182a	3.289a	
CP4	24.22b	76.8a	544.9a	4.182a	8.288c	2.048a	3.322a	

Effect of posidonia dry matter concentration on soil total forms of elements

For each chemical property of soil mixtures, columns of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). C, control (soil plus manure); CP1, CP2, CP3 or CP4, control and posidonia 0.3, 0.6, 0.9 or 1.2 g, respectively.

#### CONCLUSIONS

Four different rates of posidonia biomass were applied into a soil amended with manure, and after an Incubation experiment the effects on the soil chemical properties were attested. These results, confirm the role of dry posidonia biomass as it is a valuable material for soil amendment, could be applied to the soil as an acceleration agent for soil organic matter biodegradation, improves soil chemical and biological properties, while that recycling constitutes a useful practice for environment protection.

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# RESEARCHES CONCERNING THE USE OF TWO IRRIGATION SYSTEMS WITH REDUCED CONSUMATION OF WATER IN ORDER TO CONTROL THE WATER STRESS AT GRAPEVINE

Ion Marian<sup>1\*</sup>, Burlacu Cristian, Pircalabu Liliana, Filip Vlad Andrei, Brinduse Elena

<sup>1</sup> Research Institute for Viticulture and Enology Valea Calugareasca \* Correspondence author. E-mail: marian1367@yahoo.com

Key words: collector pluvial, humidity sensor, water stress, grapevine

# ABSTRACT

The work has been carried out during 2016 – 2017 period, in a vineyard located in Valea Călugarească viticultural center, planted with Tămâioasă romanească variety, aiming to determine the influence of two irrigation systems (with pluvial collectors located between the vines supplemented with a system based on dripping and irrigation system based only on dripping) on the biological process at the soil level and on the physiological processes in the plant, on the quality of the grape harvest. The obtained results highlight the positive effect of the two irrigation systems on the microbiological activity in the soil. The positive effects on plants is referring to the stimulation of the physiological processes in the plant, ensuring an optimal growth and yielding ratio, increasing the grape production by 17-20 % and providing a sugar content of the grape juice that allows the obtaining of wines with good quality.

#### INTRODUCTION

In the current context of climate change, which led to the appearance in the vegetation period of the vine of some periods (longer or shorter) of water-stressed, irrigation of grapevines become a necessity, even in wine-growing areas, which, two decades ago were considered wet. Although the vine is adaptable to drought conditions (possessing a root system well developed in depth and with a high capacity for absorption) and the soil moisture to values close to the coefficient of wilting can be used, the absence of water in the soil can induce critical period in terms of physiological point of view, which can be reflected not only on the production of that year, but also on the future evolution of crops.

Considering the high water consumption required for irrigation of the vineyard during excessively dry, become a necessity the use of new methods for irrigation of the vine in order to ensure a coefficient of utilization of irrigation water higher than that obtained by using the conventional methods and which do not influence negatively the quality of grape production (Pircalabu,2004; Ojeda, 2014). In this sense, the researches carried out at ICDVV Valea Calugareasca within a demonstrative polygon with the Tamaioasa romaneasca variety aimed to establish the influence of two irrigation systems (with pluvial collectors located between the vineyards+drip irrigation

system and drip irrigation) on soil biological and physiological processes of plant growth and on the grape quality.

## MATERIAL AND METHODS

In the year 2016 an experimental plot was arranged in a plantation, three years aged, with Tamaioasa Romaneasca variety grafted on rootstock SO4-4. This variety is recognized for quality aromatic wines, recommended for Valea Calugareasca viticultural center.

The experience was monofactorial, the factor studied being the irrigation system with graduations:  $a_1 = drip$  irrigation and pluvial collector;  $a_2 = drip$  irrigation and  $a_3 =$  non-irrigated. The soil in the experimental polygon is reddish preluvosol which is part of the luvisol class.

The electricity for the functioning of the irrigation system with low water consumption has been assured by a solar generation of electricity.

The pluvial collectors have been placed between the vines, and were integrated into the automated drip irrigation system.

The collectors includes a storage vessel provided with a cover, which decreases in the rate of evaporation of water accumulated.

The geometry of the cover ensures efficient capture of rainwater that is slowly ceded to the vine through cotton wicks.

The monitoring of the humidity threshold value at the level of the vine roots was done by means of 8 sensors, conceived by INCDMTM Bucharest, evenly distributed on the experimental plot, and by 3 humidity transducers.

Irrigation was started on the August  $2^{nd}$  when soil humidity reached the ceiling of 17%, considered as the minimum threshold from which it must be triggered and was stopped on August 26, although the soil moisture level fell below the minimum threshold, given that the grapes were in the water in the version.

The following parameters were used for the characterization of the ecoclimatic conditions during the experimental period: average annual temperature, sum of the active temperature, sum of the useful temperature grades, the sum of the monthly precipitation and the number of days with precipitation.

Observations and determinations of the main physical properties (apparent density, hygroscopicity coefficient, coefficient of wilting, field capacity, active humidity range) and microbiological observations (determining the viable germ load, determining the structure of microbial communities) were made.

Determinations were made on the main physiological processes in plant (photosynthetic rate, breathing, sweating), foliar surface index (LAI) by using a LCA-4 automated analyzer.

The grape production (Kg/vine) was determined by weighing the grapes individually harvested in comparison with the control.

The mechanical composition of the grapes was determined by calculating the grain technological indices (grape structure index, grain index and yield index).

The composition of the grape juice has been evaluated on the basis of the following parameters: sugar (g / I) and total acidity.

Determinations have been made on the hydric regime of the grapevine: leaf water potential and stomatal conductance.

# **RESULTS AND DISCUSSIONS**

The ecoclimatic conditions specific to 2017 year were characterized by a high heliothermic regime of rich water resources in July and relatively low in August (Table 1).

In the table 2 are shown the main physical and hydro physical characteristics of the soil in the experimental plantation.

Table 1

Month	Sum of active	Sum of useful	Monthly average	Rainfalls	Number of days with
	temperature	temperature	temperature		rainfall
January	0	0	-3.3	7.3	2
February	20.7	0.7	1.3	32.9	3
March	145.6	25.6	9.2	9.8	5
April	226.7	56.7	10.9	97.4	9
May	571.0	261.0	18.4	56.4	9
June	636.9	336.9	21.2	82.3	6
July	710.4	400.4	22.9	86.6	11
August	748.5	438.5	24.1	36.4	6
The growing season	2893.5	1493.5	19.5	359.1	41
TOTAL	3059.8	1519.8	13.1	409.1	51

# The ecoclimatic conditions recorded during the experimental period (Valea Calugareasca, 2017 year)

Table 2

# Physical and hydrophobic soil indices within the experimental polygon

Depth cm	Apparent density (DA)	The coeficient of wilting (CO)		The field capacity (CC)	
	t/cubic meters	%	Cubic meters/ hectare	%	Cubic meters/ hectare
0-80	1.44	10.8	1244	19.9	2292

Depth cm	Useful water capacity (CU)		Minimum limit 50% CU		Momentary reserve of water in soil	
	%	Cubic meters/ hectare	%	Cubic meters/ hectare	%	Cubic meters hectare
0-80	9.1	1048	15.4	1768	25.45	2932

The hydro physical soil (CO, CC) indices from the experimental polygon on 22.06.2017 highlight that the soil can store a volume of water (CU) of 1048 cubic meters/hectare. Momentary water reserve in the soil is 2932 cubic meters/hectare, resulting in a surplus of 1164 cubic meters/hectare. Summed amount of water

accumulated in the precipitation of a rainwater collector was 64.70 (32.35 l/vine), resulting in an amount of 161.75 cubic meters/hectare. The irrigation norm used to supplement the deficit was 231.25 cubic meters/hectare for variant 1-drip irrigation and pluvial collector and 393 cubic meters/hectare for the variant 2- drip irrigation.

The analysis of the data obtained for the two irrigated and non-irrigated variants shows a significant increase of soil microbiological activity in case of irrigated variants at the sampling horizon of 0-30 and 30-60 centimeters (Table 3).

Table 3

Variants	Depth	Total	Other	Actinomycete	Yeasts +	Nitrogen
	cm	number of	Bacteria		Fungus	fixative
		live			_	bacteria
		microorga-				
		nisms /				
		g soil	%	%	%	%
V/1 irrigotod	0-30	2673200	67.34	1.68	30.86	0.12
v I– Imgaleu	30-60	3448000	92.81	0.84	6.09	0.26
V/2 irrigotod	0-30	2906500	75.69	0.83	23.40	0.09
vz– imgated	30-60	5979000	91.99	0.20	7.69	0.12
V3 – non	0-30	460000	84.78	4.35	6.52	4.35
irrigated	30-60	919200	84.86	1.22	10.01	3.92

# The microbial load of soil in irrigated and non-irrigated variants (Valea Calugareasca, 2017 year)

The most numerous group of microorganisms in case of the two systems was that of bacteria which represented 67.34-92.81% from the total number of viable microorganisms.

In case of non-irrigated variant there was observed a reduction of the total number of microorganisms with 17.21-26.66%, in case of the soil profile 0 - 30 cm and with 15.37-15.83% in case of the soil profile of 30 - 60 cm.

Regarding Actinomycetes group, there was noticed a reduction with 55.56-16.66% for the soil profile 0 - 30 cm and of 75-53.33% for the soil profile of 30 cm - 60 cm, compared to the irrigated variants.

The group of fungus was represented by 23.40%-30.86% for the total number of microorganisms, in case of the irrigated variants, for the soil profile 0-30 cm, and in area percent, less than 10% for the other variants.

Fungus belonging to the genus *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Cladosporium*, *Mucor*, *Verticillium*, *Phoma*, *Trichoderma* and *Gliocladium*, were identified.

The most representative genus for irrigated variants were *Penicillium*, *Cladosporium* si *Aspergillus*.

The irrigation systems used in the study also have an important influence on the physiological processes in the plant.

Regarding the photosynthesis process, the highest values were obtained for the two irrigated variants (Figure 1).



Figure1. The physiological processes in the plant

The influence of two irrigation systems compared to the non-irrigated control on grape production and quality is shown in Table 4.

Table 4

the production of grapes to Tamaioasa romaneasca variety											
	The	Number	The	The	Total acidity						
	average	of	production	sugar							
Variants	weight of	grapes									
	a grape				g/l tartric						
	g		Kg/ha	g/l	acid						
V1-											
drip irrigation and	204	6.9	1.408*	209,0	8.1						
pluvial collector											
V2- drip irrigation	202	6.8	1.374*	209,0	8.1						
V3-non-irigated	168	7.0	1.177	201,0	8.5						
			DL 5%	DL 1%	DL 0.1%						
			0.18	0.25	0.31						

The influence of experienced technological factors on the production of grapes to Tamaioasa romaneasca variety

Based on the data obtained from the mechanical analysis of the grapes, the main urological indices characterizing the technological abilities of the grapes were calculated (Table 5).

The higher values of the mechanical composition indices of the grapes both in terms of the mechanical composition and the quality of the berry, allowing to obtaining wines with good quality were obtained in case of irrigated variants.

Regarding the influence of the two irrigation systems on the grape yield, the variance analysis emphasized a positive effect as compared with the non-irrigated control.

Table 5

Variation of the values of mechanical berry composition indices
depending on the irrigation system

Variants	Grape harvesting index	Berry index	Composition index berry	Yield index
V1- drip irrigation and pluvial collector	77.9	57.8	7.5	7.5
V2- drip irrigation	74.2	55.5	7.4	7.4
V3-non-irigated	73.4	52.6	7.4	6.7

The must sugar concentration depended to a lesser extent on the influence of the irrigation systems.

#### CONCLUSIONS

The using of different system of irrigation are positive effects on plants, in terms of the stimulation of the physiological processes in the plant, the increasing of soil microbiological activity and the ensuring an optimal growth and yielding ratio.

The obtained results put into evidence the positive influence of irrigation, both upon the grape yield and its quality, no matters what irrigation method was used. The increasing yields of the grapes were obtained in comparison with the not irrigated witness control, averagely ranging in between the limits of 19 - 20%, on the background of a better accumulation of sugars in grapes.

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# THE BEHAVIOUR OF SOME PEACH VARIETIES (*PRUNUS PERSICA* L.) BELONGING TO THE WORLD GERMOPLASM COLLECTION, CONCERNING THEIR GROWTH AND FRUCTIFICATION IN CONDITIONS OF BANAT PLAIN AREA

Iordănescu Olimpia Alina<sup>1\*</sup>, Cosmulescu Sina Niculina<sup>2</sup>, Becherescu Alexandra<sup>3</sup>, Murgu Viorica Adriana<sup>4</sup>, Sala Florin<sup>5</sup>, Scedei Daniela<sup>6</sup>

<sup>1,3,6</sup> Banat's University of Agricultural Science and Veterinary Medicine "King Michael I" from Timişoara, Faculty and Horticulture and Forestry, Timişoara, Romania, <sup>2</sup>University of Craiova, Faculty of Horticulture, Craiova, Romania <sup>4</sup> Community Police, Timişoara, Romania <sup>5</sup> Banat's University of Agricultural Science and Veterinary Medicine "King Michael I" from Timişoara, Faculty of Agriculture, Soil Science and Plant Nutrition, Timişoara, Romania \*Correspondence author. E-mail : olimpia.iordanescu@yahoo.com

Key words: biometric parameters, coefficient of variation, fruit quality indices, peach

#### ABSTRACT

To obtain higher fruit productions and qualitative fruits suppose in addition to applying of superior technology culture a good zoning, more exactly knowledge of different behavior of varieties in agreement with climatic and soil conditions in specific area. The biological material was constituted by nine peach tree varieties from around the world. So, there were determined varieties with a high Coefficient of variation (CV) for tree diameter CV = 48.10 ('Piros Magdalena') to CV = 20.03 ('Poli'), tree height CV = 33.79 ('Gold Dust') to 6.86 ('Elbertina'), number of short formation CV = 99.60 ('Poli') to CV = 16.86 ('Springold'), number of medium formation CV = 74.32 ('Poli') to CV = 21.38 ('Springold'), number of long formation CV = 112.54 ('Poli') to CV = 47.89 to CV = 6.07 ('Sui Hui Hun' – GDRT), large diameter of fruits CV = 15.92 ('Sun Hui Hun') to CV = 3.45 (, 'GDRT'), high of fruits CV = 10.39 (Piros Magdalena) to CV = 3.14 (Springold), index size CV = 14.44 to CV = 3.83 ('Sun Hui Hun' - 'GDRT').

#### INTRODUCTION

The peach tree (*Prunus persica* var. *persica*) originary from China is a highly important species, being cultivated all over the world in different eco-climates and using a large range of varieties, clones or local cultivars (Faust & Timon 1995; Zheng et al. 2014; Wang et al. 2006). Due to its high importance, a series of studies were developed on this species, starting from germplasm and its genetical diversity (Wang et al. 2006; Verde et al. 2013; Meng & Peng 2015), to its physiological indices and biometrical parameters (Quilot et al. 2002; Bregoli et al., 2006), its relation towards ecological factors and stress (Zhang & Tian 2010; Pinochet et al. 2012), fruits' quality indices (Bonora et al. 2013; Gasic et al. 2015; Mitre 2008), culture technology elements and products' and bio-product's marketing (Bielamberg et al. 2009). The physiological growth of peach trees estimated through the annual growth shoots, through stock's biometrical parameters, trees' height, crown's diameter, etc. have been

studied in order to determine the general physiological status and the productive potential of trees related to their age, climate and soil culture conditions and the culture technology (Mounzer et al. 2008; Ikinci 2014; Basile et al. 2015). In relation to the dynamics and specificity of nutrients for horticultural species and especially for peaches, studies have been carried out to characterize some physiological indices, biometric parameters and production in quantitative and qualitative terms (Chatzitheodorou et al. 2004; Han et al. 2015; Iordănescu et al. 2014; Gruia et al. 2009). The balance between annual tree growth and fructification is controlled by current crown formation (Dumitru et al. 2006) and fruiting pruning, by fertilization, irrigation (Layne et al. 2002) and others, study models of trees' architecture and physiology being made and used successfully in this respect (Xia et al. 2004; Allen et al. 2005; Lopez et al. 2010; Bevacqua et al. 2012). Fruit size and quality indices of peaches were studied in relation to the genetic potential of different genotypes (Monet et al. 1996), with environmental and technology factors, nutrition and stress factors, too. It is known that there are a number of interdependence relationships between plant nutrition status, physiological and biometric parameters, fruit quality, for peach tree and for other fruit species. (Bregoli et al. 2006; Jivan & Sala 2014). The quality of fruits, products and by-products in horticultural species and peach has been studied in relation to competitive relations with orchard grass (Tworkoski & Glenn, 2001), the ripening moment (Ziosi et al. 2008), storage conditions (Wang et al. 2006). Considering the importance of knowledge and management of fruit quality and production, different models have been developed for the evaluation and estimation of some physiological indices (Sala et al. 2015) and of some quantitative and qualitative aspects of peach production (Lescouuret & Génard 2005; Zhang et la. 2009; Grechi et al. 2008; Lopez et al. 2011).

The present study aimed at comparative analysis and characterization of some peach varieties in terms of physiological indices, biometric parameters and specific quality indicators of fruits.

#### MATERIAL AND METHOD

**Location and experimental conditions.** The research field is placed in the fruit tree orchard belonging to the Didactic Station of Banat's University of Agricultural Science and Veterinary Medicine from Timişoara, more exactly on the 2 ha parcel cultivated with some peach and nectarine varieties that belong to the World Peach Germplasm Collection. The research developed during the climatic conditions of 2012-2014 years, the varieties being cultivated on a chernozem soil with average fertility.

**Biologic material.** The biological material consists of nine peach tree varieties from around the world, as it follows: 'Poli', 'Sun Hui Hun', 'Yinquing', 'Piros Magdalena', 'Gold Dust', 'Eureka', 'July Elberta', 'Giala di Roma Tardiva', 'Elbertina' and 'Springold' variety considered the experimental witness. The worldwide biological material was introduced in our country and multiplied at SCDP Băneasa, from where it was bought and was planted afterwards in the soil and climate conditions of Timișoara, in 2007. The used planting distance was of  $4 \times 2 m$ , the peach varieties were grafted on wax cherry, while the crown form system is free palmet. The culture technology is the standard one used in peach tree orchards.

	January	February	March	April	May	June	July	August	September	October			
	Rainfall (mm)												
2012	59.1	47.3	13.5	73.5	128.5	55.1	40.5	31	30.7	46.3			
2013	43.7	39.8	31.4	83.2	115.8	113.4	40.2	50	90.2	50.4			
2014	47.3	12.2	39.1	83.2	115.8	88.8	123.5	62.8	54	67.4			
					Tempe	rature (°	C)						
2012	1	-2.0	7.0	13.0	16.0	22.0	22.0	23.0	20.0	13.0			
2013	1.0	4.0	5.0	13.0	17.0	20.0	22.0	23.0	15.0	14.0			
2014	3.0	6.0	9.0	12.0	16.0	20.0	22.0	22.0	18.0	13.0			

Climatic conditions during the observations

**Studied parameters.** Peach varieties have been studied in terms of physiological indices and biometric parameters, expressing the physiological aspects of trees' growth and development, thus: trunk's diameter (DT), tree's height (IP), crown's diameter (DC), the number of short shoots (NFS), the length of short shoots (LFS), the number of medium shoots (NFMD), the length of medium shoots (LFMD), the number of long shoots (NFLD), the length of long shoots (LFLD). Fruits' quality was evaluated through some specific indices: fruits' weight (GF), fruits' big diameter (DMF), fruits' small diameter (Dm F), fruits' height (IF), fruits' size index (IMF), stone percentage (PSF), sugar content (CZF), dry substance content (CSU).

**Statistical analysis.** Results obtained were analyzed by correlation and regression in order to evaluate the interdependence between physiological indices, biometrical parameters and fruits' quality indices. Relationships of interdependence were described by second degree polynomial equations, the safety parameters used being represented by the correlation coefficient  $R^2$ , parameter p and sample F.

#### **RESULTS AND DISCUSSIONS**

**Results.** Recorded data on vegetative indices, biometric parameters of trees, and fruit quality indices for peach varieties revealed a correlation of these in relation to the climatic conditions during the study period. At the same time, there were observed differences between the peach varieties for each of the evaluated parameter, all the collected data being presented in table 2. For trunk diameter parameter, Sui Hun Hun variety had the lowest values  $(3.78\pm0.86 \text{ cm})$ , being a variety with low vigour, while the highest values were registered for Elbertina variety  $(5.62\pm0.84 \text{ cm})$ . Trees' height gave values that ranged from 164.11±8.63 cm for Sui Hun Hun variety and 274±8.87 cm Elbertina variety. As for crown's diameter, the minimum values were obtained from Piros Magdalena variety - 127.11±6.70 cm, whith the maximum values were measured to Poli variety - 261.67±6.73 cm. The number of small shoots was lower for Elbertina variety - 6.44±2.47 and higher for Eureka variety - 21.00±9.38. the length of small shoots had values of  $1.39\pm0.24$  m for Springold variety and  $2.30\pm0.26$  m for Yinquing variety. The number of medium shoots was lower for Piros Magdalena variety -

9.22±0.59 and higher for July Elberta variety  $36.88\pm5.05$ . The length of medium shoots was smaller for Springold variety -  $10.61\pm0.30$  cm and bigger for Elbertina variety -  $18.22\pm4.59$  cm. the number of long shoots ranged from  $8.00\pm1.53$  for Yinquing variety and  $27.66\pm3.38$  for Elbertina variety, while their length had values of  $26.78\pm1.31$  cm for Yinquing variety and  $35.44\pm5.79$  cm for Poli variety. The analysis of vegetative indices and parameters values in relation to the climatic conditions revealed relations of interdependence with rainfall. For the following parameters: trunk's diameter, trees' height, crown's diameter, for all peach varieties there were determined negative correlations towards rainfall in May, for different signification levels, from very high (r = -0.993 for July Elberta variety, trunk's diameter), to high level (r = -0.771 for Piros Magdalena, trunk's diameter) and to average level (r = -0.686 for Eureka variety, trees' height). For the same parameter, during the other months of active vegetation (March–October), with the August rainfall, the most constant correlations for all varieties and the highest intensity were obtained (r =0.941–0.999). Within the other months of the study period, differentiated manifestations of this interdependence were recorded.

Within the other studied vegetative parameters were recorded relations of interdependence with the variable intensity rainfall, from very high (r =0.995, Poli variety, the number of small shoots) to high significance (r =0.866, Elbertina variety, length of small shoots), average significance (r =0.613, Eureka variety, length of small shoots), and to low significance (r =0.354, July Elberta variety, number of long shoots). Regarding the distribution of rainfall and the correlation level for the short, medium and long shoots differentiation parameters and their dimensions, most of the varieties correlated positively with the rainfall level in July, August and September, which shows the sensitivity of the studied varieties to the lack of water in this period and the need for irrigation to ensure normal vegetation conditions. The values of fruit quality indices showed differentiated relationships of interdependence with average monthly rainfall and temperatures over the study period. Dimensional parameters, such as weight, height, big and small fruit diameter, correlated positively with precipitation in July and August (r=0.997, Piros Magdalena variety, fruits' weight; r =0.999, Eureka variety, fruits' big diameter; r = 0.999 Eureka and Piros Magdalena varieties, fruits' small diameter; r =0.996, Piros Magdalena variety, fruits' height). Analyzing the correlation coefficient values for all varieties and fruit dimensional quality indices, July to August rainfall had a greater influence on the fruits. The fruit size index, as a synthetic index, showed higher interdependence relations with the August rainfall (r = 0.853 in July, r =0.909 in August). Fruits' sugar content correlated negatively to the June-August rainfall, with higher values for Sui Hun Hun variety in June (r = -0.891), Eureka variety in July (r = -0.998) and Piros Magdalena and Gold Dust varieties in August (r = -0.893). In contrast, for the period July-August, positive correlations were found for the dry fruit substance and the monthly average temperature, with very significant correlation values for Gold Dust variety (r = 0.891, July) and Giala di Roma variety (r = 0.987, August).

Table 2

Physiological indices, biometric parameters and fruit quality indices for the studied peach varieties 2012 - 2014

	1	1	<u> </u>	peu		00 2012				<b>.</b>	
			Sui	Yinauin	Piros	Gold		July	Giala di	Elbertin	Springo
		Poli	Hun	q	Magdale	Dust	Eureka	Elberta	Roma	2	ld Id
			Hun	9	na	Dusi		Liberta	Tardiva	a	iu.
рт	2012	3.97	2.30	2.40	3.03	3.83	4.10	2.40	3.07	4.00	3.40
וט	2013	5.23	3.77	3.13	4.13	5.47	4.67	4.50	4.33	6.03	5.50
	2014	6.13	5.27	4.33	6.13	5.60	6.03	4.80	4.70	6.83	6.27
10	2012	208.33	150.33	216.67	151.00	180.67	192.00	190.33	188.67	260.33	182.67
IP	2013	215.00	162.00	226.00	163.33	187.00	198.33	217.00	201.67	271.33	201.00
	2014	230.00	180.00	248.33	183.33	205.00	218.33	231.00	221.67	290.67	220.00
	2012	250.00	175.50	200.33	116.00	181.67	143.33	188.17	160.83	192.83	149.00
DC	2013	261.67	188.17	212.67	126.17	195.50	152.20	207.67	173.50	208.50	160.33
	2014	273.33	200.83	230.67	139.17	223.83	170.83	225.00	184.33	234.17	173.33
	2012	4.00	3.00	12.67	11.67	18.00	15.33	6.67	10.00	4.67	8.00
NFS	2013	5.33	19.33	12 00	7 67	29.33	39.33	12.33	10.00	11.33	10.00
	2014	17.00	16.00	11.33	11 00	7.33	8.33	17.33	2 00	3.33	9.33
	2012	2 17	1 67	2 77	3.00	1 17	1.67	2 00	1.83	3 17	1.00
LFS	2013	1.83	1.67	2.23	1.67	1 47	1.50	2.00	2.00	2 17	1.83
	2010	1.00	1.50	1.89	1.83	2 17	2.50	1.00	1 33	1 17	1.00
NEM	2014	11.67	17.67	16.00	9.00	15.00	11 33	29.67	13.00	16.00	11 33
	2012	24.00	7.67	10.00	10.33	10.00	16.00	20.07	10.00	23.33	14.00
D	2013	24.00	11 00	15.00	9.33	19.00	25.67	46.67	15.67	18.00	14.00
	2014	13 33	17.00	10.67	12.00	23.33	11 33	40.07	13.07	12.00	11.00
LFMD	2012	19.00	17.00	17.00	12.00	20.00	17.00	14.07	14.00	27.22	10.00
	2013	16.00	19.00	16.00	14.67	20.00	19.00	12.00	14.00	27.55	10.00
	2014	10.07	18.00	16.00	14.07	10.00	10.00	13.07	15.00	14.07	10.50
NFLD	2012	5.00	8.00	7.00	0.00	18.00	7.33	12.00	15.33	21.00	13.00
	2013	4.00	0.00	6.00	9.33	14.07	14.33	20.33	11.00	30.00	11.00
	2014	20.33	13.67	11.00	14.67	20.33	21.67	42.00	29.00	32.00	15.00
LFLD	2012	36.33	35.33	26.00	25.00	31.67	30.00	36.33	30.00	36.00	36.00
	2013	25.00	29.50	25.00	35.50	27.00	32.33	33.67	27.17	37.17	32.00
	2014	45.00	36.00	29.33	29.00	36.00	34.33	33.67	38.00	30.00	35.00
GF	2012	41.33	45.33	93.93	51.67	39.32	51.33	41.00	62.67	54.15	37.34
	2013	59.02	46.20	99.21	54.93	39.45	55.35	46.70	67.22	66.84	42.45
	2014	58.86	100.46	82.93	91.84	65.13	86.03	55.54	63.14	73.70	59.90
DMF	2012	42.33	42.33	51.33	45.00	40.00	46.67	43.67	49.17	46.00	42.33
	2013	48.67	43.67	52.39	46.00	41.50	47.07	44.44	50.00	61.67	43.39
	2014	49.00	56.33	53.89	56.00	48.29	56.00	47.67	51.17	60.81	49.97
Dm F	2012	40.67	40.67	50.33	42.67	38.33	45.67	42.00	45.00	44.33	40.33
DIIII	2013	46.67	45.83	50.72	43.33	40.17	46.00	43.16	47.33	58.00	42.27
	2014	48.33	54.67	52.67	55.17	46.19	52.67	46.33	49.50	58.05	47.00
IE	2012	43.67	47.00	57.33	45.33	40.67	45.67	42.67	47.00	36.00	44.67
	2013	48.00	49.17	59.11	46.33	42.67	43.33	43.39	47.50	36.00	45.44
	2014	46.33	61.00	57.45	55.33	45.75	55.67	47.67	47.50	36.50	46.97
	2012	42.22	43.33	53.00	44.33	39.66	46.00	42.77	47.05	42.11	42.44
	2013	47.77	46.22	54.07	45.22	41.44	45.46	43.63	48.28	51.89	43.70
	2014	47.89	57.33	54.66	55.50	46.74	54.78	47.22	49.38	51.78	47.97
PSF	2012	12.00	16.47	8.46	8.00	7.79	7.49	8.37	6.29	9.00	10.97
	2013	11.50	16.11	8.23	9.80	7.79	7.40	8.22	6.45	8.80	11.03

		Poli	Sui Hun Hun	Yinquin g	Piros Magdale na	Gold Dust	Eureka	July Elberta	Giala di Roma Tardiva	Elbertin a	Springo Id
	2014	10.50	15.80	8.27	8.50	7.60	7.20	8.00	6.20	8.50	10.27
CZE	2012	9.16	9.61	13.89	13.08	13.93	14.31	10.17	14.07	10.67	10.03
02F	2013	10.25	9.11	13.95	12.61	13.43	14.04	9.45	13.65	12.16	8.96
	2014	11.55	9.53	10.60	10.46	11.13	10.56	10.70	11.63	8.41	10.13
COL	2012	11.00	11.40	15.43	14.67	15.47	16.17	11.93	15.60	12.40	11.80
030	2013	12.00	10.93	15.50	14.23	15.00	15.57	11.26	15.20	13.80	10.79
	2014	13.23	11.33	12.33	12.20	12.83	12.30	12.43	13.30	10.27	11.90

**Discussions.** This study evaluated the behavior of nine peach varieties in the pedoclimatic conditions of the Banat Plain, in terms of indices and parameters reflecting annual increases, biometric parameters of tree trunk and crown and fruit quality indices. Similar studies of vegetative growth were also carried out in other researches of different peach varieties and cultivars in relation to rootstocks and pedoclimatic cultivation conditions (Weibel 2003; Forcada et al. 2014; Baciu et al. 2013). Gleen et al. (2011) studied different peach production systems in terms of yields and economic values, interactions between tree density and architecture, cutting strategies and soil management. DeJong et al. (1996) have developed a computer simulation model on annual vegetative and generative peach growths. Based on the environmental parameters, the model simulated the photosynthetic rate and the growth of the vegetative and generative organs. The sensitivity of peach to water stress was studied by Rahmati et al. (2015) in semiarid culture conditions, assessing annual growth, fruit growth and rhythm of growth, and physiological indices directly involved, such as photosynthesis, gas exchange, sweating. Annual vegetative increases are very sensitive to moisture deficiency and reduce much more to water stress compared to fruit growth (Egea et al. 2013). Peach roots were considered less affected under water stress compared to annual shoots and fruits' growth (Hsiao and Xu 2000).

Lopez and DeJong (2007) found a significant influence of spring thermal conditions during the blooming period and for 30 days after flowering on the annual growth rate, the thermal values of the period being useful in estimating the fruit production. Similar results on the relationship between climatic conditions and annual increases and peach productivity were also reported by Atkinson et al. (2013). Studies on the variation of peach growth and productivity were also performed by Matei et al. (2012); lordănescu et al. (2015) in relation to different varieties, the planting system and organic and mineral fertilization, with positive correlations that are statistically safe. Naor (2006) evaluated the relationship of peach tree in different stages of vegetation, with the water stress caused by controlled irrigation. Regarding peach fruit quality parameters and indices, many studies have observed these aspects in relation to different varieties, environmental and technological factors (Fuentes-Pérez et al. 2014; Cirilli et al. 2016) confirming the results obtained in this study.

#### CONCLUSIONS

As a result of the research conducted on lesser known and cultivated peach varieties in our country under the pedoclimatic conditions of Banat Plain area, the varieties can be grouped as follows: *depending on the culture system*, for intensive

orchards with high density of trees on the surface unit (Giala di Roma Tardiva, Eureka, Gold Dust, Sun Hui Hun, Piros Magdalena), for intensive orchards with medium density of trees on the surface unit (Yinquing și Poli) and for family orchards or gardens (July Elberta and Elbertina); *depending on the dominant fruiting shoots,* Elbertina, Giala di Roma Tardiva, July Elberta, Poli need extensive pruning, while Eureka and Sun Hui Hun need less pruning; *depending on fruit weight and size index value*, Yinquing, Sun Hui Hun, Piros Magdalena, Eureka have large fruits, for fresh consume, but also for industrialization and Gold Dust, July Elberta, Poli have smaller fruits; *depending on sugar and dry substance content*, Giala di Roma Tardiva, Eureka, Gold Dust, Yinquing have a high content of these elements, while Sun Hui Hun, July Elberta, Elbertina have a lower content.

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# THE INFLUENCE OF THE PLANTING DISTANCE ON THE DEVELOPMENT OF THE CHERRY ROOT SYSTEM

Ivanov I., Balan V., Balan P.

Agrarian State University of Moldavia Correspondence author. E-mail: v.balan@uasm.mdmailto:cameliapopescu@yahoo.com

Key words: Cherry, variety, root, planting distance

#### ABSTRACT

This study presents the distribution of roots in the soil in commercial cherry orchards (Prunus avium L.) depending on the planting distance. The study was carried out in the southern fruit tree area of the Republic of Moldova on the Ferrovia cherry variety, aged 3 years, grafted on Gisela 6, planted at the distance of 5x1.5 m, 5x2 m and 5x2.5 m and crowns after the Crown naturally ameliorated with low volume. The weight and length of the roots were determined on horizontal sections at 0-25 cm, 26-50 cm, 51-75 cm, 76-100 cm, 101-125 cm and 126-150 cm from the trunk, at 0-20 cm, 21-40 cm, 41-60 cm and 61-80 cm. Each soil layer has been rooted, washed, dried, and distributed in size categories: below 1 mm, 1-3 mm, 3-5 mm, over 5 mm. The length of roots is determined by fibrous roots (95.4-95.8%), and mass - by skeletal roots (65.6-69.3%). Most roots after length (69.8-82.9%) and after meal (88.8-92.8%) are distributed on a depth of 0-60 cm.

#### INTRODUCTION

The density of cherry trees in modern orchards is constantly changing due to vegetal rootstocks, new varieties and new tree management systems. (Whiting & amp; Ophardt 2005, Calabro et al., 2009, Balan 2015). Considering that the planting distance of cherry trees is determined by the vigor of the variety and of the rootstock, the crown shape, the soil fertility and the equipment, it has been felt that it is necessary to study the development of the root system and the distribution of the roots in the soil at different planting distances of cherry trees. (Long et al., 2014; Musacchi & Serra, 2015).

#### MATERIAL AND METHODS

The experience was organized in the southern fruit tree area of the Republic of Moldova in the spring of 2009 at S.R.L. Terra Vitis in the village of Burlacu Cahul district with cherry rootstock Gisela 6 grafted with cherry varieties Bigarreau Burlat, Ferrovia, Lapins. Planting distances were 5x1.5 m, 5x2 m, 5x2.5 m. The low crown shape was used: Crown naturally ameliorated with low volume, and Slender Spindle.

The root system development study was carried out on the Ferrovia variety formed after the reduced-volume natural crown. Three trees were chosen for the study, in each variant, with uniform increases, after the diameter of the trunk, the height and diameter of the crown.

The root mass and form were studied at the end of vegetation (1/4 of the tree nutrition surface was dug) in terms of their spreading in the soil layers vertically and horizontally. The weight and length of the roots were determined on horizontal sections at 0-25 cm, 26-50 cm, 51-75 cm, 76-100 cm, 101-125 cm and 126-150 cm from the trunk, at 0- 20 cm, 21-40 cm,

41-60 cm and 61-80 cm. In each layer of soil, the roots were selected, washed, dried, and distributed in size categories: below 1 mm, 1-3 mm, 3-5 mm, over 5 mm.

# **RESULTS AND DISCUSSIONS**

In order to obtain large and regular productions in intensive plantations, it is necessary to guide the growth of both crown and roots (Babuc 2012). Analyzing the data obtained (Table 1), it is found that cherry trees of the Ferrovia variety at the planting distance of 5x1,5 m have the root system less developed than at the larger distances taken in the study.

Table 1

The influence of the planting distance of the root system development Ferrovia cherry variety grafted on Gisela 6, age of 3 years

Planting		Length of ro	ots, cm/tree	)	Mass of roots, g/tree							
distance,	Under	1.2 mm	2 E mm	Over	Under	1.2 mm	3-5 mm	Over				
m	1mm	1-3 mm	5-5 mm	5mm	1mm	1-3 11111		5mm				
5x1,5	17240	29320	1198	1032	76,8	130,2	105,6	290,0				
5x2	25436	29633	1508	1084	83,2	157,5	142,4	336,4				
5x2,5	21712	35665	1240	1248	73,6	154,6	156,0	359,2				
Media	21462	31539	1315	1121	77,8	147,4	134,6	328,5				

By increasing the density of the trees to a surface unit from 800 to 1333 tree/ha, the root length decreases from 59867 cm to 48790 cm. The reduction of the root system in the over grown plantations takes place on roots with a diameter of less than 1 mm and a 1-3 mm. 3-year-old cherry trees make up 38.7% of roots below 1 mm in diameter, 56.9% in diameter 1-3 mm and only 4.4% in diameters over 3 mm in diameter.

The weight of the roots also varies according to the planting distance. The largest root mass was recorded at the planting distance of 5x2.5 m and consisted of 743 g/tree. The size of the root mass is based on roots with a diameter of more than 3 mm. From the values shown, 67.3% make up the roots with a diameter of over 3 mm, 21.4% of 1-3 mm and 11.3% of the diameter below 1 mm of the total mass. So, the root length of the root system increases from the diameter of the fibrous roots by less than 3 mm in diameter, and the mass - from the skeletal and semi-skeletal roots with a diameter of more than 3 mm.

Analyzing the distribution of the roots in depth (Table 2) it can be emphasized that most of the roots, both in length and after mass, are found in the soil layer 0-60 cm. Thus, 3 years old cherry trees at the planting distance of 5x1.5 m in this layer are placed 82.9% by length and 72.7% by mass. Analogously, the roots and the other planting distances studied are distributed in depth. The highest density of fibrous, skeletal and semi-skeleton roots was recorded in the 21-60 cm soil layer.

These roots are the basis of feeding the trees and have a more directionally less horizontal (Babuc 2012). At the depth of 0-20 cm and 61-80 cm, a small amount of roots is placed. Thus, at the planting distance of 5x1,5 m, the total length of the deep roots is placed as follows: 0-20 cm - 5,3%; 21-40 cm - 32.0%; 41-60 cm - 45.6%; 61-80 cm - 17.1%. The mass of the roots in depth changes in analogy to the length.

It has been found that in young cherry trees in the 0-20 cm layer there is an insignificant amount of roots up to 3 mm in diameter and missing roots with a diameter of more than 3 mm. These findings lead to the conclusion that the soil works in young orchards are made superficially up to the depth of 15-20 cm, due to both the cutting of the roots caused by the repeated works and the frequent summer drying of the superficial soil layer.

The data presented demonstrate that the planting distances taken in the study practically do not influence the distribution of the roots in depth to the cherry trees of 3 years old.

Most of the skeleton roots are found in superficial soil layers, and as the depth

increases, it shrinks abruptly. The deep distribution of the fibrous roots is more uniform than the skeletal roots.

Table 2

Soil		Length of ro	ots, cm/tree	ł	Mass of roots, g/tree						
layer,	Under	1.2 mm	2.5 mm	Over	Under	1.2 mm	2.5 mm	Over			
cm	1mm	1-3 11111	3-3 11111	5mm	1mm	1-3 11111	3-0 mm	5mm			
Planting distance, 5x1,5 m											
0-20	1192	1413	-	-	4,0	2,6	-	-			
21-40	7434	7393	540	248	25,2	28,4	54,8	132,4			
41-60	6490	14600	374	784	22,0	81,2	50,8	157,6			
61-80	2124	5914	284	-	25,6	18,0	-	-			
Total	17240	29320	1198	1032	76,8	130,2	105,6	290,0			
Planting distance, 5x2 m											
0-20	3074	2098	-	-	7,6	9,1	-	-			
21-40	7788	8409	844	312	26,4	36,4	70,8	68,4			
41-60	10916	11550	476	772	36,8	86,4	59,2	268,0			
61-80	3658	7576	188	-	12,4	25,6	12,4	-			
Total	25436	29633	1508	1084	83,2	157,5	142,4	336,4			
	•		Plantin	g distance, s	5x2,5 m	•					
0-20	1298	645	-	-	4,4	2,8	-	-			
21-40	7080	7299	440	288	24,0	31,6	53,6	132,0			
41-60	7316	15708	748	960	24,8	68,0	91,6	227,2			
61-80	4484	10720	52	-	15,2	46,4	10,8	-			
81-100	1534	1293	-	-	5,2	5,8	-	-			
Total	21712	35665	1240	1248	73,6	154,6	156	359,2			

Influence of the planting distance on the distribution of roots in the soil in the Ferrovia variety, grafted on Gisela 6, 3 years old.

The 3-year cherry tree root system did not occupy the entire nutritional area for them (Table 3). Most roots, both in length and after the meal, are at a distance of 0-75 cm from the trunk.

In this area, at the planting distance of 5x1,5 m, 65,2% of the length and 82,6% of the root mass are placed at the distance of 5x2 m respectively, 66,2% and 81,9%, and at the distance of 5x2,5 m respectively 60.9% and 71.3%. If we analyze the data presented in the table and figure it is found that all the planting distances the length and the root mass decrease from the trunk to the periphery.

The root mass decreases more suddenly than their length. Decreasing the distance between trees in a row helps to increase the amount of roots around the trunk (0-50 cm), reaching 47.2% of the length and 55.5% of the mass at a distance of 5x1.5 m.

Table 3

Influence of the planting distance on the distribution of the roots in the soil in the Ferrovia variety, grafted on Gisela 6, 3 years old.

Planting distance, m	L	ength of roo	ots, cm/tre	e	Mass of roots, g/tree					
	Under	1.2 mm	3-5	Over 5	Under	1.2 mm	2.5 mm	Over 5		
	1mm	1-3 11111	mm	mm	1mm	1-3 11111	5-5 mm	mm		
Planting distance, 5x1,5 m										
0-25	4130	7300	160	364	10,4	30,2	5,2	100,5		
26-50	4248	5545	712	496	14,4	24,0	92,4	52,4		
51-75	2124	6423	108	80	7,2	28,8	24,2	100,4		
76-100	2018	2495	48	92	6,8	10,8	4,0	32,4		
101-125	2478	3861	-	-	8,4	16,8	-	-		
126-150	2235	3696	-	-	7,6	16,0	-	-		
Amount	17233	29320	1028	1032	54,8	126,6	125,8	285,7		

Planting distance, 5x2 m										
0-25	4130	7393	128	312	14,0	29,0	9,2	80,4		
26-50	6018	6376	712	584	20,4	27,6	89,6	212,0		
51-75	2832	8010	88	112	9,6	44,4	19,2	16,0		
76-100	4248	3327	180	76	14,4	14,4	24,4	28,0		
101-125	4956	2772	-	-	16,8	12,0	-	-		
126-150	2360	756	-	-	8,0	7,6	-	-		
Amount	24544	28634	1108	1084	83,2	135	142,4	336,4		
	-		Planting of	listance, 5x2	,5 m					
0-25	5664	9980	120	468	19,2	43,2	10,0	192,0		
26-50	4484	6375	620	332	15,2	17,6	82,4	56,0		
51-75	2714	5544	160	108	9,2	24,0	14,0	40,0		
76-100	2832	2679	60	120	9,6	11,6	8,0	12,4		
101-125	2596	3972	220	180	8,8	16,8	24,4	48,0		
126-150	3422	7207	60	40	11,6	31,2	17,2	10,8		
Amount	21712	35757	1240	1248	73,6	144,4	156,0	359,2		

#### CONCLUSIONS

The roots of the cherry trees, grafted on the Gisela 6 rootstock, and shaped like a reduced-sized natural crown, advance from the trunk radially evenly in all directions. As the distance between tree trees increases, the length and root mass increase and their density in the soil decreases. The length of roots is determined by fibrous roots (95.4-95.8%), and the mass - of the skeleton roots (65.6-69.3%). The majority of the roots by length (69.8-82.9%) and after the meal (88.8-92.8%) are distributed over a depth of 0-60 cm.

The extension and distribution in the soil of the radicular system allow to make the following findings regarding some agro technical works in modern cherry plantations: In young plantations with the soil maintained as a field, the soil loosening works can be executed up to the depth of 15- 20 cm with the risk of cutting at most 5.3% of the roots by length and 1.1% by mass with a diameter of up to 3 mm; The young trees, the roots near the trunk (up to 75 cm) are higher in the superficial layer of 0-20 cm, it is better to avoid the proximity of tree rows to less than 75 cm.

The administration of nitrogen fertilizers on the row of trees, on a 1.5 m wide strip, in that the fibrous roots are denser in this sector of the nutrition of the trees. The migration of phosphorus and potassium into the soil, being difficult, the administration of these fertilizers is incorporated into the soil at a distance of 50-75 cm at a depth of 20 cm. Determining the depth (need) of water supply to the soil must be the depth at which most roots are distributed, especially 0-60 cm.

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# EFFECT OF EXTRACTION TEMPERATURES AND SOLVENT ON THE ANTIOXIDANT POTENTIAL OF GREEN ARABICA AND ROBUSTA COFFEE BEANS

Kamvoukou Christina- Anna<sup>1</sup>, Gougoulias Nikolaos<sup>2</sup>\*, Giannoulis Kyriakos<sup>2</sup>, Papachatzis Alexandros<sup>2</sup>, Papathanasiou Theodoros<sup>2</sup>, Malamis Asterios<sup>2</sup>

<sup>1</sup>School of Pharmacy, Aristotle University of Thessaloniki, Greece <sup>2</sup> Department of Agricultural Technology, Technological Educational Institute of Thessaly, 41110 Larissa, Greece \*Correspondence author. Email: ngougoulias@teilar.gr

Key words: Antioxidant activity FRAP; Antiradical activity DPPH; Coffee; Total phenols

#### ABSTRACT

Two different varieties green coffee (Robusta and Arabica) were studied for total polyphenol content and for antioxidant properties. Different solvents and temperature extraction conditions were employed. The total phenol content ranged from 4.73 to 60.31 mg (GAE)/g DW, the antiradical activity DPPH• ranged from 26.2 to 158.1 µmol (Trolox)/g DW and the antioxidant activity FRAP ranged from 115.8 to 811.9 µmol (Trolox)/g DW. Extraction with 80% aqueous ethanol at 45°C temperature proved to be a optimum method to extract phenolic antioxidant compounds from green coffee. The Robusta variety exert higher antioxidant activity FRAP and higher antiradical activity (DPPH•), compared to the Arabica variety, for all extraction treatments. The extracts green coffee can find applications as food additives, either in the form of powder or as extracts highly concentrated.

#### INTRODUCTION

The reactive oxygen and nitrogen containing free radicals (ROS and RNS), formed in the human cells as strong oxidants, can cause oxidative damage in the biological molecules. These changes of the biological molecules are the basis for many diseases (Halliwell 1991). Epidemiological studies demonstrate that the oxidative destruction of biomolecules can be reduced by using endogenous and exogenous antioxidants such as polyphenols, etc.(Pellegrini et al. 2003). They're in a diet; reduce the risk of cardiovascular and some cancer diseases (Manach et al. 2004).

The coffee plant, is an evergreen shrub, where it belongs to the family Rubiaceae, while when cultivated reaches about 2.5 meters high. Coffee contains a tremendous number of phenolic compounds; the most abundant phenolic compounds are chlorogenic acids, ferulic acid, tannins, proanthocyanidins, lignans, and tocopherols, which exert strong antioxidant effect (Borelli et al. 2002, Farah & Donangelo 2006, Gonzalez 2001). Other compounds in coffee affect its color and aroma (Cammerer & Kroh 2006, Lopez-Galilea et al. 2006).

Studies have shown that coffee consumption is related with the reduction of liver cancer, parkinson's disease, alzheimer's disease and the prevention of diabetes mellitus type 2 (Dorea & Costa 2005, Hidgon & Frei 2006, Lopez-Garcia et al. 2006).

The aim of the present study is to determine the content of phenolic compounds and the antioxidant activity, of the green Robusta and Arabica coffee beans at different solvents and temperature extraction conditions.

#### MATERIAL AND METHODS

## Sample preparation:

Two, commercial available, species of green coffee were purchased from the local market in Larisa, Robusta coffee beans and Arabica coffee beans. The green coffee were dried in a dark place at room temperature, finely ground and kept at 4oC. The sample was extracted using four different conditions.

#### **Extraction procedures:**

(i) 4 g of the finely ground sample were 2-fold treated by 25 ml 80% aqueous ethanol at 45 °C. At first treatment the samples were incubated for 4h in the extractant at stirring and the second one continued stirring for 2h at 45°C. The extract was gathered after centrifugation and filtration through Whatman No. 4 paper.

(ii) 4 g of the finely ground sample were 2-fold treated by 25 ml 80% aqueous ethanol at ambient temperature. At first treatment the samples were incubated for 24 h in the extractant at stirring and the second one continued stirring for 2h at ambient temperature. The extract was gathered after centrifugation and filtration through Whatman No. 4 paper.

(iii) 4 g of the finely ground sample was extracted with 50mL of boiling water at 150 rpm for 30 min. The extract was gathered after centrifugation and filtration through Whatman No. 4 paper.

(iv) 4 g of the finely ground sample was extracted with 50mL of water at room temperature for 24 h and filtered through Whatman No. 4 paper.

#### Methods of analyses:

Determination of total polyphenolics (TP). Total polyphenolic content was determined with the Folin-Ciocalteu (F.-C.) reagent according to the method by (Singleton and Rossi 1965) using the microvariant proposed by (Badenschneider et al. 2015) and the results were expressed as gallic acid equivalent (GAE) in mg/g dry weight.

Determination of ferric reducing antioxidant power (FRAP). The antioxidant activity of the methanol extracts was determined on the basis of the method by (Benzie and Strain 1999) and was expressed as Trolox equivalent (TEAC) in  $\mu$ mol /g dry weight.

Determination of antiradical activity (DPPH•). The antiradical activity of the methanol extracts was determined according to the method by (Brand-Williams et al. 1995) using the stable free radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH•). The activity was evaluated as Trolox equivalent (TEAC) in µmol/g dry weight.

#### Statistical analysis:

Data were analyzed using the MINITAB (Ryan et al. 2005) statistical package. The experiment had four replications. Analysis of variance was used to assess treatment effects. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.
#### **RESULTS AND DISCUSSIONS**

The extraction of the green coffee with different solvents and different temperatures results in the extraction of phenol compounds which vary between 4.73 and 60.31 mg (GAE)/g DW (Table 1). In all treatments studied, the green coffee Robusta characterised by higher TP content compared to green coffee Arabica. Our results are in agreement with the data obtained by other authors who have established differences in total phenols content in the different green coffee species (Hudáková et al. 2016, Odžaković et al. 2016, Pérez-Hernández et al. 2012).

The use of solvents at high temperatures, compared to the respective solvents at room temperature, showed a higher content of total phenols in the green coffee Robusta and Arabica (Table 1). Extraction yield of total phenols in the green coffee Robusta and Arabica shows the following order:

80% aqueous ethanol ( $45^{\circ}$ C) > 80% aqueous ethanol (room temperature > boiling water > Water (room temperature).

Table 1

Solvent	Conditions	TP – (mg GAE/g) DW	
		Robusta Arabica	
80% Aqueous ethanol	45°C	60.31a	44.53b
80% Aqueous ethanol	Room temperature	39.01c	26.27d
Water	Boiling water	16.86e	7.62f
Water Room temperat		12.79g	4.73i

Total phenols content of green Arabica and Robusta coffee beans

For each extraction treatments characteristics with the same letter of table do not differ significantly according to the Tukey's test (P=0.05).

The antiradical activity (DPPH•) of the two varieties green coffee studied ranges from 26.2 to 158.1 µmol (Trolox)/g DW (Table 2). The use of solvent 80% aqueous ethanol at temperature conditions 45oC exert the higher antiradical activity (DPPH•) with 158.1 µmol (Trolox)/g DW, in the green coffee Robusta. On the contrary, the use of water as solvent at room temperature exert the lower antiradical activity (DPPH•) with 26.2 µmol (Trolox)/g DW, in the green coffee Arabica.

The Robusta variety exerts the higher activity, compared to the Arabica variety, for all extraction treatments. The variation in the antiradical activity (DPPH•) reported and by other authors for the green Arabica and Robusta coffee beans (Kiattisin et al. 2016, Sentkowska et al. 2016).

The antioxidative assay FRAP of the two varieties green coffee studied ranges from 115.8 to 811.9  $\mu$ mol (Trolox)/g DW (Table 3). Extraction solvent 80% aqueous ethanol at temperature conditions 45oC in the green coffee Robusta, exert the higher antioxidant activity FRAP with 811.9  $\mu$ mol (Trolox)/g DW. On the contrary, extraction solvent water at room temperature in the green coffee Arabica, exert the lower antioxidant activity FRAP with 115.8  $\mu$ mol (Trolox)/g DW (Table 3).

#### Table 2

Antiradical activity	DPPH• of gi	reen Arabica	and Robusta	coffee beans
----------------------	-------------	--------------	-------------	--------------

Solvent	Conditions	DPPH• - (µmol Trolox /g) DW	
		Robusta Arabica	
80% Aqueous ethanol	45°C	158.1a	104.8c
80% Aqueous ethanol	Room temperature	120.1b	82.3d
Water	Boiling water	93.6e	63.9f
Water Room temperature		39.0g	26.2i

For each extraction treatments characteristics with the same letter of table do not differ significantly according to the Tukey's test (P=0.05).

The Robusta variety exert higher activity (as Trolox equivalent) compared to the Arabica variety, for all extraction treatments (Table 3). The variation in the antioxidant activity FRAP reported and by other authors for the green coffee varieties (Pokorná et al. 2015).

Table 3

#### Antioxidant activity FRAP of green Arabica and Robusta coffee beans

Solvent	Conditions	FRAP- (µmol Trolox /g) DW	
		Robusta Arabica	
80% Aqueous ethanol	45°C	811.9a	763.2b
80% Aqueous ethanol	Room temperature	615.2c	510.0d
Water	Boiling water	301.5e	239.7f
Water	Room temperature	135.3g	115.8i

For each extraction treatments characteristics with the same letter of table do not differ significantly according to the Tukey's test (P=0.05).

#### CONCLUSIONS

The results obtained showed differences in total phenols composition in the green Robusta and Arabica coffee beans. Phenolic compounds, antioxidant and antiradical activity of green coffee depends strongly on the variety.

The higher extraction temperatures at each solvent increased the extraction yield of total phenols in the green coffee Robusta and Arabica.

Green Robusta and Arabica coffee beans are a source of bioactive components that could be included in functional foods composition. The extracts green coffee can find applications as food additives, either in the form of powder or as extracts highly concentrated.

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#### EFFECT OF CYCOGAN ON GROWTH AND FLOWERING OF BOUGAINVILLEA SPP.

Mandă Manuela<sup>1</sup>\*, Nicu Carmen<sup>1</sup>

<sup>1</sup><sup>University</sup> of Craiova, Craiova, e-mail: manda\_manu@yahoo.com

Key words: Bougainvillea, Cycogan, control, growth, flowering

#### ABSTRACT

The effect of growth inhibitor treatments on the growth and development of Bougainvillea plants has been studied by many researchers, and the results indicate that different species and varieties react differently to their application. The main objective of this work was to investigate the effects of Cycogan treatments on the growth and development of three species of Bougainvillea sp. The best results in terms of the retardant effect of Cycogan on the plants were obtained in: B. buttiana "Raspberry Ice" - Cycogan 0.1% and 0.2%; B. spectabilis "Orange King" - Cycogan 0.1% and 0.2%; B. buttiana "Alba" - Cycogan 0.1%; B. spectabilis - Cycogan - 0.2%; B. glabra - Cycogan 0.2% and 0.3%. The flowering was positive influenced by Cycogan treatments in B. glabra, B. spectabilis "Orange King" and B. buttiana "Alba".

#### INTODUCTION

The Bougainvillea genus, originally from South America, is from the *Nyctaginaceae* family and includes 14 species, of which the following three are of particular interest: *Bougainvillea spectabilis* Willdenow, *B. glabra* Choisy and *B. peruviana* Humboldt and Bonpland. *Bougainvillea* can be a shrub (*B. peruviana H. & P.*), a small tree (*B. praecox* Griseb.) or a liana (B. *spectabilis* Willd., *B. glabra* Choisy) (Randhawa & Mukhopadhyay 1986, Kobayashi 2007).

*Bougainvillea* has green or variegated leaves; the flowers are small and tubular, usually white or yellow, supported by three persistent, brightly coloured bracts, these being the main decorative element (Probert et al 1993).

Bougainvillea is a very popular evergreen landscape plant, used for decorating pergolas, fences, walls, buildings, but also as a plant in pots or suspended baskets. Bougainvillea x buttiana and B. glabra are ideal for topiary art and bonsai because they are more compact (Cirillo et al. 2014, Whistler 2000). In our country, Bougainvillea can be used in the decoration of spacious, well-lit interiors, but it is also present in gardens, balconies and terraces during the warm season. Because of the vigorous growth habit, bougainvilea needs a lot of labor-intensive pruning when being used as a potted plant. PGRs could be alternatives to frequent pruning on bougainvillea to reduce labor costs (Jain et al. 2014). The effect of growth inhibitor treatments on the growth and development of Bougainvillea plants has been studied by many researchers, and the results indicate that different species and varieties react differently to their application (Shao et al. 2006, Saifuddin et al. 2009b, Jain et al. 2016). Thus, the treatment with ethephon stimulated the Bougainvillea flowering (Fang-Yin & Chang, 2011). In the

"San Diego Red" variety, the CCC and B-9 treatments proved to be effective in stimulating flowering in a short daytime period (Hackett & Sachs 1967). Aldrich & Norcini (1996) studied the effect of PGRs on the production of *Bougainvillea* "Barbara Karst". Foliar treatments with uniconazole (10 ppm), maleic hydrazide (2808 ppm), daminozide (5000 ppm) and paclobutrazol 200 ppm) were applied. The PGR treatment has not actually slowed the growth or has not increased the quality or marketability of Bougainvillea plants "Barbara Karst". Moreover, the daminozide reduced the number of ramifications, and the maleic hydrazide was phytotoxic.

Based on these considerations, the present study aims to present the effect of Cycogan (1000, 2000, 3000 ppm) treatments on the growth and development of three species of *Bougainvillea spp*.

#### MATERIALS AND METHOD

The biological material was represented by rooted cuttings of 3 species of the *Bougainvillea* genus, namely: *B. glabra, B. spectabilis, B. spectabilis* "Orange King" and *B. buttiana* with the "Alba" and "Raspberry Ice" varieties existing in the Floriculture discipline collection.

Cycogan 400 LC 100 ml is a growth regulator that acts on plant as a growth inhibitor but it is capable of producing a wide range of physiological changes in different plant species and varieties.

The experiment was placed in the didactic greenhouse of the Floriculture discipline of the A.I. Buia Botanical Garden from Craiova on 22.05.2015. The greenhouse is temperate, with average temperatures ranging between 18 °C and 20°C. Two experimental factors were considered: Factor A - variety with 5 graduations: a1. B. glabra, a2. B. spectabilis, a3. B. spectabilis "Orange King", a4. B. buttiana "Alba", a5. B. buttiana "Raspberry Ice"; Factor B - the treatments with different concentrations of Cycogan (C), with 4 graduations: b1. Water (Ct untreated); b2. C 0.1% (1000 ppm); b3. C 0.2% (2000 ppm); b4. C 0.3% (3000 ppm). The combination of the two factors resulted in 20 experimental variants.: V11(Ct1) - B. buttiana "Raspberry Ice"/w; V12 - B. buttiana "Raspberry Ice"/C 0.1%; V13 - B. buttiana "Raspberry Ice"/C 0.2%; V14 - B. buttiana "Raspberry Ice"C 0.3%; V21 (Ct 2) - B. spectabilis "Orange king"/w; V22 - B. spectabilis "Orange king"/C 0.1%; V23 - B. spectabilis "Orange king"/C 0.2%; V24 - B. spectabilis "Orange king"/C 0.3%; V31 (Ct3) - B. buttiana "Alba"/w; V32 - B. buttiana "Alba"/C 0.1%;V33 - B. buttiana "Alba"/C 0.2%; V34 - B. buttiana "Alba"/C 0.3%; V41 (Ct 4) - B. spectabilis/w; V42 - B. spectabilis /C 0.1%; V43 - B. spectabilis /C 0.2%; V44 - B. spectabilis /C 0.3%; V51 (Ct 5) - B.glabra/w; V52 - B. glabra /C 0.1%;V53-B. glabra /C 0.2%;V54 - B. glabra /C 0.3%. The rooted cuttings were planted in a 2:1 mixture of peat + perlite in pots of 18 cm (a size recommended in the literature). Three foliar treatments with Cycocel were applied at a two-week interval.

The observations and determinations were carried out may-october 2015 period and consisted on: the average plant height, the average shoots length, the average internodes length, the average leaf length and the percent of blooming plants under the influence of applied treatments.

#### **RESULTS AND DISCUSSIONS**

The growth retardants (PGRs) are commonly used in floriculture to reduce plant growth, thus creating much more compact shrubs and beautiful foliage, but they are also used for stimulating flowering (Mandă & Nicu 2016). The graph 1 shows that the plants responded differently to Cycogan treatments for the studied species and varieties. The average height of the plants treated with Cycogan in different concentrations for *B. buttiana* "Raspberry Ice" and *B. glabra*, had lower values in comparison to the untreated control plant. The retardant effect was stronger in *B. glabra*, causing a significant reduction in the average plant height from 37.5 cm (V51Mt) to 23.5 cm (V54). There was found that by applying treatments with different concentrations of Cycogan in *B.buttiana* "*Alba*", the effect was not to reduce the vegetative growth. The values corresponding to the average plant height were superior to the untreated control plant, ranging between 35 cm (V32) and 54 cm (V33) in comparison to 34 cm (V31-Mt). There was found that by applying treatments with different concentrations of Cycogan in *B. spectabilis*, the retardant effect was only seen in the variants treated with Cycogan 0.2% (V43), and in *B. spectabilis* "Orange King" at Cycogan 0.1% concentrations, respectively 0.2% (V22, V23). The growth was stimulated at the plants treated with Cycogan 0.3%, obtaining higher values than in comparison to 34 - 39 cm).

Regarding the average shoots length, the *B. buttiana* "Raspberry Ice" and *B. spectabilis* "Orange King" recorded lower values than at the untreated control plant in the case of concentrations of 0.1% (18 cm, respectively 21 cm) and 0.2% (16 cm - 22 cm). The retarding effect of Cycogan treatments was manifested in *B. glabra* at all three concentrations, but the best results were obtained at the variants treated with Cycogan 0.1% and 0.2% (V52, V53). In *B. buttiana "Alba"*, the retarding effect was shown only in the plants treated with Cycogan 0.1%, and in *B. spectabilis* the Cycogan treatments acted as growth stimulators, as shown in figure 2. The treatments with Cycogan 0,3% had a stimulating effect of vegetative growth in the treated variants compared to the untreated control plants (23-43cm in comparison to 20-34cm).

From the analysis of the data presented in graph 2, the average internodes length was considerably influenced by the application of growth retardants to all the studied species. The application of the Cycogan treatments in B. spectabilis "Orange King" determined the elongation of the internodes in all treated variants compared to the untreated control plant. The average length of the internodes was between 1.8 cm (V22) and 2.1 cm (V24), and the lowest value was recorded by V41Mt (1.6 cm). The values of this parameter in B. spectabilis increase up to a concentration of 0.2%, then decrease, and there is a reduction in the average length of the internodes at concentrations of 0.3%. The values obtained at the end of the experimental period in the *B. buttiana* "Alba", were superior to the control plant at concentrations of 0.2% and 0.3%. Only the concentration 0.1% determined a reduction in the length of the internodes compared to the untreated control plant (from 1.8 cm to 1.5 cm). The average length of the internode in the B. buttiana "Raspberry Ice" was higher in comparison to the control plant at the concentration of 0.1% or equal to that of the untreated control plant at the concentration 0.2%. There was a slight reduction in the length of the internode only at the concentration of 0.3% in comparison to the untreated control plant (from 0.9 cm to 0.8 cm). Inferior or equal values of the average length of the internodes (0.8 cm and 1.1 cm, respectively) were obtained in B. glabra, at the Cycogan-treated variants compared to the untreated control plant (1.1 cm).

The graph 3 shows that the average length of the leaves varied very little for each studied species due to the Cycogan treatments. The application of the growth retardant in *B.buttiana* Raspberry Ice and *B. buttiana* "Alba" determined a decrease of leaf length with increasing Cycogan concentration, from 6.3 cm (V11Mt) to 5.9 cm (V14) for *B. buttiana* "Raspberry Ice" and from 6.9 cm (V31Mt) to 6.4 cm (V33) for *B. buttiana* "Alba". Minimal values of the average leaf length were obtained in *B. glabra* at the Cycogan 0.1% treated variant (V52 - 4.6 cm) in comparison to the untreated control plant (V51-5.1 cm). The treatment with Cycogan 0.2% and 0.3% resulted in an

increase of the average leaf length. The average leaf length was superior in *B. spectabilis* in comparison to the control plant, in the case of the concentration 0.1%, and in *B. spectabilis* "Orange King" for the concentrations of 0.1% and 0.3%. The concentration 0.2% determined a reduction of the average leaf length compared to the untreated control plant (from 6.4 cm to 5.9 cm at *B. spectabilis* "Orange King" and from 8.1 cm to 7 cm at *B. spectabilis*). The only species with phytotoxicity (discoloured leaf) was *B. buttiana* "Alba" (V34 - C 0.3%).

Regarding *Bougainvillea* flowering, the influence of Cycogan treatments are analyzed for each species, knowing that the different Bougainvillea species/varieties are blooming at a certain amount of light intensity. In the didactic greenhouse of the Floriculture discipline, *Bougainvillea* is located within a sunny exposure with the intensity of light ranging from 12,000-18,000 lux in May and September.

Under these circumstances, only *B. glabra, B. spectabilis* "Orange King" and *Bougainvillea Buttiana* "Alba" flourished in October 2015. The flowering took place in all experimental variants, in *B. spectabilis* "Orange King", *B. glabra*, the best results (75%) were recorded by V23-0.2%, respectively V52-0.1% in terms of percentage of flowering plants per variant. The flowering was observed in *B. buttiana* "Alba" at all the treated variants, the best results on the percentage of flowering plants were recorded by V33-0,2% (75%). The flowering was not influenced in *B. spectabilis* by Cycogan treatments, the only flowering being observed in the untreated control plant (graph 4).





Graph 1. The influence of Cycogan treatments on the plant growth

Graph 2: The influence of Cycogan treatments on the average internodes length



Graph 3: The influence of Cycogan treatments on the average leaf length





#### CONCLUSIONS

All the analyzed growth parameters in the *Bougainvillea* species were influenced differently for each species, with no determination for an optimal concentration at which Cycogan could have a retardant effect for all the studied species / varieties. The best results in terms of the retardant effect of Cycogan on the plants were obtained in: *B. buttiana* "Raspberry Ice" - Cycogan 0.1% and 0.2%; *B. spectabilis* "Orange King" - Cycogan 0.1% and 0.2%; *B. buttiana* "Alba" - Cycogan 0.1%; *B. spectabilis* - Cycogan - 0.2%; *B. glabra* - Cycogan 0.2% and 0.3%. The only species with phytotoxicity (discoloured leaf) was *B. buttiana* "Alba" (V34 - Cycogan 0.3%). In terms of flowering, only *B. glabra*, *B. spectabilis* "Orange King" and *B. buttiana* "Alba" flourished in October 2015. The *B. spectabilis* flowering was not influenced by Cycogan treatments, the only flowering being observed at the untreated control plant.

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#### EFFECTS OF TEMPERATURE ON GROWTH RATE, LIPID AND PROTEIN CONTENT OF THE MICROALGAL SPECIES Nannochloropsis oculata

M. N. Metsoviti<sup>1\*</sup>, N. Katsoulas<sup>1</sup>, I. T. Karapanagiotidis<sup>2</sup>, G. Papapolymerou<sup>3</sup>

<sup>1</sup>Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Volos, Greece <sup>2</sup> Department of Ichthyology and Aquatic Environment, University of Thessaly, Volos, Greece <sup>3</sup> Department of Civil Engineering, Technological & Educational Institute of Thessaly, Larissa, Greece \* Correspondence author. mametsov@uth.gr

Key words: microalgae, Nannochloropsis oculata, productivity, growth rate, nutrient composition

#### ABSTRACT

This paper presents the effects of three different temperatures (20, 25 and 30 °C) on the growth rate, productivity and nutrient composition of the microalgal species Nannochloropsis oculata. The experiments were conducted in closed photobioreactors of 25 L capacity, where the temperature and the light intensity were controlled. The absorbance at 665 nm was measured daily. Total productivity was determined at the end of the experiments and the specific growth rates in exponential growth phase ( $\mu_{exp}$ ) were calculated. Additionally, the nutrient composition of the algal biomass in lipids and proteins was determined. It was found that N. oculata grew best at 30°C. The protein content was similar among the three different temperatures, while the lipid content increased with the increase of temperature.

#### INTRODUCTION

Microalgae are unicellular photosynthetic organisms that use light and carbon dioxide, with higher photosynthetic efficiency than plants, for the production of biomass. As aquatic relatives of plants, they thrive in aerated, liquid cultures where the cells have sufficient access to light, carbon dioxide and other nutrients (Rosenberg et al. 2008). Their biodiversity is enormous. There are species that prosper in freshwater and others in saline water, such as the marine microalgal species *Nannochloropsis oculata*.

Microalgae can be cultivated in open or closed systems. Open-field cultivation is usually conducted in an open pond or an open container that is exposed to the environment. Cultivation in a closed system can be carried out in photobioreactors. In closed systems, the productivity of biomass is higher than it is in the open systems and the risk of contamination of the cultivation is lower. However, investment and operating costs, as well as energy requirements, are higher than in open systems (Rösch & Posten 2012).

The microalgae culture systems are quite complex and are influenced by different factors, such as temperature, carbon dioxide, pH, light intensity, mixing grade, salinity, as well as the nutrient composition of the culture medium (nitrogen, potassium, phosphorous and micronutrients) (Dean et al. 2010).

Cultivation in stress conditions includes culture in inadequate nutrients in the culture medium, very high or low light intensities and low pH. These conditions lead to

a reduction in the rate of growth and as a result to a decrease in productivity (Wijffels et al. 2010).

Environmental and culturing condition variations (temperature, light intensity, nutrient content of the culture medium) can alter the chemical composition of the biomass of microalgae produced at the end of the cultivation (Gouveia & Oliveira 2009). For instance, as the nitrogen concentration increases in the culture medium, the protein content increases and the lipid content decreases (Metsoviti et al. 2016). It should be mentioned that, the lipid production of a microalgal species can be enhanced, by cultivation in different temperatures (Sibi et al. 2016).

Microalgae are used in pharmaceutical, in biodiesel production, in biodegradation, as nutritional supplements and as feed for animals and fish, replacing other ingredients of the feeds (Pulz & Gross 2004). In aquaculture, various species of microalgae are used for fish larval nutrition during a brief period either for direct consumption in the case of molluscs and peneid shrimp or indirectly as food for the live prey fed to fish larvae (Muller-Feuga 2000).

The present study was conducted in order to investigate the effects of three different temperatures on the growth rate, total productivity and nutrient composition in lipids and proteins of the marine microalgal species *Nannochloropsis oculata*. For this purpose, *N. oculata* was cultivated in a closed photobioreactor at three different temperatures, 20, 25 and 30 °C in the same culture medium and with the same light intensity. The total production on a dry basis (g of dry algal biomass/ 25 L of culture medium), the specific growth rate in the exponential growth phase ( $\mu_{exp}$ ) and the maximum absorbance readings ( $\alpha_{max}$ ) for the three different temperatures, were determined as well.

#### MATERIAL AND METHODS

Microalgal culture: The microalgal species *N. oculata* was obtained from the Experimental Phycology and Culture Collection of Algae from the University of Goettingen in Germany (EPSAG).

Cultivation system: All experiments were conducted in a closed photobioreactor of 25 litres capacity (Figure 1), at three different temperatures, 20, 25, 30 °C and were illuminated with red and white led lamps (each, 120  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) on 12:12 h light-dark cycles. The temperature was controlled to within ± 0.1°C. The duration of each experiment was 25 days.

Microalgal culture media: The culture medium was the same in all experiments and contained the following ingredients, suggested by Andersen et al. 1991: Each litre of the culture medium contained: 0.2g KNO<sub>3</sub>/L, 0.02 g K<sub>2</sub>HPO<sub>4</sub>/L, 0.02 g MgSO47H<sub>2</sub>O/L, 30 ml of soil extract/L, 5  $\mu$ g vitamin B<sub>12</sub>/L and 5 ml/L, of a solution containing the following micronutrients: (1 mg ZnSO<sub>4</sub>7H<sub>2</sub>O, 2 mg MnSO<sub>4</sub>4H<sub>2</sub>O, 10 mg H<sub>3</sub>BO<sub>3</sub>, 1 mg Co(NO<sub>3</sub>)<sub>2</sub>6H<sub>2</sub>O, 1 mg MoO<sub>4</sub>2H<sub>2</sub>O, 0.005 mg CuSO45H<sub>2</sub>O, 700 mg FeSO<sub>4</sub>7H<sub>2</sub>O and 800 mg EDTA)/L. Additionally, synthetic salt was added in the culture medium so that the salinity of the water was maintained at 35 ‰.

MIcroalgal biomass concentration: The microalgal concentration was determined daily by optical density measurements at 655nm with the use of a spectroscopy UV/Vis instrument. Three samples were collected daily and all measures were carried out in triplicate. Additionally, at the end of the experiment total productivity of each culture was determined (in g/25 L), after collection of the biomass by a condensation process, which was based on flocculation, natural sedimentation, removal of the supernatant, collection of the condensates and removal of moisture and the specific growth rates in the exponential growth phase ( $\mu_{exp}$ ) were calculated according to the relation:

$$\mu_{exp} = \ln \left( \alpha_2 / \alpha_1 \right) / \left( t_2 - t_1 \right)$$

where,  $\alpha_1$  and  $\alpha_2$  are the absorbances at the beginning and the end of exponential growth phase at time 1 (t<sub>1</sub>) and 2 (t<sub>2</sub>), respectively.



Figure 1. Closed photobioreactor, where the experiments were conducted.

Protein and lipid content: Crude protein of the microalgal biomass was determined by Kjeldahl method, using a conversion factor of 6.25 (AOAC 1997). Lipids were extracted from the samples with 1:1 chloroform/methanol with Folch et al. (1957) method. According to Ryckebosch et al. 2012, chloroform–methanol 1:1 was shown to be the best solvent mixture for extraction of total lipids from microalgae.

Statistical analysis: Results are expressed as mean  $\pm$  standard deviation. Data were analyzed using a one-way analysis of variance (ANOVA). The significant differences between treatments (P<0.05) were determined using Tukey's multiple comparison test.

#### **RESULTS AND DISCUSSIONS**

Figure 2 illustrates the absorbance readings versus the cultivation time of *N. oculata* during the cultivation at three different temperatures (20, 25 and 30 °C). Table 1 presents the specific growth rates in exponential growth phase ( $\mu_{exp}$ ), the maximum absorbance readings ( $\alpha_{max}$ ) and total biomass productivities on a dry basis (in g/25L) for *N. oculata* grown at three different temperatures while, Table 2 shows the crude protein and crude lipid contents (%) of *N. oculata* cultivated at the three different temperatures.

It was found that the growth rate of *N. oculata* (Fig. 2 and Table 1), was strongly dependant on the cultivation temperature. The increase in temperature from 20°C to 25°C and to 30°C, led to consequent increases in specific growth rates in the exponential growth phase ( $\mu_{exp}$ ), in the maximum absorbance readings ( $\alpha_{max}$ ) and in total productivities.



Figure 2. Growth curves of the microalgal species *N. oculata* grown at three different temperatures.

Specifically, the total productivity in algal biomass was doubled at  $30^{\circ}$ C, compared with the productivity at  $20^{\circ}$ C. Thus, it appears that the cultivation of *N. oculata* is favored at  $30^{\circ}$ C, compared to lower temperature regimes.

#### TABLE 1

Specific growth rates in exponential growth phase ( $\mu_{exp}$ ), maximum absorbance readings ( $\alpha_{max}$ ) and total productivities (g/25 L) for *N. oculata* grown at three different temperatures.

$T \rightarrow$	20°C	25°C	30°C
μ <sub>exp</sub>	0.24	0.28	0.35
α <sub>max</sub>	0.19	0.59	0.82
Productivity (g/25 L)	30.2	52.3	60.3

TABLE 2

Lipid and protein content (% of dry weight) of *N. oculata* grown at three different temperatures. Values represent means  $\pm$  st. deviation (n=3). Values in the same row bearing different superscript indicate statistically significant difference (P < 0.05).

Nutrients	20°C	25°C	30°C
Lipids (%)	$9.0 \pm 0.2^{a}$	13.2 ± 0.5 <sup>b</sup>	15.7 ± 0.4 <sup>°</sup>
Proteins (%)	16.9 ± 0.3 <sup>a</sup>	17.4 ± 0.4 <sup>a</sup>	17.1 ± 0.1 <sup>a</sup>

As far as the nutrient composition of *N. oculata* is concerned, the protein content of the algal biomass remained constant at the three different temperatures (Table 2). This may be due to the fact that the protein is a significant structural and metabolic component of algal cells, so that their protein content might be more resistant to temperature alterations.

On the other hand, the lipid content of the algal biomass increased significantly with the increase of the temperature from  $20^{\circ}$ C to  $25^{\circ}$ C and to  $30^{\circ}$ C. These findings are in agreement with those reported by Converti et al. (2009), in which the lipid content of *N. oculata* was doubled when cultured at  $25^{\circ}$ C compared to the temperature of  $20^{\circ}$ C. The increase in lipid content by rises in temperature has also been shown for other microalgal species such as *S. obliquus* (Vitova et al. 2015).

Total lipid content in microalgae increases to a certain extent as the temperature increases and reaches an optimal level (Sibi et al. 2016). The optimal value of temperature where the highest biomass and lipid production is achieved varies from species to species and as a result, it is difficult to generalize on specific influence of the environmental factors on growth, biochemical composition and enzyme activities in different microalgal species (Gigova et al. 2012). Unfortunately, a higher temperature regime that could have resulted in a possible peak in lipid content was not tested in the present study.

It should be stated, however, that total lipid content does not signify lipid quality and that culture of microalgae at higher temperatures may also alter their fatty acid compositions. It is known, for example, that higher temperatures decrease the production of unsaturated lipids in *Dunaliella salina* (Thompson, 1996). Therefore, future research on the effects of temperature on the lipid content of microalgae should also consider alterations in lipid quality, especially if their use is intended as nutrient supplements.

#### CONCLUSIONS

Growth of the microalgal species *N. oculata* at  $30^{\circ}$ C was found to be the best temperature regime in terms of growth rate, absorbance and productivity compared to  $20^{\circ}$ C and  $25^{\circ}$ C. Total lipid content of *N. oculata* was increased concomitantly with increases in temperature, but protein content was unaffected.

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### **AMPELOGRAPHIC CHARACTERISTICS OF CHARDONNAY 76**

Nakov Zdravko<sup>1</sup>, Ivanov Miroslav<sup>1</sup>, Simeonov Iliyan<sup>1\*</sup>

\* Institute of Viticulture and Enology, Pleven, Bulgaria, e-mail: iliannsimeonov@gmail.com

Key words: vine, variety, clone, ampelographic description

#### ABSTRACT

During the period 2008-2011, an ampelographic description of the introduced French clone Chardonnay 76 was made at the Institute of Viticulture and Enology – Pleven. The study found that Chardonnay clone 76 belonged to the group of medium-ripening white wine varieties. In the soil and weather conditions of the region of Pleven, its grapes reach technological maturity during the first ten days of September (07.09.). The cluster is medium sized, cylindrical-conical in shape, with one small wing, semi-compact or compact. The berry is small to medium-sized, yellow-greenish, almost spherical. The vines have medium to strong growth, good fertility and medium yield. The clone is not liable to putting forth catkins and milerandage. It has good resistance to low winter temperatures. It is sensitive to fungal diseases and especially to powdery mildew (oidium). It develops well and gives yield grafted to Berlandieri x Riparia SO4 rootstock. Chardonnay 76 has a very good sugar accumulating capacity - 23.20% sugars while keeping enough titratable acids - 7.09 g/dm<sup>3</sup>. Chardonnay 76 wine is yellow-greenish in colour with a pleasant floral fruity aroma, pronounced acidity, dense, harmonious and long-lasting aftertaste.

#### INTRODUCTION

The origin of Chardonnay variety has not been fully clarified however according to Viala and Vermorel (1905) and Galet (1958; 1976), it was a French variety grown since the old age in the regions of Burgundy and Champagne. The variety belongs to the Western ecological and geographical group. Quality white dry wines and products for sparkling wines are made from its grapes. Because of its valuable economic qualities, Chardonnay variety is widely distributed in most wine-growing countries in Europe, North and South America, South Africa and Australia. In Bulgaria the variety was introduced from France, studied by Nedelchev and Kondarev (1936), and later it was zoned to be distributed (Stoev, 1960).

During its many years of development and growing under different soil and climatic conditions, various mutational changes occurred in the population of Chardonnay variety. Most of them were of a negative nature, reducing the yield and deteriorating grapes quality. That necessitated the study of the intra-diversity variety and the application of the clonal selection method to improve some of the significant economic qualities of the variety. Many clones were obtained by the clonal selection method in Italy (Moretti, 1994), France (Roychev, 2012; Galet, 1994), Russia (Guseynov et al., 2011), Germany and other vine-growing countries. In Bulgaria, a clone of Chardonnay 6/48 (Nakov, 2006) was selected and approved in 1994. For enriching the grapevine gene pool of the country and diversifying the range of wines, a

French clone of Chardonnay variety was introduced at the Institute of Viticulture and Enology in Pleven in 1991.

*The objective* of this study is to present detailed ampelographic characteristics of Chardonnay Clone 76 introduced from France.

#### MATERIAL AND METHODS

Chardonnay 76 was selected in France by the method of clonal selection. The ampelographic study of the variety was carried out during the period 2008 - 2011 in the clonal section established for that purpose in the Experimental Base of the Institute of Viticulture and Enology – Pleven. The vines were grafted to Berlandieri x Riparia SO4 rootstock and were planted at a distance of 3.00/1.30 m. They were grown on Moser training system with stem height of 0.80 m. The vines were mixed pruned with equal individual loading - 32 winter eyes (6 x 2 eyes and 2 x 10 eyes).

The botanic description (young shoot, mature shoot, developed leaf, inflorescence, cluster and berry), the phonological observations (onset of flowering, onset of grapes ripening and technological maturity), the fertility indicators (ratio of developed winter eyes and fruit shoots with 1, 2, 3 clusters, ratio of developed and fruit shoot) and yield (average mass per cluster and average yield per vine) was done in accordance with the approved methodology in the country for clonal selection (Katerov et al., 1990).

During the grapes ripening phase, the dynamics of sugar accumulation was monitored. At technological maturity the quality parameters (sugars and titratable acids) were determined and a mechanical analysis was made to establish the texture and the structure of the cluster and the berry. From each harvest at the Experimental cellar of IVE - Pleven, 30 kilograms were processed according to the classical technology for the production of white dry wines by the method of micro-vinification (Yankov, 1992). The young wines were subjected to a chemical analysis according to the common methods used in the wine industry (Ivanov, 1979) and an organoleptic evaluation by the 100-score system was made.

#### **RESULTS AND DISCUSSIONS**

**ORIGIN**. Chardonnay Clone 76 was separated from Chardonnay varieties by the method of clonal selection in France.

**DISTRIBUTION**. In Bulgaria Chardonnay 76 is distributed in the super-elite mother plantation of IVE – Pleven.

#### **BOTANIC DESCRIPTION**

**Young shoot**. The crown is very mossy to cottony, with a pinkish border. The first leaf is gray-green, quite mossy on the top side, and cottony on the underside. The second leaf is waxy-yellowish, mossy on the top side and intensely mossy on the underside. The rest of the leaves are golden-yellowish, with a bronze tinge, mossy on the top side and intensely mossy with bristles on the underside. The young shoot is gray-greenish, with wine-red strips, slightly mossy.

**Mature shoot**. The internodes are medium long, medium thick, slightly flattened, slightly angular. The bark is light brown. The nodes are darker in color. The mature shoot has a small core and solid wood.

**Developed leaf**. The leaf blade is medium large, wider than longer, three to five-lobed, folded upward. The top side is slightly reticulated wrinkled, with arachnoidal moss, while the bottom surface - slightly mossy-bristly. The outlines of the middle section form an obtuse angle. The upper lateral notches are shallow, open, in the form of an acute angle, and rarely moderately deep, lyrated. The bottom side notches are shallow, barely noticeable, open, in the form of an acute angle or missing. The tail

notch is open, lyrated or arched, equilateral. The top teeth are small to medium-sized, straight, triangular, while the end ones are small, transient-domed. The veins are green, on top - with scarce bristles and underneath - mossy. The petiole is short, green, somewhat pale wine-red, slightly mossy-bristly. The autumn colouring of the petiole is yellow.

**Inflorescence**. It is monoecious, with 5 stamens, rarely 4 or 6 stamens.

**Cluster**. It is medium large (13.3/8.1 cm), cylindrical-conical, with one small wing, semi-compact or compact. The rachis is short, medium thick, woody at the base. the average mass per cluster is 125.0 g (Fig. 1).



Fig. 1. Cluster of Chardonnay 76

**Berry**. It is small to medium large (13.61/13.60 mm), almost spherical, sometimes deformed because of the cluster compactness. The skin is yellowish-green, thin and elastic, with slight wax coating. The texture is juicy and the taste – harmonious, neutral. The average mass of 100 berries is 146.7 g.

#### AGROBIOLOGICAL CHARACTERISTIC

**Vegetation period**. Chardonnay 76 is a medium ripening white wine variety. Under the climatic and soil conditions of the region of Pleven, its grapes reach technological maturity in the first ten days of September (07.09) and depending on the weather conditions of the year it varies from the end of August (26.08.) to mid September - (16.09.). The vegetation period duration is about 145 days. Flowering starts in the last ten days of May - 21.05. and ranges from 17.05. to 24.05. The onset of grapes ripening occurs at the end of July (29.07.) and varies from 21.07. to 04.08. depending on the weather conditions of the year (Table 1).

Table 1

Phenological observations in Chardonnay 76 from 1998-2011 periode

Phenophases	Data
Beginning of flowering	21.05.
Beginning of ripening of the grapes	29.07.
Technological ripeness in grapes	07.09.

**Growth strength**. Vines have medium intense growth. By the end of the vegetation the shoots mature well.

**Fertility and yield**. Chardonnay 76 is distinguished with good actual fertility – 84.12 % (80.00 % - 88.00 %) developed winter eyes and 79.47 % (67.31 % - 89.74 %) fruit shoots. From the fruit shoots 37.50 % (31.59 % - 43.75 %) have 1 cluster; 56.60 % (50.00 % - 62.86 %) - 2 clusters and 5.90 % (0.00 % - 10.23 %) – 3 clusters. The fertility ratio per developed shoot is 1.28 (0.96 - 1.46) and of a fruit shoot – 1.61 (1.43 - 1.69). The average yield per vine is 3.086 kg, varying from 2.264 kg to 3.710 kg (Table 2).

Table 2

Doveloped	Fruiting shoots:			Fertility coefficient in:		
buds	total	with 1 cluster	with 2 clusters	with 3 clusters	developed	fruit shoot
%	%	%	%	%	Shool	
84.12	79.47	37.50	56.60	5.90	1.28	1.61

Actual fertility indexes of Chardonnay 76 from the 1998-2011 periode

**Putting forth catkins and milerandage**. The clone is distinguished by good pollination and fertilization and it is not liable to putting forth catkins and milerandage.

**Disease resistance**. It is not resistant to fungal diseases and especially to powdery mildew. In years of rainy autumn grapes are attacked strongly by gray rot.

**Agrotechnical specifics**. Chardonnay 76 requires mixed pruning with individual loading per vine of 32 winter eyes (6 spurs x 2 eyes and 2 canes x 10 eyes). When grown on Moser training system and planting density 256 vines/dka (3.00 x 1.30 m.), the clone gives optimum yield with very good grape quality.

**Suitable rootstoks**. Chardonnay 76 has good affinity to Shasla x Berlandieri B and Berlandieri x Riparia SO4 rootstocks.

**Response to environmental conditions**. The clone has good resistance to low winter temperatures and recovery capacity, allowing it to be grown on various stem training systems in all vine-growing regions of the country.

#### TECHNOLOGICAL CHARACTERISTICS

**Grapes mechanical composition**. According to the cluster mechanical composition Chardonnay 76 is a typical wine variety, with high content of solid fraction. The rachis ratio in the cluster is 4.16 % (3.26 % - 4.96 %) and of the berries - 95.84 % (96.74 % - 95.04 %). The skins in the berry are 9.68% (7.72 % - 11.50 %), the seeds - 4.24 % (3.26 % - 5.67 %) and the mesocarp - 86.08 % (82.83 % - 88.49 %). The theoretical yield is 82.50 %, varying for the individual vintages from 79.03 % to 85.61 % (Table 3).

**Chemical composition**. Chardonnay 76 is characterized by very good sugaraccumulating capacity – 23.20 % sugars that vary depending on the weather conditions from 22.40 % to 23.90 %. The titratable acids of the grapes are kept relatively high - 7.09 g/dm<sup>3</sup> (6.15 g/dm<sup>3</sup> - 8.25 g/dm<sup>3</sup>). Chardonnay 76 wine is yellowish-green in colour with a pleasant floral and fruity aroma, pronounced acidity, dense, harmonious and long-lasting aftertaste (Table 3).

#### GENERAL ASSESSMENT OF THE CLONE

Chardonnay 76 is medium ripening white wine clone. It was selected in France by the method of clonal selection from the population of Chardonnay variety. The cluster is medium large, cylindrical-conical, with one small wing, semi-compact or compact. The berry is small to medium large, almost spherical, sometimes deformed because of the cluster compactness.

Table 3

Cluster mechanical composition				
Weight, g	125.00			
Length, cm	13.30			
Width, cm	8.10			
Rachis, %	4.16			
Berries, %	95.84			
Berry mechanical composition				
Weight of 100 berries, g	146.70			
Length, mm	13.61			
Width, mm	13.60			
Skins, %	9.68			
Seeds, %	4.24			
Flesh, %	86.08			
Yield and chemical analyses of grapes				
Average yield per vine, kg	3.086			
Theoretical yield, %	82.50			
Sugars, %	23.20			
Titratable acids, g/dm <sup>3</sup>	7.090			
Chemical composition and tasting score of white wine				
from Chardonnay 76				
Alcohol, vol. %	12.82			
Sugars, g/dm <sup>3</sup>	1.60			
Titratable acids, g/dm <sup>3</sup>	6.84			
рН	3.16			
Sugar free extract, g/dm <sup>3</sup>	22.78			
Total phenolic compounds (TPC), g/dm <sup>3</sup>	0.45			
Tasting score	77.80			

Yield, mechanical and chemical analyses of grapes and wine of Chardonnay 76 from the 1998-2011 periode

The skin is yellowish-green, thin and elastic, with slight wax coating. The texture is juicy and the taste – harmonious, neutral. Vines have medium to intense growth and average high fertility. It is not liable to putting forth catkins and milerandage. It is not resistant to fungal diseases and especially to powdery mildew. In years of rainy autumn grapes are attacked strongly by gray rot. The vines have good resistance to low winter temperatures and recovery capacity. Chardonnay 76 has good affinity to Berlandieri x Riparia SO4 rootstocks. When grafted to this it is obtained medium high yield with very good grapes quality. Under the soil and weather conditions of Pleven region Chardonnay 76 has very good sugar-accumulating capacity by keeping relatively high titratable acidity. The grapes are suitable for the production of quality white dry wines. Chardonnay 76 wine is yellowish-green in colour with a pleasant floral and fruity aroma, pronounced acidity, dense, harmonious and long-lasting aftertaste.

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# RESEARCH ON THE BEHAVIOR OF SOME HYBRIDS LETTUCE IN SOLARIUM

Neață Gabriela<sup>1</sup>, Hoza Gheorghița<sup>1\*</sup>, Dobrescu Aurelia<sup>1</sup>, Basarabă Adrian<sup>1</sup>,Dinu Maria<sup>2</sup> Mohammed Jasin Mohammed<sup>1</sup>, Hadi Hoobi Shallat<sup>1</sup>

<sup>1</sup> University of Agronomical Sciences and Veterinary Medicine Bucharest, Horticulture Department, Bd.Mărăşţi, 59, Bucharest, Romania <sup>2</sup> University of Craiova, Horticulture Department, Craiova, Romania \* Correspondence author: , e-mail:hozagh@yahoo.com

Key words: Lactuca sativa L., hybrids, growth regulator

#### ABSTRACT

The research was conducted in the solarium of the Department of Horticulture at USAMV Bucharest in 2017. Six F1 hybrids of Lactuca sativa L were used: Touareg, Alanis, Analena, Shangore, Centore and Sotalis. The behavior of lettuce was followed when applying gibberelinic acid (AG3) at different doses during vegetation. Phenological observations, biometric measurements, agrochemical determinations of lettuce quality, and production were recorded. The results were statistically interpreted.

#### INTRODUCTION

Achieving a high level of agricultural crop production implies the combined use of classical chemical or organic fertilizers as well as growth regulators that ensure optimal supply of plants with nutrients.( Pavlou et al.2007).

Cultivation of crops is done under conditions of maximum economic efficiency without producing negative effects on the quantity, commercial quality and the food value of the production (http://www.giberelina.ro).

Foliar fertilizers apply to most crop plants to provide complete and balanced fertilization, stimulate rooting, growth and fructification by accelerating the absorption of nutrients in the soil.( Tamme et al., 2009).

The discovery of stimulating growth regulators (gibberellins) has resulted in spectacular effects with outstanding results in agricultural and horticultural production. ()The main changes that occur in the metabolism of plants under the action of gibberellins are intensification of sweating and increased water consumption, respectively faster absorption of nutrients and delaying aging processes of vegetal tissues.

The purpose of the research was to track the lettuce's behavior when applying gibberellin acid (AG3) at different doses during vegetation.

#### MATERIAL AND METHODS

The experience was carried out in 2017 in a USAMVB solarium, the Department of Vegetables.

The biological material used was represented by 6 F1lettuce hybrids: Touareg, Alanis, Analena, Shangore, Centore and Sotalis.(Table 1).

Table 1.

			Iable
		Characterization of cultivated lettuce hybrids	
No	Hybrid	Characteristics	Producer
1	TOUAREG	It has resistance to the attack of aphids, LMV (the virus of the mosaic virus) as well as the hand. The bright, bright green caps are compact and can reach great size. The Touareg has a good tolerance to the floral stems and to the marginal necrosis of the leaves. Leaves of equal, fine, color and shape of the captain make the Touareg an excellent variety for marketing and fresh consumption. Touareg is recommended both for open-field cultivation and protected areas.	Seminis
2	ALANIS	For crops in the field or tunnels and solariums, throughout the year, the captains are large, well- closed, compact, light green, shiny. Alanis has shown good tolerance to plant disease ( <i>Botrytis, Sclerotinia</i> ) and good tolerance to floral stems, resistance to LVW ( <i>Lettuce mosaic virus</i> ) and to <i>Bremialactucae</i> , I-25	Seminis
3	SHANGORE	Butterhead autumn lettuce for late-winter autumn crops. The caps are compacting, semi-finished. The base of the captains is closed, smooth and shining. It is noted for its high resistance to <i>Bremialactucae</i> BI 1-24. The plants grow rapidly, the leaves are erect. Forms large captains (400-600g) even on weak soils. Easily cut off with erect leaves	Syngenta
4	ANALENA	Analena is a large lettuce for unprotected culture, set up early in spring and autumn. The leaves are large, of medium thickness and form large caps in a very short time. The light green color of the leaves makes this variety a very attractive one. Presents high mold resistance ( <i>Bremia</i> ).	Syngenta
5	CENTORE	The best choice for spring late winter. Shows closed and compact captains, high hand strength (HR) Bl 1- 24. It is not pretentious to soil and low temperatures. The crop cycle is fast, it is resistant to drying the inner leaves. The base of the captains is smooth and well closed. The internal structure of the head is compact, the leaves are light and erect.	Enza Zaden
6	SOTALIS	It is a variety of lettuce with a very nice presentation for spring and autumn crops. It has medium and good disease resistance (BI: 1-28, Nr: 0). The head is compact, uniform in light green and weighing 700-900g. Excellent for marketing and fresh consumption.	Enza Zaden

The lettuce was planted on March 6, 2017, the planting scheme being 0.4 x 0.3m = 8plt / m2 of seedlings prepared in the multiplier of the same university.

The behavior of lettuce was followed by the application of gibberellic acid (AG3) in different doses respectively: gibberellic acid 25mg / I and gibberellic acid 50mg / I applied twice during vegetation. (Table 2).

During the vegetation, phenological observations were made. Biometric measurements, respectively mean weight of head, weight of the lettuce part, plant diameter, weight and volume of the roots were recorded at the harvest and production was recorded.

At the same time, analyzes were made regarding the quality of lettuce, nitrate content, phosphate nitrate and lettuce potassium. The methods used in the analyzes were: Griess method, spectrophotometric for nitrates, nitrites and Duval reagent and spectrophotometric phosphorus dosing, and for flame spectrophotometry. These methods are according to the standards in our country: ISO 9001.(Metodologia de analiza a solului si plantei. ICPA, 1987).

#### **RESULTS AND DISCUSSIONS**

Table 2

No	Hibrid	Tratment
1	TUADEC	Control
1	TUAREG	
2	_	Gibbereilic acid 25mg/i
3		Gibberellic acid 50mg/l
6	ALANIS	Control
7		Gibberellic acid 25mg/l
8		Gibberellic acid 50mg/l
9	SHANGORE	Control
10		Gibberellic acid 25mg/l
11		Gibberellic acid 50mg/l
12	ANALENA	Control
13		Gibberellic acid 25mg/l
14	-	Gibberellic acid 50mg/l
15	CENTORE	Control
16		Gibberellic acid 25mg/l
17		Gibberellic acid 50mg/l
18	SOTALIS	Control
19		Gibberellic acid 25mg/l
20		Gibberellic acid 50mg/l

Scheme of experimental variants

Determination of the average diameters of lettuce showed that the smallest diameters were obtained at Centore hybrids where the application of gibberellic acid caused the decrease of this characteristic from 33cm to control at 31cm to 50mg / I gibberellic acid and 32cm, respectively, at the application of 25mg / I gibberellic acid.(Figure 1`)

Beneficial results were obtained in several hybrids, namely Shangore, Analena and Sotalis, at which the lettuce diameters reached 41 cm at the application of 50 mg / I gibberellic acid. Touareg and Alanis responded differently to the increasing doses of gibberellic acid, the lettuce diameters increased at 25mg / I and then dropped to the maximum dose of 50mg / I.

The analysis of lettuce weights in the experimental variants showed differently, so at a lower dose of 25mg / I we have an increase in weight in all hybrids but then at 50mg / I in a decrease in weights and recorded production respectively. (Figure 2)

The biggest increases in lettuce weight were recorded at Shangore (373g / plt) and Centore (376g / plt).



Figure 1. The diameter of lettuce in experimental variants

The statistical interpretation of the results (Table 3) shows that the application of 25 mg / Gibberellic acid resulted in increased production with very significant differences compared to control, eq Alanis, Shangore and Centore hybrids. When only 50mg / I gibberellic acid was applied, the results were either insignificant (Tuareg, Shangore) or only significantly statistically ensured.



Figure 2. The medium weight of lettuce plants on variant

At the harvest, lettuce quality, nitrates, phosphates and potassium contents were also analyzed (Table 4.). Nitrates have been absorbed in varying amounts in plants. The highest values were obtained when applying 25 mg / I gibberellic acid to Analena and Sotalis hybrids. (Wang et al., 2002)

If we analyze the results in terms of the Maximum Allowable Concentration at lettuce cultivated in the greenhouse of 3000 ppm nitrates, the lettuce content of all the variants under investigation was below this value, so the lettuce is good for consumption. (Santamaria, 2006). In the case of phosphorus, the absorbed quantities are small, not exceeding 200 ppm, and the potassium is absorbed in high amounts, enrolling in the literature values for these elements.

Statistical Interpretation of Production Outputs in the 6 Hybrids

No	Hybride	Tratment	Produc-	Dife-	Percent	Signifi-
			tion	rence	%	cant
			kg/m2	±		
1	TUAREG	Control	2.43	-	100.00	-
2		Gibberellic acid 25mg/l	2.82	0.34	116.05	*
3		Gibberellic acid 50mg/l	2.57	0.14	105.76	ns
6	ALANIS	Control	2.32	-	100.00	-
7		Gibberellic acid 25mg/l	2.8	0.48	120.69	***
8		Gibberellic acid 50mg/l	2.72	0.40	117.24	**
9	SHANGORE	Control	2.4	-	100.00	-
10		Gibberellic acid 25mg/l	2.99	0.59	124.58	***
11		Gibberellic acid 50mg/l	2.52	0.12	105.00	ns
12	ANALENA	Control	2.3	-	100.00	-
13		Gibberellic acid 25mg/l	2.59	0.29	112.60	*
14		Gibberellic acid 50mg/l	2.84	0.54	123.47	***
15	CENTORE	Control	2.19	-	100.00	-
16		Gibberellic acid 25mg/l	3.01	0.82	137.44	***
17		Gibberellic acid 50mg/l	2.67	0.48	121.91	***
18	SOTALIS	Control	1.94	-	100.00	-
19		Gibberellic acid 25mg/l	2.28	0.34	117.52	*
20		Gibberellic acid 50mg/l	2.23	0.29	114.94	*

DL 5% = 0.232kg/m2

DL 1%=0.345kg/m2

DL 0.1% = 0.454kg/m2

Table 4.

### The lettuce quality characteristics of the experimental variants

No	Hybride	Tratment	N-NO3,	P-PO4,	K,ppm
			ppm	ppm	
1	TUAREG	Control	494	198.12	3940
2		Gibberellic acid 25mg/l	646	152.40	2340
3		Gibberellic acid 50mg/l	760	129.54	5260
6	ALANIS	Control	513	251.46	3940
7		Gibberellic acid 25mg/l	646	106.68	3560
8		Gibberellic acid 50mg/l	722	144.78	1880
9	SHANGORE	Control	836	182.88	1700
10		Gibberellic acid 25mg/l	418	83.82	2300
11		Gibberellic acid 50mg/l	418	121.92	2100
12	ANALENA	Control	760	198.12	2260
13		Gibberellic acid 25mg/l	1120	167.64	2060
14		Gibberellic acid 50mg/l	950	144.78	2300
15	CENTORE	Control	646	289.56	2840
16		Gibberellic acid 25mg/l	494	152.4	1640
17		Gibberellic acid 50mg/l	703	121.92	2180
18	SOTALIS	Control	513	220.98	2380
19	]	Gibberellic acid 25mg/l	1254	144.78	2480
20	]	Gibberellic acid 50mg/l	418	121.92	1500

#### CONCLUSIONS

1. Determination of average lettuce diameters showed that beneficial results were obtained in several hybrids, namely Shangore, Analena and Sotalis, at which lettuce diameters reached 41 cm when applying 50 mg / I gibberellic acid.

2. Touareg and Alanis responded differently to the increasing doses of gibberellic acid. Lettuce diameters increased at 25mg / I and then dropped to a maximum dose of 50mg / I.

3. The analysis of the lettuce weights in the experimental variants showed differently, so at a lower dose of 25mg / I we have an increase in weight in all hybrids but then to 50mg / I in a decrease in weights and the recorded production respectively. Lettuce weight gains were recorded at Shangore (373g / plt) and at Centore (376g / plt).

4. The statistical interpretation of the results shows that the application of 25 mg / Gibberellic acid resulted in increased production with very significant differences compared to control, eg Alanis, Shangore and Centore hybrides. When only 50mg / I gibberellic acid was applied, the results were either insignificant (Tuareg, Shangore) or only significantly statistically ensured.

5. Nitrates were absorbed in varying amounts in plants. The monkey values were obtained by applying 25 mg / I gibberellic acid to the Analena and Sotalis hybrids.

6. If we analyze the results in terms of the Maximum Allowable Concentration of lettuce cultivated in greenhouses of 3000ppm nitrates, the lettuce content of all the variants under investigation is below this value, so the sludge is good for consumption.

7. In the case of phosphorus, the absorbed quantities are small, not exceeding 200 ppm, and the potassium is absorbed in high amounts, enrolling in the values presented by the literature for these elements.

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#### COMPARATIVE STUDY ON POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY IN SOME SWEET PEPPER CULTIVARS DURING DEVELOPMENT IN HYDROPONIC CULTURE

Papathanasiou Theodoros<sup>1</sup>, Gougoulias Nikolaos<sup>1</sup>\*, Kamvoukou Christina- Anna<sup>2</sup>, Efthimiou Spyridoula<sup>1</sup>, Papachatzis Achilleas<sup>3</sup>

<sup>1</sup> Department of Agronomy Technology, Technological Educational Institute of Thessaly, 41110 Larissa, Greece <sup>2</sup>School of Pharmacy, Aristotle University of Thessaloniki, Greece

<sup>3</sup>School of Agriculture, Aristotle University of Thessaloniki, Greece \*Correspondence author. email: ngougoulias@teilar.gr

Key words: FRAP activity; Pepper fruits; Stages ripening; Total phenols

#### ABSTRACT

The changes of the total phenolic content and of the FRAP activity during development and ripening three sweet peppers cultivars in hydroponic culture they studied. For all cultivars of sweet peppers studied, from early development stages up to the mature green color, the total phenols content and antioxidant activity did not show any increase. From mature green color up to mature red color of fruits, all varieties studied showed a significant increase the levels of the total phenolics content and FRAP assay values. In particular, total phenolics content for the Dolmy, Yahoo and Florinis peppers increased by 220%, 195% and 260% respectively. FRAP assay values for the Dolmy, Yahoo and Florinis peppers were found to be 2-fold, 1.8-fold and 2.9-fold times raised respectively. It is advisable to consume sweet peppers at the mature red stage for better effects consumer health.

#### INTRODUCTION

The free radicals are strong oxidisers which damage the biological molecules, causing the occurrence of many diseases in the human organism (Halliwell 1991). Studies demonstrate that the oxidative destruction of biomolecules can be protected by using endogenous and exogenous antioxidants which are contained in fruits, vegetables etc. (Ou et al. 2002, Pellegrini et al. 2006, Wu et al. 2004).

Phenolic compounds are secondary metabolites which are synthesized by plants and contained in a high proportion in the plants and the fruits (Gougoulias 2012). Many phenolic compounds contribute to shaping of the taste and color of fruits and plants, while others are good sources of natural antioxidants and have protective role against some diseases (Gambacorta et al. 2009, Shahidi 2000). The intake of polyphenols with food reduces the risk of cardiovascular, carcinogens and other diseases (Manach et al. 2004, Scalbert and Williamson 2000).

Sweet peppers fruits (Capsicum annuum L.) it is natural reservoir of nutrients and of natural antioxidants with high antioxidant activity (Gougoulias et al. 2016). The cultivation systems, fertilization, irrigation and variety, affect the levels of the antioxidant activity in the peppers fruits (Ayodele et al. 2015, Guinoza et al. 2015).

The aim of this study was the identification of the phenolic content and antioxidant activity in three sweet peppers cultivars during the development and ripening, in a hydroponic culture.

#### MATERIAL AND METHODS

Pepper plants (*Capsicum annuum* L.) obtained from a commercial nursery of Larisa, were transplanted and ware cultivated in hydroponic system at the Technological Education Institute of Larisa, Greece, during February 2016 to July 2016 in an area of  $200 \text{ m}^2$ . The crop substrate consisted from coconut slabs in double rows, with a distance of plants for each slab 30 cm. Three cultivars of the sweet peppers ware cultivated. The pepper fruits and the basic characteristics of each variety are presented in Table 1.

Table 1

Cultivars	Flavor	Shape	Flesh	Color of
			thickness	harvest
Dolmy F1	Sweet	Bell	High	Green
Yahoo F1	Sweet	Elongated	High	Green
Florinis NS 700	Sweet	Elongated	High	Red

Basic characteristics of pepper fruits

For experimental purposes, drip irrigation technique was used in order to supply the open hydroponic system. The crop was fertilized through a stably chemical nutritive solution at the rates of 58.9 ml/min for 3 minutes, repetitively 4 times a day (Table 2). Nutrients were kept separately according to their chemical properties in three tanks. The nutritive solution was prepared automatically mixing equal solution quantity from each tank. The final nutritive solution consisted of Ca<sup>2+</sup> = 253.5 meq/L, K<sup>+</sup> = 380.1 meq/L, Mg<sup>2+</sup> = 97.2 meq/L, NH<sub>4</sub><sup>+</sup> = 27.5 meq/L, H<sup>+</sup> = 168 meq/L, Fe<sup>2+</sup> = 0.9 meq/L, NO<sub>3</sub><sup>-</sup> = 421.9 meq/L, PO<sub>4</sub><sup>3-</sup> = 214.9 meq/L and SO<sub>4</sub><sup>2-</sup> = 290.3 meq/L.

Table 2

Chemical properties of the coco palm substrate and the nutrient solution were used in experiment

Chemical Property	* Coco palm (fresh mass)	Nutrient Solution influx	Irrigation water
рН	6.78 ± 0.31	6.45 ± 0.28	7.7
Electrical conductivity, (dS/m)	0.69 ± 0.38	2.23±0.31	0.55

\* Coconut pH and electrical conductivity, determined in (1:5) coconut / water extract. Data represent average means and SE deviation. (n) = 8.

Four pepper fruit for each variety with uniform size, shape and color were harvested for analysis at each development stage. The development stages and maturation of pepper fruits are presented in Table 3.

**Preparation of the methanol extracts:** Ten g of the peppers samples were two rounds treated by 20 ml of 80% aqueous methanol. Samples were incubated for 24 h in the extractant at stirring; the supernatant material was removed. The pellet was re-treated with aqueous methanol for 2 h at stirring at ambient temperature. The

extract was gathered after centrifugation/filtration and the volume was made up to 50 ml with aqueous methanol and used for further chemical analysis (Kanner et al. 1994).

Table 3

Stages	Cultivars			
	Dolmy F1	Yahoo F1	Florinis NS 700	
	Size and color			
S1	Small, green	Small, green	Small, green	
S2	Medium, green	Medium, green	Medium, green	
S3	Big, green	Big, green	Big, green	
S4	Maturation, green	Maturation, green	Maturation, green	
S5	30% red	30% reddish-brown	30% red	
S6	Full red	Full reddish-brown	Full red	

The development stages and ripening of the pepper fruits during cultivation

(S1), 10 days after flowering

**Methods of analyses:** Total polyphenolic (TP) content was determined with the Folin-Ciocalteu (F.-C.) reagent according to the method by (Singleton and Rossi 1965) using the microvariant proposed by (Badenschneider et al. 2015) and the results were expressed as gallic acid equivalent (GAE) in  $\mu$ g/g fresh weight.

The antioxidant activity of ferric reducing antioxidant power (FRAP) of the methanol extracts was determined on the basis of the method by (Benzie and Strain 1999) and was expressed as ascorbic acid equivalent (AAE) in µmol/g fresh weight

**Statistical analysis:** Data were analyzed using the MINITAB (Ryan et al. 2005) statistical package. The experiment had four replications. Analysis of variance was used to assess treatment effects. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

#### **RESULTS AND DISCUSSIONS**

Among the sweet peppers cultivars studied the six stages development, in the bell Dolmy pepper the total phenols content ranged from 432.2 to 1380.5  $\mu$ g (GAE) / g FW, in the elongated Yahoo pepper the total phenols content ranged from 377.5 to 1256.9  $\mu$ g (GAE) / g FW, and in the elongated Florinis pepper the total phenols content ranged from 540.8 to 2283.8  $\mu$ g (GAE) / g FW (Table 4).

The Dolmy and Yahoo cultivars from the start of development of fruits (first stage) up to the mature green color (fourth stage) they showed reduction of the total phenols content. The total phenols content in the Florinis peppers did not show statistically significant differences from the start of development of the fruits up to the mature green color. Subsequently, from mature green color up to mature red color of fruits, all varieties studied showed a significant increase the levels of the total phenolics content. The composition of total phenols in the Dolmy, Yahoo and Florinis peppers increased by 220%, 195% and 260% respectively. To the same conclusions arrived some researchers which studied the change in the content total phenolic during ripening for some sweet pepper cultivars of India (Deepa et al. 2007).

Finally, during the mature green color of the sweet peppers or of the mature red color, the Florinis variety showed a higher total phenols content compared to Dolmy and Yahoo varieties in the respective stages (P < 0.05). Also, our results at the stage of full maturation (mature red color) have higher values than those reported by

other authors for some hot peppers cultivars of Greece (Gougoulias et al. 2017). These differences, in the variability of total phenols, likely due to the different cultivars, in soil and climatic conditions of each region.

Table 4

Changes of the total phenolics content during development and ripening of sweet pepper cultivars

Development stages and ripening	TP-μg (GAE)/g FW			
	Dolmy F1	Yahoo F1	Florinis NS 700	
S1 (small size)	615.9b	512.1c	607.1bc	
S2 (medium size)	458.1d	433.8d	665.6b	
S3 (big size)	509.4c	377.5e	540.8c	
S4 (maturation green)	432.2d	426.9d	611.6bc	
S5 (30% red color)	621.6b	578.8b	2283.8a	
S6 (full color)	1380.5a	1256.9a	2197.2a	

For each different variety of pepper fruits, columns of table with the same letter do not differ significantly according to the Tukey's test (P=0.05).

The results of the FRAP antioxidant assay of three sweet pepper cultivars studied, reveal that the antioxidant activity FRAP from the start of the development of fruits (first stage) up to the mature green color (fourth stage), showed no statistically significant difference for all cultivars studied (Table 5). Significant increase of the FRAP activity in all sweet peppers cultivars reveal from the mature green color until the mature red color. FRAP assay values for the Dolmy, Yahoo and Florinis peppers were found to be 2-fold, 1.8-fold and 2.9-fold times raised respectively. To the same conclusions arrived some researchers which studied the change in the antioxidant activity FRAP during ripening for some sweet pepper cultivars of iran (Ghasemnezhad et al. 2011). Also, the ferric reducing antioxidant power (FRAP) of sweet peppers reveal that the Florinis cultivar in the mature red color, exert the highest activity (as ascorbic acid equivalent) with 23.8  $\mu$ mol (AAE)/g FW, compared to the other sweet peppers cultivars (P < 0.05).

Table 5

Changes of the antioxidant activity FRAP during development and ripening of sweet pepper cultivars

Development stages and ripening	FRAP activity -µmol (AAE)/g FW		
	Dolmy F1	Yahoo F1	Florinis NS 700
S1 (small size)	8.2bc	7.5b	7.9b
S2 (medium size)	7.2c	6.7c	8.8b
S3 (big size)	7.6c	6.5c	7.7b
S4 (maturation green)	7.1c	7.4b	8.3b
S5 (30% red color)	8.8b	7.9b	23.2a
S6 (full color)	14.3a	13.1a	23.8a

For each different variety of pepper fruits, columns of table with the same letter do not differ significantly according to the Tukey's test (P=0.05).

#### CONCLUSIONS

The results of the hydroponic cultivation showed that in the Dolmy, Yahoo and Florinis sweet peppers, from early development stages up to the mature green color, the total phenols content and antioxidant activity did not show any increase.

For all cultivars of sweet peppers studied, from the mature green color to the mature red color, the composition of total phenols and FRAP assay values they increased significantly. Finally, it is advisable to consume sweet peppers at the mature red stage for better effects consumer health.

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#### INFLUENCE OF GROWTH REGULATORS ON PRODUCTIVITY OF PLANTATION AND QUALITY OF APPLE FRUITS

Pesteanu Ananie<sup>1</sup>

<sup>1</sup>Faculty of Horticulture, State Agrarian University of Moldova Mircesti str. 48, Chisinau, Republic of Moldova; a.pesteanu@uasm.md

Key words: Apple, economic efficiency, profitability, relative efficiency.

#### ABSTRACT

The experimental plot is placed in the orchard "Codru-ST" Ltd. founded in 2012 year. The study subject of the experience was ldared apple variety grafted on M 9 rootstock. The distance of plantation is 3.5x 0.8 m. The research was conducted during the period of 2015 year. The tested agents was Humifield, WG, 0.2/0.2/0.2/0.2/0.2/0.2 kg/ha; Nertus PlantaPeg, 0.6/0.6/2.0 l/ha; Nertus PlantaPeg, 1.0/1.0/2.5 l/ha, which was sprayed in different period. During the research, it was studied the influence on the growth regulators on productivity of plantation and quality of apple fruits. It was established that the The Nertus PlantaPeg growth regulator can be included in the technology system to improve apple growth and fructification processes and reduce the etiquette release rate of fruit, applied 3 times by foliar spraying. The first treatment to be carried out in the "pink button" phase at a dose of 0.6 - 1.0 l/ha, the second - in the binding phase - the intensive growth of fruits (fruit diameter 20-25 mm) at a dose of 0.6 - 1.0 l/ha, and the third - 1-2 days before harvesting in the dose 2.0 - 2.5 l/ha.

#### INTRODUCTION

The action of the production factors is generally limited after achieving a certain level of production (Balan et al., 2001). Thus, mineral fertilizers can be administered up to a certain limit, over which there are regression phenomena of increased production, water can be administered up to the physiological limit specific to apple culture. That is why new and new means discovered by man are needed for the continuous growth of productions (Babuc, 2012; Cimpoieş, 2012).

The discovery of growth-enhancing substances (phytohormones, growth regulators, etc.) has made it an effective tool for producers to manage and control growth, plant development and apple production (Babuc et al., 2013; Suman et al., 2017).

In addition to regulating growth and development processes, growth regulators also influence other biochemical processes and biological phenomena such as flowering and fertilization of flowers, fruit binding, yield of photosynthesis, differentiation of fruit buds, resistance to fall of leaves and fruit (Ghena et al., 1999).

Polyethylene glycol, fulvic acide and salts of humic acids are those compounds that increase the resistance of trees during vegetation to stress factors such as drought, high temperatures, insufficiency of mineral elements, disease of various diseases and pests. The products in question allow their action to have a positive effect for a longer period after the treatments (Burzo et al., 1999).

Fulvic acids and salts of humic acids improve the activity of the root system in

the soil, transforming the minerals that are harder to reach for the trees, in an easily added form, which through the absorbent roots are supplied to the air side (Burzo et al., 1999; Neamţu et al., 1991).

#### MATERIALS AND METHODS

The research was carried out during the year 2015 in the superintensive apple orchard founded in the autumn of 2012 at the enterprise "Codru ST", with "knip boom" crown trees.

The object of study of the experience was Idared apples grafted on the weak M9 rootstock. The crown was driven after the improved thinning system. Planting distance was  $3.5 \times 0.8$  m.

To determine the influence of growth regulators on fructification and not to imbalance the physiological processes inside the plant and to obtain constant and quality productions, the following variants were tested (tab. 1).

Table 1

Scheme of experiments to determine the biological efficiency of the growth regulator in apple trees

Variants	Application method	
Control - without treatment	-	
Humifield, WG, 0.2/0.2/0.2/0.2/0.2/0.2 kg/ha	Spraying 6 times. The first treatment - until flowering, II - after flowering, the next 4 with 10-14 days between treatments.	
Nertus PlantaPeg, 0.6/0.6/2.0 l/ha	Spraying 3 times. The first treatment - in the pink "button" phase, the second - in the binding phase - the intensive growth of the fruit (fruit diameter 20-25 mm), and the third - 1-2 days before the harvest.	
Nertus PlantaPeg, 1.0/1.0/2.5 l/ha		

Trees treatment was done with the portable sprinkler in the hours without wind, in the morning. The amount of solution per tree when treated with growth regulators on apple trees was 0.3 liters, based on the number of trees per unit area and the recommended water quantity of 1000 l/ha.

The amount of fruits, the average weight of a fruit, the production of a tree and a surface unit, their redistribution by diameter were established during the harvest period. The results were reported in the control variant and the standard version. The apple firmness evaluation was performed using the FT 327 penetrometer, which fixes the opposing pulp resistance to the penetration of a 1 cm2 plunger. The degree of emission of ethylene was determined using the ICA 56 ethylene analyzer.

The main results obtained were statistically processed by the dispersion analysis method after Б. Доспехов.

#### **RESULTS AND DISCUSSIONS**

Fruit production is one of the main indexes that are taken into account in determining the efficiency of one or another technological element applied in the superintensive culture system.
The investigations show that the number of fruits in the crown of the trees included in the research differs on the variants in the study (tab. 2). If, in the control variant, the number of fruit was 52 pcs/tree, then, in the variant treated with the growth regulator Humifield WG in dose of 0.2 kg/ha was 54 pcs/tree. When treating variants with the Nertus PlantaPeg growth regulator, the studied index consisted of 55 - 56 pieces/tree.

The difference between the control variant and the variant treated with Humifield WG in dose of 0.2 kg/ha was 2 pcs/tree, but between the third and fourth variants, which was treated with the Nertus PlantaPeg growth regulator, respectively 3 and 4 pieces/tree.

This increase in the number of fruits in the tree crown was due to improved metabolism of growth hormones, positive influence on inflorescence fertility, fertilization and fruit binding process following treatment with Humifield WG and Nertus PlantaPeg growth regulators during the pink button period and after flowering.

The average weight of a fruit on variants in the study underwent changes compared to the control variant. The lowest average fruit weight was recorded in the control variant, where the study index was 173.9 g. When using the Humifield WG growth regulator in dose of 0.2 kg/ha, the average weight of a fruit constituted 176.3 g, or an increase of 2.4 g compared to the control variant, without treatment.

Table 2

	Fruit	Average	Fruit pro	In %, compared	
Variants	quantity, buc/tree	weight, g	kg/tree	t/ha	to the witness
Control	52	173,9	9,04	32,28	100,0
Humifield, WG, 0,2 kg/ha	54	176,3	9,52	34,00	105,5
Nertus PlantaPeg, 0,6 l/ha	55	179,5	9,87	35,24	109,1
Nertus PlantaPeg, 1,0 l/ha	56	178,5	9,99	35,69	110,5
DL 5%	2,4	7,3	0,45	1,63	-

The influence of the growth regulator on the average quantity, average weight and fruit production of Idared apple trees

The use of the Nertus PlantaPeg growth regulator, which besides fulvic acids and salts of humic acids in its composition also has polyethylene glycols (PEG 400 and PEG 1500) has positively influenced the average fruit weight. In the third variant, treated with the Nertus PlantaPeg growth regulator, the average weight of a fruit was 179.5 g, or an increase of 5.6 g compared to the control variant and 3.2 g with the standard Humifield WG in the dose 0.2 kg/ha. The increase in the Nertus PlantaPeg growth regulator dose, the fourth variant, lowered the average weight of a fruit to 178.5 g or 0.6% compared to the third variant, but it recorded higher values compared to control variant by 2.6%.

This slight difference in the average weight in variants 3 and 4 is due to approximately the same number of fruits in the apple tree crown and maintenance of

the physiologically active balance following treatment with the Nertus PlantaPeg growth regulator. The results outlined above are also confirmed by statistical processing.

The fruit production per tree and on a surface unit correlates directly with the number of fruits in the tree crown and their average weight. The smallest fruit production was recorded in the control variant, constituting 9.04 kg/tree or 32.28 t/ha.

When treatment with the Humifield WG growth regulator was made, the fruit production was 9.52 kg / tree or 34.00 t/ha, or a 5.5% increase compared to the control variant without treatment.

The largest fruit production was recorded in the variants treated with the Nertus PlantaPeg growth regulator. If in the third variant the fruit production constituted 9.87 kg/tree, then in variant four the studied index increased to 9.99 kg/tree, or 1.2%. The recorded fruit production at a surface unit on the above mentioned variants constituted 35.24 and 35,69 t/ha respectively. Treatments with Nertus PlantaPeg growth regulator increased fruit production by 9.1-10.5% compared to control variant and 3.6-5.0% versus Humifield, WG.

The insignificant difference between the third and fourth variants treated with the Nertus PlantaPeg growth regulator is also provided by statistical data.

The results show that the Nertus PlantaPeg growth regulator used to prevent water stress, plant temperature oscillations and maintaining the physiological balance between growth and fructification in both doses has positively influenced the number of fruits in the tree crown, the average weight of a fruit and plant productivity.

It is very important that apples are harvested at the optimum time. The data of the investigations carried out (tab. 3) shows that the firmness of the apple pulp on the variants studied at the time of the harvest constituted 7.2-7.6 kg/cm<sup>2</sup>. The smallest firmness of the pulp was recorded in the control variant without treatment - 7.2 kg /  $cm^2$ .

When treating growth regulators, the pulp firmness increased. In the variant treated with Humifield, WG growth regulator, the firmness of the pulp increased and constituted 7.4 kg/cm<sup>2</sup>, or 0.2 kg/cm<sup>2</sup> higher compared to the control variant. In the variants treated with Nertus PlantaPeg, it was recorded, approximately the same values of pulp firmness, 7.6 kg/cm<sup>2</sup>, or an increase of 0.4 kg/cm<sup>2</sup> compared to the control variant and 0.2 kg/cm<sup>2</sup> versus the Humifield, WG variant. The increase in the dose did not affect the firmness of the pulp.

The dry substance content of the Idared variety, on variants in the study, is 15.4 - 15.8%. The smallest value of dry substance weight was recorded in the control variant - 15.4%.

The treatments with growth regulators increased the index in the study. In the variant treated with Humifield, WG, the amount of dried substance in the fruit constituted 15.7%, or an increase of 0.3% compared to the control variant.

When using the Nertus PlantaPeg growth regulator at 0.6 / 0.6 / 2.0 l/ha and 1.0 / 1.0 / 2.5 l/ha, the amount of dried substance in the fruits was 15, 8%. Treatments on both variants with the Nertus PlantaPeg growth regulator increase 0.4% of the dry substance content compared to the control variant and 0.1% versus the standard variant. Therefore, the standard treatments with the growth regulator have influenced the increase in the amount of dry substance in the fruit.

The content of soluble dry substance is a sort of variety, after which the optimum harvesting time can be determined. The investigations show that the quantity of soluble dry substances in the Idared variety on the variants in the study constituted 13.6 - 14.0%.

Table 2

Influence of the growth regulator on the firmness, biochemical indices and the amount of ethylene emanated from Idared apples

Variants	Firmeness, kg/cm <sup>2</sup>	Dry substance , %	Soluble dry substance, %	Titratable acidity,%	The amount of ethylene emitted, µl/kg/h
Control	7,2	15,4	13,6	0,50	0,013
Humifild, WG, 0,2 kg/ha	7,4	15,7	14,0	0,47	0,012
Nertus Planta Peg, 0,6 l/ha	7,6	15,8	13,9	0,46	0,003
Nertus Planta Peg, 1,0 l/ha	7,6	15,8	14,0	0,46	0,004

The smallest value of the weight of the soluble dry substances was recorded in the control variant without treatment - 13.6%. In the case of treatment with growth regulators, we record an increase in the study index to 13.9 - 14.0%. That is, when treated with the Humifield, WG standard growth regulator, the amount of fruit-soluble dried substance increases by 0.4% compared to the control variant.

Treatments with the Nertus PlantaPeg growth regulator on both variants increases by 0.3 - 0.4% compared to the control variant, and compared to the standard variant the same values were recorded. Therefore, treatments with growth regulators in the study have positively influenced the increase in the amount of soluble dry substance.

The share of titratable acids in fruits is in direct dependence on the amount of soluble dry substance. Concomitantly, with the decrease of the quantity of dry substances soluble in fruits the share of titratable acids increases, registering the highest value in the control variant - 0.50%.

When, the treatment was made with the Humifield, WG growth regulator, the amount of titratable acid in the fruit constituted 0.47%, and with Nertus PlantaPeg decreased, constituting 0.46%.

Ethylene is considered as the main fruit maturing hormone. This substance is synthesized in all plant organs and in larger quantities in apples.

The amount of ethylene emitted by fruit immediately after harvest differs on the variants in the study. The largest quantity of ethylene emitted was recorded in the control variant without treatment -  $0.013 \mu l/kg/h$ .

In the variant treated with the Humifield, WG growth regulator, the amount of ethylene emitted by the fruits is  $0.012 \mu l/kg/hr$ .

When using the tested growth regulator Nertus PlantaPeg, which has the ability to form a protective film on the fruit, the amount of ethylene emitted from fruits has diminished on the variants in the study up to  $0.003 - 0.004 \,\mu$ l/kg/hr.

This demonstrates that treatment with the Nertus PlantaPeg growth regulator inhibited the amount of ethylene emitted by fruit by 0.009-0.010  $\mu$ l/kg/hr compared to the control variant. Increasing the dose of Nertus PlantaPeg has insignificantly influenced the indicator in the study.

#### CONCLUSION

The Nertus PlantaPeg growth regulator can be included in the technology system to improve apple growth and fructification processes and reduce the etiquette release rate of fruit, applied 3 times by foliar spraying. The first treatment to be carried out in the "pink button" phase at a dose of 0.6 - 1.0 l/ha, II - in the binding phase - the intensive growth of fruits (fruit diameter 20-25 mm) at a dose of 0.6 - 1.0 l/ha, and III - 1-2 days before harvesting in the dose 2.0 - 2.5 l/ha.

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## POSSIBILITIES OF OPTIMIZATION OF THE COMPLEX FERTILIZATION IN CORNICHON CUCUMBERS CROP

Petcuci Andrei-Cătălin<sup>1</sup>, Atanasiu Nicolae<sup>1</sup>, Neață Gabriela<sup>1</sup>, Câmpeanu Gheorghe<sup>1</sup>, Cornea Călina Petruța<sup>1</sup>, Petcuci Alexandra<sup>2</sup>

<sup>1</sup> University of Agronomical Sciences and Veterinary Medicine Bucharest, Horticulture Department, Bucharest, Romania, <sup>2</sup>Dow AgroSciences Corespondence author: E-mail:alexandramaria costea@yahoo.com

Key words: cornichon cucumbers, fertigation, complex fertilization

#### ABSTRACT

Cucumber is a vegetable appreciated and wanted very much not only by consumers but also by the farmers. In the evolution of the cultivated area, cucumbers have gained the extension of the protected crop even at higher altitude, using the conditions of the appearance and extension of plastic-houses or cold or warm glass-houses. These culture systems are appreciated by the farmers due to short length of the crop cycles that occupy these constructions not more than 2.5-4 months in case of cornichon cucumbers. For obtaining the maximum of production and quality of the cornichon cucumbers crop, very important is to establish a fertirigation of a complex fertilization program that is efficient and optimum depending on the crop characteristics.

#### INTRODUCTION

Cucumbers are known and cultivated in southern and south-east Asia and northern Africa for more than 4000 years. Nowadays, due to the technological evolution, cucumbers are grown on large geographic areas between the Equator and the large latitudes of the Scandinavian countries (for example in Finland).

Resistance and / or tolerance to diseases is a technological asset of cucumbers that can reduce the number of phytosanitary treatments, while lowering the costs of these works, resulting in a production with fewer inputs and a higher profit.(Atanasiu, 1999, Popescu et al., 2002)

Elasticity of production is also remarkable for small fruit cucumbers: fruits of this species can be harvested when they reach either very small size (3-5 cm long) or large size (over 10-14 cm). From a crop of cornichon cucumbers, due to this technological trait, fruits intended for industrialization or fruit intended for fresh consumption can be harvested.

#### MATERIAL AND METHODS

The following variants were studied in order to develop the previously presented and argued experience.

Experience has been assembled on two sites:

- On the ground, in a cold plastic house, in Brezoaele, Dâmboviţa County

- On peat used as rootstock in an unheated greenhouse from the technical equipment of the sector of greenhouses District 4, ADP Bucharest.

The experience was located in a traditional tunnel-type plastic house, with a base width of 5.40 m and in a cold greenhouse. The plastic house, located in the Brezoaele area (Dâmboviţa County), is located on brown-red soil, with a moderate degree of favorability for the cultivation of cornichon cucumbers. In the cold greenhouse, District 4, Bucharest, the peat rooting substrate was used.

At the same time, analyzes were made regarding the quality of lettuce, nitrate content, phosphate nitrate and lettuce potassium. The methods used in the analyzes were: Griess method, spectrophotometric for nitrates, nitrites and Duval reagent and spectrophotometric phosphorus dosing, and for flame spectrophotometry. These methods are according to the standards in our country: ISO 9001.(Metodologia de analiza a solului si plantei, ICPA, 1987).

Table 1

Var. no.	Culture substrate	Variety
V <sub>1</sub> (mt)		Kybria F <sub>1</sub>
V <sub>2</sub>	On soil, in plastic house	Lenara F <sub>1</sub>
V <sub>3</sub>		Mirabelle F <sub>1</sub>
V <sub>4</sub> (mt2)		Kybria F <sub>1</sub>
V <sub>5</sub>	On peat, in cold glasshouse	Lenara F <sub>1</sub>
V <sub>6</sub>		Mirabelle F <sub>1</sub>

Experimental variants. Influence of the assortment and the culture substrate on the production of cornichon cucumbers in cold plastic houses in autumn culture

Basic fertilization was carried out in the autumn by the application of 3 kg of well fermented manure to square meters of land.

Cornichon cucumber culture was established at 80/40 cm distances, providing a density of  $3.15 \text{ plants/m}^2$  in the case of the plastic house, and at distances of 160 (between peat bags) / 40 cm, with 2 plants per the peat bag, which ensures the same density. This density has been established since the very good light conditions in the first part of the crop cycle favor both vegetative growth and fructification of cucumbers.

Phaseal fertilization during vegetation was applied to the soil, along with watering, using recommended doses of the Universol, richer in nitrogen before fruiting and richer in potassium during the fruiting period.

The plastic house was not equipped with water spray equipment to increase atmospheric humidity and maintain it inside the building. The consequence of this situation was unfavorable for culture, influencing negatively the level of production (Horgos, 1999).

#### **RESULTS AND DISCUSSIONS**

Additional fertilization has a special contribution. The first one is done 10-15 days after the rising, and the second one 15-20 days after the first.

The best soils for cucumber culture are fertile, loose, permeable soils. For early production, the most obvious are light soils. Heavy, clay and soils are unsuitable. Also avoid soils too wet or too dry and those that make the crust.

Cucumber culture soil should have a good structure; high content of organic matter, with groundwater at 80-90 cm. Ground water should not be over 70 cm. The soil reaction must be slightly acidic to neutral (pH 6.5-7).

Soil fertilization for cucumber cultivation is indispensable. The type and amount of fertilizer depends on the type of soil and its natural fertility. Cucumbers in general need a lot of organic matter. That is why large crops are obtained at a massive fertilization (40-60 tones / ha) with well-decomposed waste. Poorly decomposed garbage cannot be used by cucumbers unless it was given to the precursor crop. in this case, the fertilization is supplemented with 250-300 kg of superphosphate, 100-150 kg of ammonium nitrate and 150-200 kg of potassium salt. The need for nutrients is high in cucumbers and cannot be covered only by mineral fertilization.

- The cucumber requirements for nutrients are as follows:
- Medium requirement for phosphorus;







 High demand for potassium and nitrogen.
Figure 1 – *Traditional plastic house,* Brezoaele, Dâmboviţa county
Figure 2 – *Cold glasshouse,* District 4, Bucharest Cucumber culture cannot be practiced on the same field for several consecutive years or after other cucurbits, as soil fatigue occurs very rapidly and disease and pests are greatly multiplied. It is recommended not to return to the same place with a cucumber culture until after at least 4 years. It is recommended a place for cucumbers where last year crops have been planted that improve the soil structure, then those that leave free-weeds soils like onions, potatoes, tomatoes, late beans, then spinach and early salad.

To achieve the described experience, the following complex fertilizers were used:

Table 2

Some Chemical and Technological Particularities of Complex Fertilizers Universol Albastru and Universol Violet, Brezoaele, Dâmboviţa County, 2016 Composition %

No	Macroelements	Macroelements
NO	Universol albastru	Universol violet
1.	Total N – 18, from which	10, from which
	N-NO <sub>3</sub> – 10.0	7.2
	N-NH <sub>4</sub> – 7.7	3.0
2	Urea (when necessary) – 0.5	0.5 (when necessary)
3	$P_2O_5 - 11$	10
4	K <sub>2</sub> O – 18	30
5	MgO – 2.5	3.3
6	SO <sub>4</sub> - 8.3	15.5
7	CaO – 0.0	0.0
	Microelements	Microelements
	Universol Albastru	Universol violet
8	Bor – 0.01	0.01
9	Cu – 0.01	0.01
10	Fe – 0.10	0.10
11	Mg – 0.04	0.04
12	Mo – 0.001	0.001
13	Zn – 0.01	0.01
	E.C. la 21°C – 1.3	1.5
	Maximum solubility at 25°C – 350	300

Recommended dose rates: 10-15 g/m<sup>2</sup>/week

#### Table 3

### Nutrient content in differentiated nutrient solution on phenophases A - cornichon cucumber in cold greenhouses on peat substrate, autumn culture, Phenophase I - emergence - first harvest,

No	Nutrient	Concen- tration mg/L	Total Universol albastru, 1 g/L	KNO₃ 0.35 g/L	CaNO <sub>3</sub> 0.50 g/L	MgSO 4 0.25 g/L	Concen- tration mg/L
1	N (total)	180-225	180	0.35	80	-	305
2	К	225-300	180	160	-	-	340
3	Са	145-185	-	-	137	-	137
4	Mg	38-42	21	-	-	40	61
5	Р	40-50	110	-	-	-	110
6	SO <sub>4</sub>	40-60	-	-	-	80	80
7	Fe	0.06	0.06	-	-	-	0.06
8	В	0.01	0.01	-	-	-	0.01
9	Cu	0.01	0.01	-	-	-	0.01
10	Mn	0.04	0.04	-	-	-	0.04
11	Мо	0.001	0.001	-	-	-	0.001
12	Zn	0.001	0.001	-	-	-	0.001

complex fertilizer – Universol albastru

Table 4

Nutrient content in nutrient solution differentiated on phenophases - B - cornichon cucumber in cold greenhouses on peat substrate, autumn culture, Phenophase II - from first to last harvest, complex fertilizer – Universol violet

No.	Nutrients	Concen- tration mg/L	Total Universol violet, 1 g/L	KNO₃ 0.35 g/L	CaNO <sub>3</sub> 0.50 g/L	MgSO₄ 0.25 g/L	Conce n- tration mg/L
1	N (total)	180-225	90	45	50	-	185
2	К	225-300	270	160	-	-	430
3	Ca	145-185	-	-	137	-	137
4	Mg	38-42	33	-	-	40	73
5	Р	40-50	90	-	-	-	90
6	SO <sub>4</sub>	40-60	-	-	-	80	80
7	Fe	0.06	0.06	-	-	-	0.06
8	В	0.01	0.01	-	-	-	0.01
9	Cu	0.01	0.01	-	-	-	0.01
10	Mn	0.04	0.04	-	-	-	0.04
11	Мо	0.001	0.001	-	-	-	0.001
12	Zn	0.001	0.001	-	-	-	0.001

The nutrient solution plant has three pools:

Basin A - alkaline reaction

- Calcium nitrate - Ca - Ca (NO3) 2 - additional + urea + ammonium nitrate - max 15%

- Potassium nitrate - KNO3

- Fe chelated DTPA
- Basin B acidic reaction
- Potassium nitrate KNO3
- Potassium sulphate K2SO4
- Magnesium sulphate MgSO4
- Magnesium nitrate Mg (NO3) 2
- Monopotassium phosphate KH2PO4
- Ammonium nitrate NH4NO3
- Phosphoric acid H3PO4 or nitric acid
- Microelements manganese sulphate

- copper sulphate

-boron

-molybdate sodium

Basin C - nitric acid

In the Brezoaele experience, fertirigation and Universol Galben were used in the first vegetation phases at up to 10 g/m<sup>2</sup>/ week (3 g / plant) - this replaces classical fertilization with phosphorus chemical fertilizers.

Harvesting must be done when cucumbers are young. For pickled cucumbers are indicated fruits of 8-12 cm length. Harvesting is repeated at 2-3 days, because the fruit ages very quickly and loses its quality. You do not have to rotate or twist gobs because the plant suffers. If they are left unharvested and aged, the plants no longer bind fruit.

From 1000 m<sup>2</sup> of culture we can get 1000-2000 kg of early cucumbers, 2000-3000 kg of summer cucumbers and 1500-2000 kg of autumn cucumber.

Comparing the results obtained with the three hybrids cultivated on the soil, we can see that the best results were obtained at Kybria with a production of  $12.9 \text{ kg}/\text{m}^2$ 

The production at Lenara and Mirabelle came close to Kybria's production on the ground.

Comparing the results obtained with peat culture all Kybria has the highest production of 17.40kg / m<sup>2</sup>.

Comparing the experimental variants cultivated on the soil with the peat cultivars, it can be seen that all peat variants yielded distinct significant results at Mirabelle and very significant in Kybria and Lenara.

#### CONCLUSIONS

1. Comparing the results obtained with the three hybrids cultivated on the soil, we can see that the best results were obtained at Kybria with a production of 12.9 kg /  $m^2$ 

2. The production at Lenara and Mirabelle came close to Kybria's production on the ground.

Comparing the results obtained with peat culture all Kybria has the highest production of 17.40kg /  $m^2$ .

3.Comparing the experimental variants cultivated on the soil with the peat cultivars, it can be seen that all peat variants yielded distinct significant results at Mirabelle and very significant in Kybria and Lenara.

Table 5

Production per plant and per square meter

	ces	%	69.8	66.4	66.0	100.00	94.3	91.6
tes from	Differen	Kg/m <sup>2</sup> Signification	-5.31 00	-5.84 000	-5.92 ∞	1	*** 6'6+	+14.6 ***
Differenc	ces	%	100.00	95.60	94.90	143.9	135.70	131.80
	Differen	Kg/m <sup>2</sup> Signification		-0.53 ns	-0.61 ns	+5.31 ***	+4.32 ***	+3.85 **
	Production - Kg/m <sup>2</sup>		12.09	11.56	11.48	17.40	16.41	15.94
	Production Kg /plant		3.87	3.70	3.68	5.58	5.26	5.11
	o/ fruit	2	56.4	57.1	52.3	61.1	59.2	56.4
	No of fruite	plant	68.7	64.8	70.4	91.3	88.9	90.6
	Hybrids		Kybria F1	Lenara F1	Mirabelle F1	Kybria F1	Lenara F1	Mirabelle F1
	Crop technology		On soil in plastichouse	crop		On peat in cold	glassnouse	
	Variant			V2	٨3	V4 (mť2)	V5	90

DL 5%= 1.29kg/m<sup>2</sup> DL 1%= 2.37kg/m<sup>2</sup> DL 0,1%= 4.05kg/m<sup>2</sup>

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# INFLUENCE OF SEVERAL TECHNOLOGICAL LINKS APPLIED IN THE ECOLOGICAL SYSTEM ON THE QUALITY OF GRAPES PRODUCTION

Pîrcălabu Liliana<sup>1</sup>\*, Tudor Georgeta, Ion Marian

<sup>1</sup><sup>\*</sup>Research Institute for Viticulture and Enology Valea Călugarească \* Correspondence author. E-mail: lilianapircalabu@yahoo.com

Key words: soil maintenance systems, black furrow, total mulching

#### ABSTRACT

The researches were carried out in two vineyards grown with the Feteasca neagra and Cabernet Sauvignon varieties, with two experimental factors: the soil maintenance system (black furrow, total mulching with straws, partial mulching with marc compost, permanent herbage with grasses spontaneous flora) and the system for the control of pests and diseases (ecologically and conventional). In the Feteasca neagra variety, the highest yields of 3.83 kg/vine (ecological control system) were obtained in the case of soil maintenance by mulching with marc compost compared with 4.23 kg/vine (conventional control system). In terms of total mulching with straws the production was 3.76 kg/vine (ecological control system), compared to 4.08 kg/vine (conventional control system) compared to 3.89 kg/vine (conventional control system).

#### INTRODUCTION

In accordance with the concept of organic viticulture allocation technological inputs should be performed at a level that will produce reasonable yields, while ensuring adequate protection of the ecosystem vineyard.

The viticultural ecosystem has evolved over time to an anthropic ecosystem where the anthropic factor has gained a more and more obvious regulatory function. In these conditions, the functioning of the viticultural ecosystem and implicitly its productive capacity can be negatively affected by the anthropic factors, represented by the applied crop technologies (Dejeu and Matei, 1996, Heidi Resenthal Duminy, 2004)).

Different soil maintenance systems (mechanically, manually, biologically) and to control vines and pests (ecologically, conventionally), each with their advantages and disadvantages, generates in time differentiated effects on the soil, the plant and the environment, in the production of grapes obtained and in their quality and sanogenity.

#### MATERIAL AND METHODS

The researches were carried out in two vineyards grown with the Feteasca neagra and Cabernet Sauvignon varieties, with planting distances of  $2 \times 1$  m and the bilateral Cordon form. The cutting system practiced is mixed in two-three eyes taps and a cutting load of 10-12 eyes per vine, with 12-15 eyes/square meter loadings.

The soil in the experimental polygon is eumezobazic brown, with a sandy loam texture, pH weak acid (6.1), well supplied with humus (2.9%) and useful mineral elements (N, P, K).

The device included two experimental factors: the soil maintenance system (black furrow, total mulching with straws, partial mulching with marc compost, permanent herbage with grasses spontaneous flora) and the system for the control of pests and diseases ecologically (E) and conventionally (C).

#### **RESULTS AND DISCUSSIONS**

The experimental results obtained showed that under the ecoclimatic conditions specific to 2016 year, characterized by a high heliothermic regime of rich water resources, the experimental technological variants had a different impact on the quality of the grape production.

Compared to normal (1985-2015), the average monthly air temperature varied between + 3.0°C (April), + 0.6°C (Jun), and -1.9°C (May).

The vegetation period (April) started with average temperatures higher than normal (14.7°C to 11.7°C) and a high water regime (65 l/square meter to 44.8 l/square meter) multiannual (Table 1).

Table 1

Month	Monthly a temper	thly average Maximum M mperature absolute a		Minimum absolute
	Normal	2016	temperature	temperature
April	11.7	14.7	29.3	3.6
May	17.5	15.6	29.2	6.1
June	21.5	22.1	33.8	10.5
July	23.6	23.7	33.4	14.4
August	23.3	22.7	35.0	12.2
September	18.1	19.0	32.1	6.5
October	12.2	12.1	25.4	5.4

#### The thermal regime during April-October

In 2016, the air hygroscopicity was higher by 6.6% in May, 2.2% in June, 5% in October and lower hygroscopicity by 0.7% in April, 1.1% in August, 5.6% in September and the amount of monthly rainfall recorded to the normal (1985-2015) positive differences 20.2 mm (April) 14.9 mm (May), 15.5 mm (August) and 11.6 mm (September) and negative difference of -5.6 mm (July), (Table 2).

Table 2

#### The pluviometric regime during April-October

Month	Rainf	Number of days with rainfall	
	Normal	2016	above 10 mm
April	44.8	65.0	2
May	67.3	82.2	2
June	81.5	81.8	5
July	75.8	70.2	2

August	62.7	78.2	4
September	54.4	66.0	3
October	46.2	120.0	4

Thus, with regard to the control systems under study, the use of conventional pest and disease pest control has ensured the highest yields. The average weight of a grape was varied with oscillations between 93.65 g (conventional control system-C) and 94.43 g (ecological control system-E) the Feteasca neagra and Cabernet Sauvignon range between 93.57 g (conventional control system-C) and 84.75 g (ecological control system-E), (Table 3).

Table 3

# Production of grapes obtained in 2016 year

Variety	kg/vine		The average weight of a grape g		Weight of 100 berry g	
	Е	С	Е	С	Е	С
Feteasca neagra	3.59	4.23	94.43	93.65	161.6	173.6
Cabernet Sauvignon	3.04	3.14	84.75	93.57	135.2	140.1

Regarding the influence of experienced technological factors on the quality of grape production, expressed by the sugar content and the acidity of the must, it is found that the use of the conventional control system ensured the highest concentrations of sugars in grapes. The sugar accumulation potential in the must, a variety characteristic influenced by climatic factors during the grape maturation period, was variable, with values between 194.6 g/L (conventional control system) and 190.8 g/L) and the Cabernet Sauvignon variety with limits of 206.3 g/L (conventional control system) and 187.8 g/l (ecological control system), (Table 4).

Table 4

Quality wine harvest obtained in 2016

Variety	Sugars g/L		Total acidity g/L H₂SO₄	
	E	С	Е	С
Feteasca neagra	191	195	8.6	8.6
Cabernet Sauvignon	188	206	4.9	4.6

With regard to soil maintenance systems, the effects were differentiated, depending on the specific conditions, the highest yields were obtained in the case of soil maintenance by mulching with marc compost 3.83 kg/vine(ecologic control system) and 4.23 kg/vine (conventional control system) in the Feteasca neagra variety, total mulching with straws 3.76 kg/vine(ecologic control system) and 4.08 kg/vine (conventional control system), black furrow 3.71 kg/vine(ecological control system) and 3.89 kg/vine (conventional control system), while the lowest yield was obtained in the case of maintenance of soil by permanent herbage 3.59 kg/vine (ecological control system) and 3.71 kg/vine (conventional control system), (Table 5).

#### Table 5

The influence of experienced technological factors on the production of grapes and its qualities

a) Feteasca ne	eagra		-					
Graduation	kg/vine		The average weight of a grape (g)		Sugars g/L		Total acidity g/L H <sub>2</sub> SO <sub>4</sub>	
	Е	С	Е	С	Е	С	Е	С
Black furrow	3.71	3.89	95.7	94.6	197.6	198.5	4.7	4.7
Total mulching with straws	3.76	4.08	91.2	92.0	191.4	196.3	4.8	4.9
Partial mulching with marc compost	3.83	4.23	90.1	95.6	187.8	186.2	4.9	4.6
Permanent herbage	3.59	3.71	94.4	92.0	190.9	199.8	4.8	4.7

b) Cabernet Sauvignon

Graduation	kg/vine		The average weight of a grape (g)		Sugars g/L		Total acidity g/L H <sub>2</sub> SO <sub>4</sub>	
	Е	С	E	C	E	С	E	С
Black furrow	3.00	3.07	90.5	86.9	196.8	195.4	8.4	8.4
Total mulching with straws	3.05	3.08	86.9	91.9	192.1	190.6	8.5	8.6
Partial mulching with marc compost	3.06	3.14	89.5	93.6	191.4	189.2	8.5	8.8
Permanent herbage	2.88	3.04	86.8	84.7	198.6	195.5	8.4	8.5

The highest grape sugar contents were obtained when the soil is maintained by permanent herbage 190.9 g/L (ecological control system) and 199.8 g/L (conventional control system) and the black furrow 197.6 g/l (organic control system) and 198.5 g/l (conventional control system).

Regarding the influence of experimental technological factors on the degree of attack of the main cryptogamic diseases: Plasmopara viticola, Uncinula necator and Botrytis cinerea (Table 6), it was found that due to climatic conditions specific to the 2016 year (abundant rain and high hygroscopicity) showed the degree of attack on the grape, with values ranging between 3.35% for the ecological control system and 2.87% for the conventional control system at Feteasca neagra, and for the Cabernet Sauvignon the variations were between 4.05% for the ecological control system and 2.98 % at the conventional control system.

The attack of Uncinula necator on grapes was 3.93% for the ecological control system and 2.63% for the conventional control system for the Feteasca neagra variety and between 2.26% for the ecological control system and 1.82 for the conventional control system for the Cabernet Sauvignon variety. As for the Botrytis cinerea, it had an attack rate of 1.97% (Feteasca neagra) and 2.26% (Cabernet Sauvignon) in the

ecological control system, and in the case of conventional control system the attack rate was 1.27% (Feteasca neagra) and 1.82% (Cabernet Sauvignon).

Table 6

Variety	Plasmo vitico	opara ola	Uncir neca	nula ator	Botrytis cinerea		
	Е	С	Е	С	Е	С	
Feteasca neagra	3.35	2.87	3.93	2.63	1.97	1.27	
Cabernet Sauvignon	4.05	2.98	4.51	2.13	2.26	1.82	

The degree of attack of the main diseases under the conditions of 2016

Analyzing the sensory profile of the obtained wines it is found that the experimental technological links influenced differently the 14 characteristics that make up the sensory profile. In the case of Feteasca neagra, the best wine quality indices were obtained in the conditions of soil maintenance as a black furrow and by total mulching with straws (Figure 1).



Figure 1. The influence of the soil maintenance system on the technological typicality of the Feteasca neagra wines

In the case of the Cabernet Sauvignon variety, the best wine quality indices were obtained under the conditions of soil maintenance as a black furrow and by total mulching with straws (Figure 2).

In the case of Feteasca neagra and Cabernet Sauvignon wine, the best wine quality indices were obtained under the conditions of soil maintenance as a black furrow and by total mulching with straws and the ecological control system.





#### CONCLUSIONS

With regard to the soil maintenance system, the highest sugar content in grapes was obtained in the case of soil maintenance by permanent herbage and black furrow.

The experimental data show that the permanent herbage exerts a negative influence on the production of the vines, through the competition of the perennial herbs in the use of water and mineral substances on the vine.

The use of the conventional control system has ensured the highest concentrations of sugars in the grapes.

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## NOROCEL - A NEW SEEDLESS GRAPE VARIETY CREATED IN ROMANIA

Popa Camelia<sup>1</sup>, Cichi Daniela Doloris<sup>2\*</sup>, Ciobotea Cristina Magdalena<sup>1</sup>

<sup>1</sup> National Research and Development Institute for Biotechnology in Horticulture Stefanesti-Arges, Bucharest Street - Pitesti, No.37,Stefanesti, Romania <sup>2\*</sup> University of Craiova, Faculty of Horticulture, Department of Horticulture & Food Science, Craiova, Romania \* Correspondence author. E-mail: danielacichi@yahoo.com

Key words: seedless grapes, agrobiological descriptors

#### ABSTRACT

The present paper presents the results of the observations and determinations regarding the evaluation of the fertility and productivity potential of a new grapevine variety, Norocel, obtained at I.N.C.D.B.H. Stefanesti (Romania). Under the specific conditions of the study years and the area investigated, the new variety Norocel proves superior to the Perlette variety in terms of resistance to frost under some conditions of critical temperatures of – 17.5 °C, potential fertility and productivity of shoots, the average weight of a bunch, the production per plant and qualitative potential.

#### INTRODUCTION

The ever-increasing of growing grape consumer requirements makes it necessary to intensify research into the improvement of grapevine, with a focus on the creating new *Vinifera* genotypes, with superior quality and productivity characteristics with high ecological plasticity, high tolerance to diseases, pests, high resistance to stress factors (Cichi Daniela Doloris et al. 2013, 2014; Ciobotea Cristina et al.2014; Popa Camelia et al.2009; Rotaru Liliana et al.2008).

At present, the Romanian market is experiencing a shortage of seedless grape varieties, but also with the existence of a limited number of varieties with extra season and early maturation, but especially of those with complex biological resistance (Damian Doina et al. 2006; Necula Cezarina et al.2014). In this context, at National Research and Development Institute for Biotechnology in Horticulture Stefanesti - Arges (I.N.C.D.B.H., Romania) was created a new variety of seedless grapevine, *Norocel*, registered in the *Official Catalog of the Crop Plants Varieties of Romania* and proposed for protection by patent in 2017.

#### MATERIAL AND METHODS

The researches were carried out between 2015 - 2016 in the experimental fields of National Research and Development Institute for Biotechnology in Horticulture Stefanesti - Arges (N.R.D.I.B.H., Romania), vineyard Stefanesti Arges, Muntenia and Oltenia Hills. The climate favourability assessment was carried out as a synthesis of several climatic factors with an impact on the bioproductive behaviour and quality of the grapevine.

Seedless variety *Norocel* (Figure1) was obtained by conducted hybridization of varieties (*Augusta x Askari*) x *Black Pearl*, by Camelia Popa and Gheorghe Smaranda at N. I. R. D. B. H. Stefanesti - Arges being enrolled in the *Official Catalog of the Crop Plants Varieties of Romania* in 2017 and proposed for protection by patent in 2017.

To assess the bio productive and qualitative potential of the new seedless grape variety Norocel and the Perlette variety - as the witness variety, a series of agrobiological and technological descriptors were determined in accordance with the O.I.V. (2002) methodology and I.C.V.V (1988).

To this end, observations and determinations were made on the following agrobiological descriptors: proportion of fertile shoots (%), the absolute (c.f.a) and relative fertility (c.f.r) coefficients, relative (l.p.r.) and absolute productivity (l.p.r) index. The observations and determinations of the technological descriptors was concerned: single bunch weight (g), production (kg/vine), sugar content of must (g/L) and total acidity of must (g/L  $H_2SO_4$ ).



Figure 1. Bunches of Norocel seedless table grapes cultivar

#### **RESULTS AND DISCUSSIONS**

As for the climatic resources in the viticultural experimental centre under study, during the related period, they were analysed vs the requirements for the table grape and seedless varieties. The main viticultural climatic characteristics during the period of 2015 and 2016 years are shown in Table 1.

The percentage of fertile shoots, as indicator of the real fructification potential of varieties in a certain viticultural area, offers information on the specific genetic potential of varieties, as well as on the good use of pedoclimatic resources and on the value of the cultivation system, mostly depending on the photo-periodicity characteristics, as well as on the nutrient reserves accumulation (Cichi Daniela et al. 2011).

The comparative study of the percentage of fertile shoots to the analysed varieties reveals a number of differences between them. Thus, under the same agropedoclimatic conditions of Stefanesti Arges vineyard, the Norocel variety (68%) is characterized by superior fertility per plant, compared to seedless Perlette variety (62%), due in particular to the viability of buds, in a higher percentage for the Norocel variety (96%) compared to 31% as recorded to the Perlette variety under conditions of absolute minimum temperatures of -17.5 ° C recorded in 2016 (Figure 2).

Table 1

The climate characteristics during the vegetation period of years 2015 and 2016

Vitioultural alimatic Indiaca	Year			
	2015	2016		
Average annual temperature (°C)	12.5	12.00		
Winkler thermal index (IW)- useful balance sheet	1825	1806		
Annual precipitations ( $\sum P mm$ )	804	797.4		
$\sum P$ (mm) during active vegetation period	478.6	519.6		



Figure 2. Buds viability and proportion of fertile shoots

Fertility expressed by the values of fertility coefficients (c.f.r and c.f.a.) highlights obvious differences both between varieties and between vineyards years (Figure 3). The average values of the relative fertility coefficient were between 0.62 (Perlette) and 0.9 (Norocel), and those of the fertility coefficient absolute between 1.1 (Perlette) and 1.52 for the Norocel variety. We note the high fertility potential of the Norocel variety as compared to that of the witness variety Perlette.

The productivity potential of the shoots to the studied varieties was expressed by relative productivity indices (I.p.r) and absolute (I.p.a.).



Figure 3. Absolute (c.f.a) and relative fertility (c.f.r) coefficients



Figure 4. Relative (I.p.r.) and absolute productivity index (I.p.a.)



Figure 5. Productive and qualitative characteristics

The relative productivity index, indicating how much a shoot produces in average has, besides the value significance of the cultivation conditions, also the function to determinate the load of buds per surface unit or per stock in order to determine the planned production (Cichi Daniela et al. 2011).

The relative productivity index was determined (I.p.r. = the average weight of a bunch x c.f.r.) and the absolute productivity index was also calculated (I.p.a. = the average weight of a grape x c.f.a.).

Very good results in terms of productivity have been recorded by the Norocel variety (Figure 4), with average values of productivity indices between 479 (2016) and 559 (2015), for the absolute productivity index (I.p.a) and between 322 and 363 for the relative productivity index (I.p.r.).

The evolution of climatic factors during the grape maturity has a decisive influence on the process of grape maturation and finally on the quality of the wine-produced wine production.

Depending on the climatic supply characteristic of the wine years 2015-2016, the full maturation of the Norocel variety was achieved from 28.08-01.09 with 4-5 days later than the Perlette variety.

Regarding the qualitative potential of the two seedless genotypes, it was found that the highest sugar accumulation potential (Figure 5) was found in the Norocel variety (140 g / L and 169 g / L must) in the context of in the context of a total acidity of 3.79-3.89 g / I of H2 SO4.



Figure 6. Average production per vine

We appreciate that although the two genotypes reach full maturation approximately in the same calendar period, the Norocel variety has a high potential for accumulation in sugars, which ultimately results in a balance of the glucose-acidic index and the taste qualities of this cultivar.

The Norocel cultivar is also distinguished by superior production / plant compared to the Perlette variety (figure 6), the average production being 5.3 kg / plant in 2015 and 8.9 kg / plant in 2016.

This is also explained by the higher average weight of the berry (3.4 - 3.8 g) and the bunch (370 - 431 g) recorded to the Norocel variety compared to the seedless Perlette variety (Figure 5).

#### CONCLUSIONS

Under the specific conditions of the study years and of the investigated area, the new Norocel grape variety is superior to the Perlette variety in terms of resistance to frost under critical temperatures of - 17,5 °C, the potential fertility and productivity of the shoots, the average weight of a bunch, production/plant and qualitative potential (sugar g/L).

In this context, the new seedless - Norocel grape variety is recommended to be introduced into culture in very favourable and favourable areas for table grape varieties in the south of the country, in order to evaluate its behaviour and its expansion into culture.

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Environmental engineering

### RESEARCH REGARDING THE EFFECT OF GROWTH REGULATORS OVER THE IN VITRO MORPHOGENETIC REACTION AT SALVIA OFFICINALIS

Radomir Ana-Maria<sup>1\*</sup>, Ciobotea Cristina-Magdalena<sup>1</sup>

<sup>1</sup>National Research and Development Institute for Biotechnology in Horticulture, Stefanesti, \* Correspondence author. E-mail: radomir.anamaria@yahoo.com

Key words: Salvia officinalis, "in vitro" culture, explants, microshoots, growth regulators

#### ABSTRACT

The goal of this work was to develop a protocol for obtaining a high regeneration rate by testing the response of different explants of Salvia officinalis species on different culture media. Results showed that explants of apexes stems represent optimal source of inoculums. Research in the evaluation of morphogenetic capacity of explants on different nutrient regeneration formulas have shown that the most effective hormonal balance in the generation of shoots was 3 mg/l BAP / 1 mg/l ANA, in which case the rate of multiplication was 5,25 microshoots/explant. Shoots were multiplied by subculture on the same medium. The highest percentage of rooting (94.2%) was registered in the case of supplementing the nutrient medium with NAA in a concentration of 0.6 mg/l. The rooted plantlets were acclimatized and successfully fortified in pots registering a survival rate of 87%.

#### INTRODUCTION

Salvia officinalis (garden sage) is a perennial subshrub from the family *Lamiaceae*. This plant originates from the Mediterranean region and is widely used in pharmaceutical and food industries as well as in cosmetics (Khan et al., 2010; Garcia et al., 2013). The leaf extract of the plant possesses antibacterial, antifungal, antiviral, anti-inflammatory, antitumor and hypoglycemic activity (Longaray Delamare et al., 2007; Keshavarz et al., 2011). The pharmacology action of sage is a result of the presence of essential oil, di- and triterpenes, phenolic acids, flavonoids and tannins (Azimova and Glushenkova, 2012; Kontogianni et al., 2013). There are several studies concerning micropropagation of *Salvia officinalis* using different explants: shoot tips (Grzegorczyk and Wysokinska, 2008), nodal segments (Gostin, 2008), axillary and apical buds (Wielgus et al., 2011). However, some of the serious limitations in the above mentioned protocols were low propagation frequency, inconsistent and less number of shoots and occurrence of hyperhydricity.

The aim of this study was to develop an efficient method for micropropagation of *Salvia officinalis*.

#### MATERIALS AND METHODS

The biological material derived from *Salvia officinalis* species has been multiplied *in vitro* to yield neoplantlets regenerated by tissue culture.

Explants used to initiate tissue culture was represented by nodes and apexes stems. The defoliated shoots were first washed in tap water and were sterilized in 6% HgCl<sub>2</sub> for 10 minutes, after which 3 rinses were performed in sterile distilled water.

After sterilization of plant material, the explants were sampled and inoculated on MS culture medium without growth regulators. As a carbon source was used dextrose (40 g/l) and as a source of iron was used NaFeEDTA (32 g/l). For solidification of the culture medium was used agar (7 g/l).

The culture media were sterilized by autoclaving at 120°C temperature for 20 minutes. Before autoclaving, the pH registered in a culture medium was adjusted to 5.6-5.8.

The reactivity of explants was evaluated after a month of culture.

In order to culture proliferation, the explants by a node taken from microshoots obtained in the initiation cultures phase were inoculated on MS culture medium supplemented with various concentrations and combinations of BAP, K and ANA: 3 mg/l BAP / 0,5 mg/l ANA (V.1.), 2 mg/l BAP (V.2.), 1 mg/l BAP (V.3.), 1 mg/l K / 0,1 mg/l ANA (V.4.), 3 mg/l BAP / 1,0 mg/l ANA (V.5.). The cultures were transferred each 4 weeks on fresh media.

The differentiated microshoots, with a length of 3-4 cm, were detached from the culture, individualized and cultivated on rooting medium, represented by a variation of the MS basal medium with mineral salts reduced to half in which the auxin type varied (ANA - V.1., AIA - V.2., IBA - V.3.) and was kept constant its concentration (0.6 mg / I).

For growth, multiplication and rooting of explants have ensured in the growing room controlled conditions of temperature (22-24°C), photoperiod (16 hours) and light intensity (2 500 lx).

The *in vitro* rooted plants were transferred *ex vitro* in order to be acclimatization at their natural environment.

For the regeneration stage of the cultures, the monitored parameter was the multiplication rate calculated as the number of differentiated shoots on the explant. The percentage of rooting the shoots *in vitro* was estimated as the ratio of the number of shoots to which the rhizogenesis induction process occurred and the total number of shoots placed on the rooting substrate. The acclimatization percentage of *in vitro* regenerated plant was calculated as the ratio between the number of acclimatized plants viable and total number of *in vitro* regenerated plants.

#### **RESULTS AND DISCUTIONS**

Research on *in vitro* multiplication of the species *Salvia officinalis* have showed the complexity of the process and revealed the influence of biotic (type of explant) and abiotic (nutritive substrate) factors on the different phases of initiation, regeneration, rooting and acclimatization of regenerated plants. To establish a reproductible protocol for *in vitro* multiplication was necessary the selection of explant and of optimal culture medium to induce a higher number of shoots.

The observations made during the initiation stage of *in vitro* culture revealed that the inclusion of sage in this culture system does not pose particular problems, the use of calcium hypochlorite (a 6% solution for 10 minutes) for the sterilization of the explants proved to be efficient. The use of basal medium MS without growth hormones favored the process of shoots development and the production of neoplantlets which were then used to test the morphogenetic reaction of sage explants on different hormonal formulations of the MS medium (Figure 1).

In comparative analyzes aimed morphogenetic competence of explants from different origins (nodes and apexes stems), the results obtained showed that both types of explants analyzed have the ability to regenerate shoots under *in vitro* 

conditions, but the highest morphogenetic potential was observed in the case of apexes stems (Figure 2).



Figure 1. Minishoots inoculated on MS medium without growth regulators



Figure 2. The morphogenetic response of the apexes stems explants, after 4 weeks from initiation of *in vitro* culture

In order to proliferate the culture, the explants of a node taken from the microshoots obtained in the initiation phase of the cultures were inoculated on the MS culture medium supplemented with different concentrations and combinations of phytohormones.

The results registered in shoots regeneration experiments at sage reflected the important role that it has hormonal balance in overall of the factors that determine the expression of regenerative potential in the *in vitro* culture of the explants, a conclusive evidence in this regard being different effects of the same basal medium supplemented with various combinations and concentrations of growth hormones.

From the five variants of culture medium tested, two (V1 and V5) led to a better morphogenetic response, two (V2 and V3) allowed induction of the regenerative processes but to a lesser extent, and one (V4) did not stimulate the production of shoots. It is well known that directing of the morphogenetic processes to the development of shoots directly from the explant can be accomplished by supplementing the basic nutrient media with different types of cytokinins. In our experiments, the research was performed on three of the most important cytokinins, namely BAP, NAA and K. The results obtained showed that among the three types of cytokines used, BAP was the most effective in the shoot regeneration. Each of the four variants of nutrient media which allowed the induction of regenerative processes was characterized by the presence of BAP (V2 and V3) or BAP in combination with ANA (V1 and V5). The most appropriate hormonal balance for this purpose was found to be 3 mg / I BAP / 1 mg / I ANA (V5), in which case the value of the multiplication rate was 5.25 minishoots / explant. Replacement of BAP with K (V4) did not stimulate shoots regeneration, the multiplication rate being 0 (Figure 3).

After approximately 30 days, the shoots obtained were transferred to fresh medium that supported the regenerative processes by determining a good proliferation of shoots (Figure 4).



Figure 3. The influence of growth hormone combination and concentration on *in vitro* multiplication rate to the species Salvia officinalis



Figure 4. Biological material multiplied in vitro

From the qualitative point of view, the biological material resulting from the regeneration of explants had a normal morphology, without vitrification aspects, necrosis or callus differentiation.

Differentiated microshoots were detached from the culture, individualized and cultured on rooting medium. Knowing the beneficial effect of auxins on the efficiency of the rhizogenesis process, three variants of the rooting medium were tested in which the auxin type (ANA, AIA, IBA) varied, and its concentration was maintained constant (0.6 mg /l). From the three variants tested, the highest rate of rooting (94.2%) was registered in the V1 variant, which is characterized by the presence of the auxin NAA at a concentration of 0.6 mg / I. Variants V2 and V3, although lead to the rooting of the shoots in a fairly high percentage - 88.5%, respectively 72.4%, failed to reach a value of 94.2% (rate obtained for V1 variant) (Figures 5 and 6).







Figure 6. Rooting of Salvia officinalis microshoots on ½ MS supplemented with 0.6 mg / I ANA

In vitro rooted plants were transferred *ex vitro* in order to accommodate to septic life conditions. Their acclimatization has been successful, plants having a well developed radicular system, capable of further supporting growth and development process.

The best results with regard to acclimatization of *Salvia officinalis* plants has been obtained when have been used pills of peat type Jiffy (87% acclimated plants). When using the mixture of peat, perlite and manure in proportion of 2: 1: 1, the percentage of acclimatized plants was lower (75%), this substrate however ensures a good fortification of acclimated plants (Figure 7).



Figure 7. Acclimatization of *in vitro* regenerated plants: a) peat, manure, perlite mixture; b) pills of peat

After the acclimatization and fortification at pots, the plants were transplanted to greenhouse for further their growth and development (Figure 8). Mention the fact that plants obtained by *in vitro* propagation have preserved morphological characteristics of donor plant.



Figure 8. Salvia officinalis plants fortified in pots (a) and on soil (b)

We conclude that results obtained throughout *in vitro* regeneration phases, confirming preliminary studies and certify that for *Salvia officinalis* species, cloning by tissue culture is viable. Micropropagation technique represents an effective alternative to propagating for species under study, in order to create a permanent source to extract of some secondary metabolites for pharmaceutical industry.

## CONCLUSIONS

> The results obtained with regard to the morphogenetic competence of explants from different origins (nodes and apexes stems) revealed that the apexes stems have the highest morphogenetic potential.

> Of the three types of cytokinins used, the most effective in the production of shoots was BAP. Replacing BAP with K did not stimulate shoot regeneration.

> The highest percentage of rooting (94.2%) was recorded in the case of supplementation of the nutritional medium with ANA at a concentration of 0.6 mg/l.

 $\succ$  The yield on acclimatization was influenced by nutritive support used. The best results were obtained by using peat pills as substrate nutrient which ensured a

percentage of 87% acclimatized plants. We recommend the mixture of peat, manure and perlite in a proportion of 2:1:1 for the fortification of vitroplants in pots.

> On the basis of the results obtained was developed an original, reproducible and efficient *in vitro* multiplication protocol to the sage.

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#### RESEARCH ON DEPENDENCE OF GRAPE PRODUCTION OF TEMPERATURE IN THE REST PERIOD AT SOME WHITE WINE GRAPES IN THE SANDY SOILS FROM SOUTH-WESTERN OLTENIA

Iulian Rățoi<sup>1\*</sup>

<sup>1</sup>Research – Development Center for Plant on Sands Dabuleni, Victoria Street, Dabuleni. Correspondence author: e-mail: iulianratoi@yahoo.com

Key words: vine, negative temperature, abundant rainfall

#### ABSTRACT

Determinations were performed over the 2012-2016 period, on a plantation with the classic form of leadership (low). The state of vegetation and vine production is located permanently subject to weather conditions. In the favorable climatic conditions (2013 year), all varieties have been expressed at the level of genetic potential and of the degree of adaptability. The highest level of production was achieved at the noble variety Selena with 35.9 t/ha, followed by the resistant variety Brumăriu (34.8 t/ha). Analysis of grapes average production, conducted in the first 4 years of fruiting, highlights the special ability of fruits variety with ,,blood of hybrid" Brumăriu, 18 t/ha, a superior production of noble varieties Selena and Blasius which achived a grapes average production of 13.9 and respectively 13.8 t/ha.

#### INTRODUCTION

Climatic factors, together with a edaphic factors, are limiting factors for vines. Climatic factors as well as harmful minimum temperatures from winter and spring frosts, sometimes, determines the utmost grape production (Ţârdea C., Dejeu L., 1995), even when the taught elements of fruiting, arranged at the base of the vine, are protected by earth, because and the practiced cultural measures are extremely important in minimizing the damage caused by harmful minimum temperatures at vine (Robert G.E., 2000). In some years the damage can be total when intracellular ice crystals are formed, affecting the main buds and wood annual and multiannual even. The risk of freezing at the base of the vine is greater than to height of 100 cm, it being reduced by up to 0.36°C every 10 cm above the ground level (Sugar, D. R. et. al., 2003).

In current circumstances, when they manifest changes or climate disruption is necessary to improve the existing product range, with varieties showing superior quantitative and qualitative traits, of the current range, and adapt to sandy soil from south-western area (Vlădoianu Em., 1979; Vlădoianu Em., 1981; Anca Onache et al., 2008).

#### MATERIAL AND METHODS

The experience was founded in 2010. In 2012 year the plantation was found in the third year after planting, and in 2013 year was the first year of fruiting.

Were plannted the varieties following: Columna, Donaris, Fetească albă 1 Od., Fetească regală 21 Bl, Pinot gris, Riesling italian, Riesling de Rhin, Neuburger, Chasselas d'ore, Muscat Ottonel, Sauvignon blanc, Alb aromat, Grasă de Cotnari, Brumăriu, Blasius, Sauvignon, Selena. Planting density is 3787 vines/hectare, density which results from planting distances of 2.2 / 1.2 m.

During 2012-2016 period, the climatic conditions were unfavorable for vine in winter of 2012, 2015, 2016 years, and during the vegetation period in 2014 year due to abundant rainfall (640.7 mm in vegetative period) spreaded over a number of days, 74 of 183 total (48.5%), but and of phenomenon of dew shown daily throughout the year (table 1). In the 3 years previously named temperatures recorded were below the limit of resistance for buds and other organs of the vine, -24.3°C in 2012 year, -25.1°C in 2015 year and -23.4°C in 2016 year.

At these temperatures were left only one part of the buds whith were placed in the snow, the thickness of which was 25-30 cm. In the five years in which conducted the study, only one was normal, in terms of climate conditions for vine, 2013 year, when the absolute minimum temperature of -18.1°C, was not affected the viability of main buds.

In 2014 year, allthouth the vine did not suffer due to low temperatures, grape production was affected by the large amount of precipitation, 994 mm annual, over the average multiannual of rainfall.

In years when vines were affected by frost, cutting short fruiting was performed in short elements, of 2-3 eyes, as the existing buds above the layer snow were destroyed in fully, and with these annual and multiannual and wood. In these conditions we have secured fruit load specific to each variety.

Were effectuated observations and determinations experimentale on grape production and quality (weight of 100 grapes, the content of total sugars and total titratable acidity).

Table 1

Year	Tempera	ture ( ⁰C)		Rainfall (mm)		
	Minimum	Maximum	Annual	April-	Multiannual	Snow
				September		layer (cm)
				period		
2012	-24.3	42.6	383.5	230.8	578.5	25
2013	-18.1	38.4	451.5	307.8	588.4	15
2014	-14.1	37.6	994	640.7	546.8	25
2015	-25.1	39.2	748.9	398	554.9	30
2016	-23.4	38	748.9	297.4	554.9	30

#### The main climatic elements from 2012-2016 period

#### **RESULTS AND DISCUSSIONS**

Negative minimum temperatures registered during the rest period of the 2012, 2015 and 2016 years and abundant rainfalls of 2014 year, were constituted in harmful factors for vines in this area. In 2012 year, grape production was lower due to the fact that the vine has been in the third year after planting, in the last year of formation (table 2). This demonstrates that not all varieties have the same precocity in the bear.

In 2013 year, the favorable climate has made all the varieties to express with the genetic potential and the degree of adaptability, specific to each variety individually for the first year of fruit, in conditions of sandy soils area from south-western Oltenia. The highest level of production of grapes was done at *Selena* noble variety, with 35.9 t/ha, followed a short distance from resistant variety *Brumăriu* (34.8 t/ha). From noble varieties were also noted *Blasius* (30.0 t/ha), *Alb aromat* (29.2 t/ha) and *Chasselas d'ore* (27.6 t/ha). With a production of grapes over 20 t/ha, were found varieties *Fetească albă Cl. 1 Od.* (21.2 t/ha) and *Fetească regală Cl. 21 Bl.* (21.2 t/ha). Harder adaptable in this area and with lower genetic potential were *Muscat Ottonel* (11.7 t/ha), *Neuburger* (11.3 t/ha) and *Donaris* (10.2 t/ha).

#### Table 2

# Yield production (t/ha) of some varieties with grapes for white wine in 2012-2016 period

Variety			Year			2013-2016
	2012	2013	2014	2015	2016	Average
Riesling italian	0.7	16.7	7.6	2.3	16.3	10.7
Columna	0.7	12.9	7.2	2.9	11.7	8.7
Donaris	0.7	10.2	3.0	0.9	11.3	6.3
Fetească albă Cl. 1 Od.	0.4	21.2	7.9	3.6	6.8	9.9
Fetească regală Cl. 21 Bl.	3.9	21.2	10.2	7.5	10.6	12.4
Pinot gris	0.4	12.5	0	1.8	7.2	5.4
Riesling de Rhin	0.7	15.5	3,8	2.4	7.6	7.3
Neuburger	0.4	11.3	0	3.4	6.4	5.3
Chasselas d'oré	3.0	27.6	0	4.9	9.1	10.4
Muscat Ottonel	0.7	11.7	3.4	1.4	4.5	5.2
Sauvignon blanc	0.7	21.2	0	2.9	8.3	8.1
Alb aromat	4.2	29.2	8.3	0.5	7.2	11.3
Grasă de Cotnari	1.1	19.7	0	0.6	7.6	7.0
Brumăriu	4.2	34.8	10.6	4.9	21.9	18.0
Blasius	4.5	30.0	8.7	8.9	7.6	13.8
Sauvignon	0.7	14.0	3.4	3.6	10.6	7.9
Selena	1.5	35.9	6.4	6.8	6.4	13.9

In 2014 year, temperatures were favorable for dormant vines, but the abundant rainfall during the growing season increased vulnerability against the major fungal diseases of plants and increased their virulence, so that the pesticide treatments carried out have failed to halt their work, or if the moment was stopped the next rain or even dew diseases were reactivated.

Some varieties have shown greater tolerance and achieved grape production 8-10 t/ha, *Brumariu* variety (10.6 t/ha), which is part of resistant varieties, but also noble varieties *Fetească regală Cl. 21 Bl.* (10.2 t/ha), *Blasius* (8.7 t/ha) and *Alb aromat* (8.3 t/ha). At some varieties, more sensitive grape production was compromised, example varieties *Pinot gris, Neuburger, Chasselas d'ore, Sauvignon blanc, Grasă de Cotnari.* The adverse effect of minimum temperatures on vines in 2015 year, was shown amid of vines diseases by 2014 year. So that, in 2015 year, grapes production achieved by studied varieties was extremely low. *Blasius, Fetească regală Cl. 21 Bl.* and *Selena* varieties who realized the production of grape between 8.9 and 6.8 t/ha. On the other varieties of grape yields realized was insignificant.

In autumn of 2015 year, was protected with earth 2-3 strings from each vine, so that, the negative effect of harmful minimum temperatures in winter 2015-2016, has declined more were less depending on the variety. The grapes production achieved was over 6.4 t/ha, culminating with resistant variety *Brumăriu* which achieved a grapes production of 21.9 t/ha. Noble variety was highlighted with a *Riesling italian* variety, with a grape production of 16.3 t/ha.

Table 3

Weight of 100 grapes (g) in harvesting moment of some varieties with grapes for white wine in 2012-2016 period

Variety			Year			Multiannual	Average
	2012	2013	2014	2015	2016	interval	
Riesling italian	133	166	180	204	150	133-204	166
Columna	180	155	258	256	205	155-258	211
Donaris	182	178	244	267	263	178-267	227
Fetească albă Cl. 1 Od.	126	181	142	163	147	147-181	152
Fetească regală CI. 21 BI.	137	172	182	170	205	137-205	173
Pinot gris	92	140	156	127	125	92-156	128

Riesling de Rhin	126	152	156	159	167	126-167	160
Neuburger	154	209	202	259	205	154-259	206
Chasselas d'oré	230	211	196	316	222	196-316	235
Muscat Ottonel	146	224	196	275	183	146-275	205
Sauvignon blanc	133	171	158	192	167	133-192	164
Alb aromat	313	282	288	368	354	282-354	321
Grasă de Cotnari	208	276	276	248	333	208-333	268
Brumăriu	159	217	180	209	189	159-217	191
Blasius	218	288	392	420	395	218-420	343
Sauvignon	121	174	180	190	168	121-190	167
Selena	149	218	196	263	221	149-263	209

They also noted varieties *Columna* (11.7 t/ha), *Donaris* (11.3 t/ha), *Fetească* regală *Cl. 21 Bl.* and *Sauvignon* with 10.6 t/ha, production of grapes each. Analysis of grape average production, realised in the first 4 years of fruits, highlights the special ability of fruiting for variety with ,,blood hybrid" *Brumăriu*, 18 t/ha, a superior production of noble varieties *Selena* and *Blasius* which achived a grapes average production of 13.9 and respectively 13.8 t/ha.

Table 4

Sugar content (g/l) in the harvesting moment at some varieties with grapes for white wine in 2012-2016 period

Variety			Multiannual			
-	2012	2013	2014	2015	2016	interval
Riesling italian	209	208	197	183	198	183-209
Columna	188	166	177	165	155	155-188
Donaris	185	166	211	206	180	166-211
Fetească albă Cl. 1 Od.	184	188	163	174	178	163-188
Fetească regală Cl. 21 Bl.	187	177	174	168	187	168-187
Pinot gris	199	220	182	167	189	167-220
Riesling de Rhin	192	206	187	171	177	171-206
Neuburger	200	217	183	181	189	181-217
Chasselas d'oré	185	179	142	156	167	142-185
Muscat Ottonel	224	222	186	194	182	182-224
Sauvignon blanc	230	215	178	188	186	160-230
Alb aromat	190	188	160	168	174	160-190
Grasă de Cotnari	185	182	167	152	176	152-185
Brumăriu	177	179	174	183	193	174-193
Blasius	203	172	159	178	175	159-203
Sauvignon	196	227	191	186	184	184-227
Selena	186	222	175	180	172	172-222

With chances of reaching the assortment of sandy soils is *Fetească regală Cl.* 21 Bl., which conducted a grape average production of 12.4 t/ha. Weight of 100 grapes (g), is an indicator of quality vine. This varies depending on the variety, the load of grapes per vine, the way it was maintained plantation, climate, etc. (table 3). Overall varieties of wine have grape berries small, weighing between 1 to 2.3 g/grape, or medium grape who weight is 2.4 to 5 g. In the case of varieties studied in this period, the majority have small-berry grapes, namely 13 at number and only 4 have over 2.3 g/grape (*Blasius*, 3.4 g; *Alb aromat*, 3.2 g; *Grasă de Cotnari*, 2.7 g and *Chasselas d'oré*, 2.4 g).

The content of total sugars in the grapes in the time of harvesting, is a characteristic of the variety, but it depends on edaphic and climatic factors, the level of production and the health of the grapes, etc., and oscillated between broad limits from one year to the next (table 4).

The studied varieties have a high potential for accumulation of sugars, over 220 g/l, thus ensuring a wine with more than 12.5 vol.% alcohol, in certain years, example *Sauvignon blanc* (230 g/l), *Sauvignon* (227 g/l), *Muscat Ottonel* (224 g/l), *Selena* (222 g/l).

Those varieties have accumulated the minimum amount of total sugars as follows: Sauvignon blanc (160 g/l), Sauvignon (184 g/l), Muscat Ottonel (182 g/l), Selena (172 g/l). In years with optimal conditions for accumulation of sugars, no one variety has not accumulated under 185 g/l.

Fluctuating levels of sugars of grapes from one year to another, not offer a guarantee to obtain a constant quality for wine permanently, so that, in this area you can get topquality wines only in certain years.

Table 5

Variety			Year			Multiannual
	2012	2013	2014	2015	2016	interval
Riesling italian	3,1	4,2	5,2	4,1	5,0	3,1-5,2
Columna	3,3	3,8	4	2,7	4,5	2,7-4,5
Donaris	2,4	3,9	4,5	3,6	3,5	2,4-4,5
Fetească albă Cl. 1 Od.	2,5	3,4	4,2	2,7	4,0	2,5-4,2
Fetească regală Cl. 21 Bl.	2,6	3,8	4,4	5,0	5,6	2,6-5,6
Pinot gris	4	4,6	4,6	5,3	4,7	4-5,3
Riesling de Rhin	3,6	4,6	4,6	5,7	5,2	3,6-5,7
Neuburger	3,9	4,2	5,6	4,2	5,8	3,9-5,8
Chasselas d'oré	2,7	3,2	4,1	2,5	4,5	2,5-4,5
Muscat Ottonel	2,5	2,9	3,5	2,5	3,5	2,5-3,5
Sauvignon blanc	3,9	4,2	4,4	5,3	5,6	3,9-5,6
Alb aromat	3,1	3	3,3	2,7	3,5	2,7-3,5
Grasă de Cotnari	4	4,2	5	4,8	5,7	4-5,7
Brumăriu	4,2	4,1	2,6	5,3	4,5	2,6-5,3
Blasius	4,1	4,8	4,6	4,3	5,7	4,1-5,7
Sauvignon	3,8	4,2	4,9	4,0	5,8	3,8-5,8
Selena	3,5	4,2	4,5	3,0	3,5	3-4,5

Total titrable acidity (g/l H <sub>2</sub> SO <sub>4</sub> ) in the harve	esting moment at some varieties with
grapes for white wine in	2012-2016 period

Total titrable acidity, like the other two indicators of the quality of the wines listed above, has a level fluctuating from one variety to another, from one year to the next (table 5). In the area of sandy soils of South-western Oltenia, the value of this is less than in other areas, for variety, due to much higher temperatures (above 35°C), from July to August, when a good part of the day the main plant physiological processes are blocked. The value of total titrable acidity is influenced by the health of the grapes.

In certain years, some varieties can record total titrable acidity values between 4.5-5.5 g/l  $H_2SO_4$ , which conduce to balanced taste. In years when the acidity values are around 3-3.5 g/l  $H_2SO_4$ , obtained fade wines, and in these situations it is necessary to correct this value to improve the taste.

#### CONCLUSIONS

State of vegetation and vine production is constantly under incidence of climatic conditions. Harmful minimum temperatures to the rest period, but other climate elements, outside the optimal manifested during the growing season, producing significant damage on vegetative and generative organs, and thus the production of the year or even the next few years.

From the five years analyzed (2012-2016), optimal conditions for vines on sandy soils were just in one year. In 3 years of these, negative minimum temperatures recorded below the resistance of vine organs, they were harmful, and in a year of abundant rains during the growing season, spread over a number of days (48.5% and

dew phenomenon manifested daily, in this period, were established in factors unfavorable for this crop, which affected grape production and quality.

In favorable weather conditions, all varieties are expressed at genetic potential and degree of adaptability. The highest production was achieved at *Selena* noble variety, with 35.9 t/ha, followed by resistant variety *Brumăriu* (34.8 t/ha). They also noted the noble varieties *Blasius* (30.0 t/ha), *Alb aromat* (29.2 t/ha) and *Chasselas d'ore* (27.6 t/ha). Harder adaptable in this area and with lower genetic potential were *Muscat Ottonel* (11.7 t/ha), *Neuburger* (11.3 t/ha) și *Donaris* (10.2 t/ha).

In 2014 year, the rainfall during the growing season has increased vulnerability against the major fungal diseases of plants and reduced effectiveness of substances used in plant protection treatments, so that at some varieties that year production was compromised, example *Pinot gris, Neuburger, Chasselas d'ore, Sauvignon blanc, Grasă de Cotnari.* 

In years with harmful minimum temperatures (2016 year), protecting the earth 2-3 wood shoots from each vine, led to reduced negative effect. Grape production was achieved over 6.4 t/ha, culminating with resistant variety *Brumăriu* that has achieved grape production of 21.9 t/ha. Noble varieties was highlighted with a variety *Riesling italian* grape production of 16.3 t/ha. Analysis of grape production average, conducted in the first 4 years of fruits, highlights the special ability of fruiting for variety *Brumariu* with ,,blood hybrid", 18 t/ha, a superior production of 13.9 and 13.8 t/ha, respectively. With chances of reaching the assortment of sandy soils is *Fetească regală Cl. 21 Bl.*, which conducted a grape average production of 12.4 t/ha. The main quality indicators (weight of 100 grapes, content of total sugar and total titrable acidity), it is a characteristic of variety but also depends on climatic and edaphic conditions, and the applied technology. In certain years the some varieties as well as *Sauvignon blanc* (230 g/l), *Sauvignon* (227 g/l), *Muscat Ottonel* (224 g/l), *Selena* (222 g/l), realized wines over 12.5 vol.% alcohol and an total acidity of 4.5-5 g/l H<sub>2</sub>SO<sub>4</sub>.

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# THE HOMEOPATHIC PRODUCTS USED IN PLANT PROTECTION: AN ALTERNATIVE CHOICE

Rîndașu Ileana<sup>1</sup>, Ciceoi Roxana<sup>2</sup>, Stănică Florin<sup>1</sup>

<sup>1</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, <sup>2</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest, Research Center for Study of Food and Agricultural Products Quality, Laboratory of Diagnose for Plant Protection *\*Correspondence author. E-mail*: roxana.ciceoi@gmail.com

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#### ABSTRACT

The paper presents the history and trends of the homeopathic products used in plant protection. Also known as high-dilution or agrohomeopatic treatments, this method started with the pioneering work of Kolisko in 1923, on wheat germination and Junker in 1928, on growth of microorganisms. Their approaches can be mainly divided in two important types of studies: effects on crop growth and effects on crop disease/phytopathology and pests control, both on plant models and in field trials. An important contribution in the agrohomeopathic research is brought by the isopathic model, where the same substance is used for the induction of a stress and then, in a diluted form, for the cure. Our work synthetises the information gathered from previous reviews made in 1984-2015 and other various papers published in the past years.

#### INTRODUCTION

The medicines taken for specific symptoms cause similar manifestations in healthy organisms if taken a certain period of time (principle of similitude). The opposite is also true: the medicines that cause symptoms in healthy organisms can cure them when administered in sick individuals, in diluted, nontoxic doses.

In plants, the mechanism of action may imply metabolic changes which lead to formation of secondary metabolic products related to the mechanism of defense of plants (Lensi et al, 2010). Gangar has concluded that homeopathic medicines can influence also the genetic activity in plants, this phenomenon being explained with the help of experiments made on mungbean, gram, cotton, wheat etc.

The paper presents an analysis of the evolution of research made in agrohomeopathy, from international publications, to put in evidence the level of knowledge acquired between 1923 - 2017.

#### MATERIALS AND METHODS

For the present paper were used all the available online and offline bibliographical references we could find, using the following international databases: Web of Science - Core Collection (Journal Citation Reports, Derwent Innovations Index, Thomson Reuters), SpringerLink Journals (Springer), Scopus (Elsevier), ScienceDirect Freedom Collection (Elsevier), PROQUEST Central, HomBRex (Homeopathy Basic Research experiments database). The period analysed in this study was 1923-2017.

# **ISOPATHIC MODELS**

The first studies on agrohomeopathy were performed by Kolisko on wheat germination in 1923. Afterwards, in 1928, another researcher published a study about the effects of high dilutions on microorganisms (Junker, 1928). These two researchers were initiating a domain which lately was defined as agrohomeopathy, domain that started to develop more intensely in the end of the 60's.

One of the earliest models belongs to Nitien et al, in 1969, with homeopathic preparations of copper sulphate (*Cuprum sulphuricum* CH 15) for detoxification of green pea previously intoxicated with the same substance in high dosage, demonstrating a positive effect. Another study was performed in 2011 on wheat seedlings pretreated with high concentrations of gibberellic acid expected to facilitate the inhibitory effect of the same plant hormone in diluted doses, but the supposition was not confirmed by the experimental results (Pfleger et al).

Another model consisted of seeds of *Vigna unguiculata* pretreated with *Natrium muriaticum* and afterwards stressed with NaCl. In the pretreated group there was an increase of seed germination compared with the control group stressed with the same substance (Mondal, Sukul, 2012).

The action of *Natrium muriaticum* in dilutions 6 CH and 30 CH, in comparison with NaCl solution of 5% was also studied on *Phaseolus vulgaris*. *Natrium muriaticum* had a significative effect of increasing the vegetable growth, mainly in the 6CH dilution (Lensi et al).

A comparative study was made on two plant models: *Lemna gibba*, an autotrophic multicellular organism and *Saccharomyces cerevisiae* yeast, a heterotrophic single-celled organism, stressed with arsenic and then treated with decimal potency of *Arsenicum album*. Application of *Arsenicum album* on the *Lemna gibba* bioassay yielded the largest effects compared to control groups, confirming the hypothesis that complex organisms show stronger reactions to homeopathic treatments (Jager, Scherr, 2011).

# **EFFECTS ON PESTS**

The preparation made with *Coccinella septempunctata* from the adult beetle gives the necessary protection against different types of pests, like *Trioleurodes vaporariorum* (whitefly). Another efficient remedies against whitefly are made from the tincture of a live parasitic wasp, *Encarsia formosa* (Kaviraj, 2012) or from *Sulphur* (CH 200), if the honeydew secretions leads to colonisation with sooty mould, turning the leaves black (Maute, 2011).

An interesting study was made with the rosy apple aphid (*Dysaphis plantaginea* Pass., considered a major pest in apple orchards) on apple seedlings, with *Lycopodyum clavatum* CH 6, CH 15, CH 30 and a nosode of rosy apple aphid, in eight independent, randomized and blinded experiments in growth chambers. The number of juvenile offsprings was reduced after application of *L.clavatum* CH 15 and nosode CH 6 compared with the control group (Wyss and al).

# EFFECTS ON DISEASES, GERMINABILITY AND CROP GROWTH

In most of the cases, the research of the effects on diseases was carried out on artificially diseased organisms. (Table 1)

# Summary of phytopathological bioassays (after Betti et al)

Authors	Species/pathogen	Treatment	Working variable
Aggarval et al., 1993	Wild taro/ phytophthora colocasiae	Homeopathic treatments**	Disease symptoms, fungal growth and spore germination
Cheema et al., 1993	Tomato/tobacco mosaic virus	Clerodendrum aculeatum, CH Thuja	Disease symptoms
Betti et al., 2003	Tobacco/tobacco mosaic virus	DH As <sub>2</sub> O <sub>3</sub>	Virus-induced Hypersensitive lesions
Cheema et al., 1986	Papaya/papaya mosaic virus	Homeopathic treatments	Disease symptoms
Datta, 2006	Mulberry/ <i>m.</i> Incognita	CH Cina	Plant growth, nematode infection
Khanna and Chandra, 1989	Mango, guava, tomato/ pestalotia spp., fusarium roseum	Homeopathic treatments and adjuvants	Post-harvest losses
Khanna and Chandra, 1992	Different fungi	DH treatments	Spore respiration rate, organic acid pool in spores
Rivas et al., 1996	Wheat, tomato/ Alternaria solani	CH treatments***	Seed and spore germination
Rolim et al., 2001	Apple/ podosphaera leucotricha	CH treatments****	Powdery mildew symptoms
Saxena et al., 1987	Reed okra/ seed-borne fungi	CH Thuja, nitric acid, Sulphur, Calcarea carb., Teucrium Q	Fungal spore germination
Sukul and Sukul, 1999	Cowpea / Meloidogyne incognita	CH Cina	Plant growth, nematode infection

D, C = decimal, centesimal potency; H = Hahnemannian potency; \*\* = Kali iodatum, Arsenicum album, Blatta orientalis, Thuja occidentalis; \*\*\*= Arsenicum album, Calcarea, Cuprum, Ferrum metallicum, Lycopodium, Natrum, Phosphorus, Selenium, Silicea, Sulphur; \*\*\*\* = Kali iodatum, Lachesis trigonocephalus, Staphysagria, Sulphur, Oidium lycopersici.

Khanna and Chandra, obtained good results in the control of rot in mango, guava and tomatos, suppressing the spore germination and respiration of the fungi *Alternaria alternate, Colletotrichum gloeosporioides, Fusarium roseum* and *Gloeosporium psidii.* 

Sinha and Singh obtained significative results in the control of fungi producing aflatoxins in stored products, with *Sulphur* CH 200 which inhibited 100% the growth of *Aspergillus parasiticus*. Other remedies (*Silicea terra* and *Dulcamara*) reduced the growth of the fungi with 50% and the production of the toxin with 90%. Verma et al evaluated the effect of homeopathic remedies *Lachesis* and *Chimaphila* both in CH 200, before and after the inoculation of the tobacco mosaic virus (TMV) and obtained a reduction with 50% of virus content in tobacco leaf discs. In apple seedlings, two sprays of *Staphysagria* 100 CH at intervals of 12 days reduced powdery mildews

caused by *Podosphaera leucotricha* (Rolim et al). Rolim used also *Staphysagria*, this time in the 30 CH potency for tomato plants, in greenhouse and reduced the severity of early blight, as well as with *Phosphorus* 30 CH and isotherapics of *A. solani* in 30 and 60 CH.

A three year project on the control of dark leaf spot caused by *Alternaria* brassicicola in cauliflower made by Betti et al concluded that  $As_2O_3$  DH 35 reduced significantly the infection on cauliflower heads compared to control.

Other investigations are made on healthy plants for the determination of the stimulation of the growth and on abiotically stressed plants (table 2).

The test organism most frequently used was wheat. The most commonly used stressor was arsenic.

Table 2.

Authors	Species	Stress	Treatment	Work variable
Auquiere et al, 1988	Wheat	Ethanol Lysine	CH Ethanol, Lysine	Shoot growth, weight of shoots
Novic et al., 1990	Wheat	Dark germination	DH Aurum	Shoot growth; fresh and dry weight of shoots
Kovac et al., 1991	Wheat	NaCl, CuCl, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	<i>DH</i> NaCl, <i>DH</i> CuCl, <i>DH</i> K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Shoot growth; fresh and dry weight of shoots, grains and roots
Lehner et al., 1991	Wheat	Dark germination	DH Platinum, Mercurius, Cadmium, Plumbum, Cuprum, Aurum, Argentum nit, Sulfur	Shoot growth; fresh and dry weight of shoots, grains and roots
Lauppert, 1995	Wheat	Dark germination	DH CuSO₄	Shoot growth; fresh of shoots; dry weight of shoots, grains and roots
Betti et al., 1997	Wheat	As <sub>2</sub> O <sub>3</sub>	DH As <sub>2</sub> O <sub>3</sub>	Shoot growth
Brizzi et al., 2000	Wheat	As <sub>2</sub> O <sub>3</sub>	DH As <sub>2</sub> O <sub>3</sub>	Germination
Binder et al., 2005	Wheat	As <sub>2</sub> O <sub>3</sub>	DH As <sub>2</sub> O <sub>3</sub>	Shoot growth
Brizzi et al., 2005	Wheat	As <sub>2</sub> O <sub>3</sub>	DH As <sub>2</sub> O <sub>3</sub>	Shoot growth
Carvalho et al., 2003	Feverfew	Adaptation	DH Arnica montana	Shoot growth, parthenolide content
Carvalho et al., 2004	Feverfew	Water shortage	CH Natrum muriaticum	Shoot growth, chlorophyll and proline content
Carvalho et al., 2005	Feverfew	Adaptation	CH Arnica montana	Shoot growth, parthenolide content
Projetti et al., 1985	Lentil	CuSO <sub>4</sub>	CH CuSO₄	Root growth
Steffen, 1985	Yeast	CuSO <sub>4</sub>	DH CuSO <sub>4</sub>	In vitro growth
Tighe, 2005	Cress	NaCl	CH NaCl	Shoot growth, germination

Overview of bioassays with abiotic stress (after Betti et al)

D, C = decimal, centesimal potency; H = Hahnemannian potency; K = Korsakovian potency;

#### CONCLUSIONS

1. The difficulties of previous studies described in literature lies in the fact that no complete medical material has been developed with specific indications of phytopathology or pest control, although there are data reported by different authors. The methodology varies from case to case, using various treatments, various concentrations, and there is no standardization that allows identical treatments.

2. It is necessary to use new research models to properly assess the potential of the domain. In particular, we have found that there are only a few studies on fruit trees, most of the studies being made on wheat and vegetables.

3. There are only a few studies conducted in the field and no specific methodologies have been established for different cultures. Most studies were conducted in growth chambers and seedlings. New studies are needed to develop methodologies for treatments applied directly to cultures, but the development of the work strategy must first be done in laboratory conditions to control more easily the environment.

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# CONSIDERATIONS CONCERNING THE INFLUENCE OF VACUUM AND PRESSURE CYCLIC PROCESS UTILISED TO IMPROVE THE TENDERNESS OF ROMANIAN TRADITIONAL GOAT MEAT PRODUCTS

Roşca Adrian<sup>1x</sup>, Roşca Daniela<sup>2</sup>

<sup>1)</sup> Faculty of Horticulture, University of Craiova
 <sup>2)</sup> Faculty of Electrical Engineering, University of Craiova
 \* Correspondence author. E-mail: adrosca2003@yahoo.com

Key words: compression / piercing, vacuuming, pressuring, Warner - Bratzler method

#### ABSTRACT

The paper presents a new method to increase the tenderness' quality of the traditional Romanian product "Goat Pastrama". In order to decrease the wet curing / marinating period of the goat meat, and in the same time to increase the tenderness' quality of the product, the new tenderizing process consists in one simultaneous compression and piercing step (2000 N), one intensive cyclic vacuum process (0...- 0,85 bar), followed by certain steps of intensive cyclic pressuring (0...9 bar) process. The paper presents characteristic shear force amounts obtained by using Werner - Bratzler testing method for this type of cured - hot smoked final product tenderized due to piercing / compression step, intensive cyclic vacuum process, followed by intensive cyclic pressuring steps process, in comparison with no tenderized cured - hot smoked final product.

#### INTRODUCTION

The word *pastramă* is etymologically rooted from the Latin *pastor* who means *shepherd*, and from the Romanian *a păstra* which means "to keep" or "to preserve". But the word is maybe more ancient and come so because *Pastramă* was shepherd's meat food of lamb, sheep, goat or mutton. *Pastramă* was originally created as a way to preserve meat before modern refrigeration. Traditionally *Pas-trama* is made from the brisket (which comes from the lower chest of the steer), or from the navel (a small piece cut from the muscle known as the plate), that are the tenderless parts of the animal's carcass. For any meat *Pastrama* traditional making, the raw meat is brined, partly dried, seasoned with various herbs and spices, then medium or hot smoked.

In industrial process *Pastrama* making consists in meat curing realized by fast injection of brine usually containing preservation additives, or otherwise, in home-made or small enterprise processing, marinated for long time in concentrated salt solution (http://en.wikipedia.org/wiki/Pastrami).

In small / middle enterprise, after the brining process the meat is tenderized for 4 - 8 hours in massaging vacuum equipment (maximum relative vacuum - 0,65 ...- 0,75 bar). For the same reason, in homemade or small enterprise process, the meat is tenderized for 1 - 3 weeks in high concentration brine containing additional flavors added. Then, the brined meat is cold smoked, and finally dried for long time in 2 - 8°C ventilated air (Roşca & Roşca 2014, Roşca et al. 2014).

Tenderizing is a process that breaks down collagens in meat to make it more palatable for consumption.

There are several ways to tenderize meat: mechanical tenderization, such as piercing; the tenderization that occurs through cooking, such as braising; ten-derizers in the form of naturally occurring enzymes, which can be added to food before cooking (examples of enzymes used for tenderizing: marinating the meat with vinegar, wine, lemon juice or yogurt; brining the meat in a high concentration salt solution. Mechanical tenderization actions produce multiple cuts in the meat muscle in order to increase the surface area and thereby facilitate extraction and solubilization during the massaging phase. Softening of the muscle is also obtained, making the meat more adaptable to the cooking moulds (Tyszkiewicz & Klossowska 1996). Tenderization, pre-massage and massage are closely inter-related, and not all products require the same mechanical action. Thus the mechanical action must be intensified and adapted in order to compensate for some of the negative cones-quences that may result in the product's guality. This will depend on the rest of the process and, above all, on the presentation and final quality of the product itself. In low-injection products where meat content represents more than 80% of the final composition, meat quality is a determining factor in mastication, while in more highly injected products, this is not as important as the process and technology used (McGee 2004, Maddock 2008, Shewfelt 2000).

In order to reduce the time tenderizing process as much is possible, and to improve the final product tenderness', this paper presents a new tenderizing method based on compression / piercing step, one intensive cyclic vacuum process, followed by intensive cyclic pressuring steps process.

#### MATERIAL AND METHODS

#### Material, equipment and processing method

This paper presents experimental research concerning a new method to obtain a cured - smoked goat boneless brisket "*Pastrama de capra*" product, similar with traditional Romanian assortment made by SC AVI-GIIS SRL Mihaesti, Valcea.

In order to produce "*Pastrama de capra*", goat boneless brisket (4 pieces; 0,5 kg/piece; aprox. dimensions: length x width x height =  $120 \times 60 \times 30$  mm) was used (according Animals Slaughter Certificate: 26 months, individual small farm).

In principle, at SC AVI-GIIS SRL Mihaesti, the traditional processing techno-logy of this cured - smoked product type consists in: wet curing phase of entire pieces of the meat in 12% curing salt concentration, during 2 - 3 weeks; drying/ripening phase in cold ventilation (4 - 6°C) for 4 hours; middle smoking phase (45 - 50°C) for 2 days, followed by a cold ventilation long sequence (4 - 6°C) for 8 hours (Roşca & Roşca 2014).

According to Romanian legislation, sodium nitrite (NaNO<sub>2</sub>), potassium or sodium nitrate (KNO<sub>3</sub> / NaNO<sub>3</sub>), or any chemical alternative curing substances are not permitted in traditional cured - cooked types products usual processing, and no brine injection is allowed (Roşca & Roşca 2014).

In order to determine the efficiency of the new tenderizing and curing method proposed in this paper, one of these four pieces was used to make "*Pastrama de capra*" in similar technological condition alike "SC AVI-GIIS SRL Mihaesti" is made.

The other 3 pieces were used to obtain "Goat Pastrama" made using the proposed method.

To reduce the wet curing phase, and in the same time to improve as much is possible the final product tenderness' quality, this paper propose a tenderizing method based on certain steps: one *simultaneous piercing and compression step*, one *intensive cyclic vacuuming processing* step, finally followed by *intensive cyclic pressuring process step*.

The meat's *simultaneous piercing and compression processing* is realized by using *Multi - needle piercing and compression device* (designed and made by Unconventional Technologies and Equipment for Agro-Food Industry Laboratory - *UTEFIL* within Faculty of Horticulture, in collaboration with Laboratory for Environmental Protection in Industry - *EPIL* within Faculty of Electrical Engineering). This experi-mental device consists in two parallel plates (W1.4571 stainless steel): the lower fixed plate with 120 holes (ø5,5); the upper mobile plate with 120 needles (ø5, 20<sup>0</sup> conical sharp, W1.4571) placed in the same shape and reciprocity distance as into the industrial brine injection equipment (Figure 1) (Roşca & Roşca 2014).



Figure 1. Multi - needle piercing and compression device



Figure 2. Experimental equipment for cyclic vacuuming and cyclic pressuring process

The meat's *simultaneous piercing and compression* is realized between the two plates of the *Multi - needle piercing and compression device* that is actuated by the crosshead of universal testing machines *LBG 10* (within *EPIL*): maximum force 2000 N; speed 100 mm/min. Due to the compression force the meat's initial height decreases up to 20 mm final height.

For a better correlation of the research's results, all the four pieces (both one piece to be processed at "SC AVI-GIIS SRL Mihaesti", and the three pieces that will be processed by using the method proposed in this paper, too) were simul-taneous pierced and compressed (piece by piece), as above was presented.

In order to put in evidence the influence of cyclic vacuuming and cyclic pressuring process (*CVCP*) on meat tenderization, *experimental equipment* (*EE - CVCP*) was used (Figure 2). In principle, *EE - CVCP* is composed in a vacuuming and pressuring process hydraulic cylinder (VP-HC) consisting in a cylindrical vessel (inner ø 80; length 180 mm) made in stainless steel W1.4571 and a piston made in food grade Teflon. VP-HC is provided with a manometer gauge (0...12 bar; 1,6 class), and a manovacuumeter gauge (-1 ...1,5 bar; 2,5 precision class) when vacuuming process is actuated. In order to evacuate the liquid / gas excess before and after *VCPC*, the piston is provided with G1/4" tap connected to ø8 Rilsan tube.

*EE - CVCP* was designed and made by *UTEFIL*, in collaboration with *EPIL*. (Roşca & Roşca 2014, UTEFIL Data Base 2005-2017).

In order to actuate the vacuuming and pressuring processes into *EE* - *VCPC*, universal testing machine *LBG 10* (within *EPIL*), was used.

After meat's simultaneous piercing and compression the new tenderizing method presented in this paper proposes two main successive steps:

- Cyclic vacuuming and de - vacuuming step of the pierced and compressed meat's and a certain amount of brine (brine concentration: 1% salt solution; meat / brine proportion: 2/1), too, into a processing vessel.

The cyclic vacuuming and de - vacuuming step lasts 15 min consisting in 5 consecutive stages, each stage lasting 3 min: *slow vacuuming* up to - 0,85 bar (during 1 min), followed by *maintaining* for 2 min at this vacuum level, followed by *fast de-vacuuming* up to the ambient atmosphere (Figure 3).

During this step the blood is "squeezed" from the meat. At the end of this cyclic step, the salt solution and the "squeezed" blood are replaced from the vessel, but the meat remains into the vessel. Over the meat into the processing vessel a certain amount of new brine is poured (brine concentration: 3% salt solution; meat / brine proportion: 1/1), and the next processing step can begin.



and de - vacuuming step

- Cyclic pressuring and de - pressuring step in the processing vessel of the pierced and compressed meat and the brine at certain pressure amounts. During the pressuring process, the pressure level is 4 - 5 times higher than during brine in-jection in industrial equipment, and 3 - 4 times than the dynamic pressing / dropping during the industrial massaging process. Therefore this large amount of pressure realizes uniform tenderizing both meat's internal tissues nearby each piercing / per-foration, and the external meat's internal tissues, too.

Each *cyclic pressuring and de - pressuring* step lasts 15 min consisting in 5 consecutive stages, each lasting 3 min: *slow pressuring* (during 2 min) up to 9 bar, followed by *maintaining* for 1 min at this pressure level, followed by *fast de-pressuring* up to the ambient atmosphere (Figure 4).

In this paper were considered certain successive repetitions lasing 60 min (*CP* 60), 90 min (*CP* 90) and 120 min (*CP* 120).

All the four meat pieces (1 pieces no tenderization - *TRAD*, brined in 12 % salt concentration, during 2 weeks; each of 3 pieces *CP 60*, *CP 90* and *CP 120* (tenderized as presented above) were ripened in cold ventilation (4 -  $6^{\circ}$ C) for 4 hours; middle smoked (45 -  $50^{\circ}$ C) for 36 hours, followed by a cold ventilation long sequence (4 -  $6^{\circ}$ C) for 8 hours.



Figure 4. Diagram for cyclic pressuring and de - pressuring step

#### **Tenderness evaluation using Warner - Bratzler method**

Instrumental - objective methods are based on fundamental tests measure properties that are familiar to mechanical engineers (strength, Poisson's ratio, Young's and shear modulus). Mechanical tests cover a wide range of simple and rapid tests, including puncture, compression, extrusion, shear, and others, which measure one or more textural properties and are commonly used in quality control applications. Actual instruments tend to be more and more sensitive to small differences between samples and are able to detect trends in quality loss before they can be detected by humans. The most relevant and utilized destructive texture and tenderness tests are puncture/ penetration test, compression test, and Warner - Bratzler shear test.

In Warner - Bratzler test a blade cuts through a specimen, therefore the shear force behavior gives information about tenderness, as well as the bite characteristic products. The jig consists of a rigid frame supporting a shear bar. Interchangeable shear blades fit into the frame. There are wide known two blade types: the square cut blade that is mainly used for rectangular specimens and the notched (triangular or "V" blade) for round cross-section specimens. The jig acts in direct compression for slicing / shearing tests on products. Because of the good reproducibility of the results, this test is widely used in food texture and tenderness analyzes (Xargayó et al. 2015, Xianzhong & Shaofang 2011).

To perform interdisciplinary researches concerning general texture and tenderness analysis, universal testing machines Lloyd Instruments LRXPlus 5 (0,5 accuracy class for force and extension) within UTEFIL is used since several years ago to perform comparative texture measurements for fruits and vegetable firmness's (skin strength, ripeness' degree), and for the tenderness of row-meat and cooked-meat. The mechanical characteristics of the specimens as a function of force - deformation are recorded and displayed by using specialized NEXYGENPlus control and measure-ment software (www.lloyd-instruments.co.uk).

Due to collaboration between UTEFIL and EPIL an experimental equipment Warner - Bratzler was made: special rigid frame (made in food-grade Teflon) sup-porting a shear bar that permits interchangeable Warner - Bratzler shear blades (V and square plate cut blade made in stainless steel DIN W1.4571) to slide fit into the frame (Figure 5) (Roşca & Roşca 2011, Roşca & Roşca 2013, UTEFIL Data Base 2005-2017). During this experiment, 100 mm/min cutting speed was used.

*Goat Pastrami* obtained using goat brisket tenderized *CP 90* method, during Warner - Bratzler testing shear force, is presented in Figure 6.



Figure 5. Universal testing machine Lloyd Instruments LRXPlus 5 and Warner - Bratzler experimental equipment



Figure 6. *Goat Pastrama* made using *CP 90* method, during Warner - Bratzler shear force test

#### **RESULTS AND DISCUSSIONS**

In order to determine the influence of tenderizing process on the final pro-duct tenderness, *Goat Pastrama* pieces made by using traditional homemade method, or tenderized by using the presented processing method, respectively, were sliced according Warner - Bratzler shear force test; during these shear force tests, each of all 4 pieces of Goat Pastrama were sliced in 6 parts. Representative Warner - Bratzler shear force diagram is presented in Figure 7.

In Table 1 are presented the maximum shear force amount and the shear force average for each of the four of *Goat Pastrama* types, and in the same time, presents the percentage average decrease of shear force's (in comparison with traditional homemade *Goat Pastrama*'s tenderness *TRAD*) by using each processing method, that demonstrate the tenderness quality increase of the product.

Table 1

Sample	Maximum shear force	Shear force	Decrease of shear
code	min…max amount, N	average, N	force average, %
TRAD	281,78323,55	301,46	-
CP 60	171,22213,34	188,59	38,62
CP 90	148,11173,82	159,11	47,25
CP 120	132,82151,63	141,75	53,14

Warner - Bratzler shear force for Goat Pastrama types



Figure 7. Warner - Bratzler shear force diagram for *Goat Pastrama* made using simultaneous piercing and compression, one cyclic vacuuming and de - vacuuming step, finally followed by cyclic pressuring and de - pressuring step *CP 90* method

#### CONCLUSIONS

Due to both high vacuum level and fast vacuuming and de-vacuuming step, and high pressure level of the cyclic pressuring and de - pressuring steps, too, the pro-posed processing method represents a much more intensively tenderizing method, than home-made tenderizing method.

The main conclusion concerning the influence of simultaneous piercing and compression, one cyclic vacuuming and de - vacuuming step, finally followed by cyclic pressuring and de - pressuring step could be drawn from Table 1: in comparison with traditional homemade Goat Pastrama's tenderness, each type of the proposed method deter-mines an important tenderness' increasing (36,62...53,14%) of the this product.

The proposed method determines the shortest salt marinating / tenderizing period, only 90-120 min, in comparison with 4-8 hours in massaging vacuum equipment in industrial processing, or 2-3 weeks marinating time in homemade or small enterprise processing.

This paper opens further experimental researches focused on the influence of similar cyclic vacuuming / pressuring process (higher vacuum or pressure level, shorter or longer vacuuming and de-vacuuming steps, and pressuring and de - pressuring steps, respectively) for making traditional products made in other much more tenderless parts of animal's carcass.

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# MATHEMATICAL MODEL FOR COMPRESSION STRENGTH OF PORK SHOULDER MEAT TENDERIZED BY USING MECHANICAL PROCESSES

Roșca Daniela<sup>1</sup>, Roșca Adrian<sup>2x</sup>

<sup>1)</sup> University of Craiova, Faculty of Electrical Engineering
 <sup>2)</sup> University of Craiova, Faculty of Horticulture
 \* Correspondence author. E-mail: adrosca2003@yahoo.com

Key words: mathematical model, compression strength, Warner - Bratzler method

#### ABSTRACT

The paper presents a mathematical model for compression strength for shoulder pork meat tenderized by using mechanical process. The mathematical model is based on compression characteristic diagrams that are experimentally determined after pork shoulder meat tenderi-zation by using statically pressing. In order to decrease the wet curing / marinating period of the pork shoulder meat, the new tenderizing process consists in one statically pressing step, one simultaneous compression and piercing step, followed by certain steps of intensive cyclic vacuum processing. The paper presents characteristic shear force amounts obtained by using Warner - Bratzler testing method for a cured - hot smoked final product tenderized by pressing, piercing and cyclic vacuum processing, in comparison with no tenderized cured - hot smoked final product.

#### INTRODUCTION

Tenderization is the mechanical action of producing multiple cuts in the meat muscle in order to increase the surface area and thereby facilitate extraction and solubilization during the massaging phase. Tenderization, pre-massage and massage are closely interrelated, and not all products require the same mechanical action. Thus the mechanical action must be intensified and adapted in order to compensate for some of the negative consequences that may result in the product's quality. This will depend on the rest of the process and, above all, on the presentation and final quality of the product itself. Tenderizing process breaks down collagens in meat to make it more palatable for consumption. There are several ways to tenderize meat: mechanical tenderization, such as piercing; the tenderization that occurs through cooking, such as braising; tenderizers in the form of naturally occurring enzymes, which can be added into food before cooking (examples of enzymes used for tenderizing: marinating the meat with vinegar, wine, lemon juice, yogurt (Institute of Food Technologists 1981, Maddock 2008, Tyszkiewicz et al. 1996).

Cohesion of the muscles takes place thanks to the myofibrillar proteins which have been extracted during the manufacturing process and which are found on the surface of the muscle. These proteins form the exudates and, due to their gelling capacity, act as glue between the muscles. It has been widely demonstrated in the pertinent literature that the greater number of proteins extracted, the greater the stability between muscles and therefore the better the sliceability and masti-cation. Extraction of myofibrillar proteins is achieved through both mechanical and chemical actions. The degree to which the muscle structure is opened will determine the final quantity of proteins present in the exudates. This structure's openings is done by means of tenderization, pre-massage and massage (Maddock 2008).

By means of certain additives, aside from the above-mentioned salt and phosphates, such as carragenan and vegetable gums, muscle texture can be slightly hardened and / or "plastified", how-ever this alone will not be sufficient to compen-sate for the meat's lack of firmness. It has been observed that the mechanical action of tenderization does have a positive effect on this type of meat, because the texture is less fragile due to an increased surface of contact between muscles (Maddock 2008, Tyszkiewicz et al. 1996).

There are slight differences in the processing technology of cured - cooked products, mainly depending on the size of the meat parts used for product making. Curing brine is administered in all products, usually done by brine injection. Even distribution of the injected brine is achieved by treating the injected meat pieces in a meat tumbler, and when no tumbler is available, "resting periods" for the meat pieces are needed. For some raw - cured products smaller amounts of curing brine are injected directly into the muscle tissue to accelerate the curing process. This fast curing technique significantly shortens curing periods, as curing substances migrate in both directions, from outside to inside and from central to less central parts. But because of this accelerated process, the curing flavor remains less intensive and texture of these products remains softer then in products are kept refrigerated. Typical products of this fast-cured type are cured - smoked pork, beef, sheep and goat meat. Fast curing with injection of curing brine will therefore remain the method of choice for rapid turn - over cured - cooked meat products only (Maddock 2008, Tyszkiewicz et al. 1996).

During wet-salting process, the curing salt solution infiltrates the meat tissue and at the same time liquid from the meat tissue is extracted by the salt surrounding the meat. Depending on the size of the meat cuts, the curing process alone can last up to several weeks for equal penetration of the meat cuts with curing salt (at temperatures of about +4°C, a pork shoulder takes about two weeks, a leg of pork about four weeks) (Tyszkiewicz et al. 1996, Xargayó et al. 2011).

As exception to the common technology of using curing salt (containing nitrite or nitrate, or a mixture of both), some well - known traditional cured - raw ham products (e.g. "*Parma Ham*" and "*Jamon Serrano*" in Spain; "*Jambon Savoie*" in France; Romanian traditional products "*Ceafa Perperlita*"), are made without nitrite, using common salt only. Although no nitrite is used, a stable red color is achieved in these cured - raw ham products. This red color derives from the natural meat color intensified by the drying and ripening process (Roşca & Cirdei 2015, Tyszkiewicz et al. 1996, Xargayó et al. 2011).

# MATERIAL AND METHODS

# Material, processing method and equipment

This paper presents experimental and theoretical research concerning a new method to obtain a cured - hot smoked boneless shoulder "*Ceafa Hituita*" product similar with traditional Romanian assortment "*Ceafa Perpelita*" made by SC AVI-GIIS SRL Mihaesti, Valcea County.

In principle, the processing technology of this traditional cured - hot smoked - cooked product type consists in: wet curing phase of entire pieces of muscle meat in 12 - 15% curing salt concentration, during 2 - 3 weeks; drying / ripening phase in cold ventilation for 6 - 8 hours; cold smoke phase (20 -  $25^{\circ}$ C) for 2 - 3 days, followed by a short

sequence of hot smoke phase (80 - 85°C), for 4 - 6 hours (Roşca & Cîrdei 2015, Simion et al. 2014).

According to Romanian legislation, sodium nitrite (NaNO<sub>2</sub>), potassium/sodium nitrate (NaNO<sub>3</sub> / KNO<sub>3</sub>), or any alternative curing substances are *not permitted* in traditional cured - cooked types products usual processing, and *no brine injection* is allowed (Roşca & Cîrdei 2015, Simion et al. 2014).

In order to produce traditional cured - hot smoked - cooked product "*Ceafa Hituita*", 2 large pieces (long pieces from symmetrical both sides) of boneless pork shoulder were used (Animal Slaughter Certificate: 18 months, 95...100 kg carcass; large farm). Each of this large piece of boneless pork shoulder was cut in 2 equal small pieces (aprox. dimensions: length x width x height =  $150 \times 70 \times 45$  mm).

To determine the efficiency of the new tenderizing and curing method pro-posed in this paper, one of these small pieces was used to make "*Ceafa Hituita*" in similar technological condition alike "*Ceafa Perpelita*" is made by SC AVI-GIIS SRL Mihaesti. The other 3 small pieces were used to obtain "*Ceafa Hituita*" made by using the proposed method.

In order to improve the final product tenderness, and in the same time to reduce as much is possible the wet curing phase, this paper propose a tenderizing method based on meat *one pressing / compression step*, *one simultaneous compre-ssion and piercing* step, followed by *intensive cyclic vacuum* process.

The pressing / compression step is realized by introducing the meat between two Teflon plates; the compression force is realized by actuating the crosshead of universal testing machines LBG 10 (Environmental Industrial Engineering Laboratory - *ElL*, within Faculty of Electrical Engineering), with maximum force 3500 N and speed 200 mm/min. Due to the compression force the meat's initial height decreases up to 20...25 mm final height. During the compression step, the testing machine's software plotted compression force - deformation diagram.

The meat's *simultaneous compression and piercing* processing is realized by using the experimental *Multi - needle compression and piercing device* (Figure 1). The experimental equipment consists in two parallel plates (W1.4571 stainless steel): the lower fixed plate with 120 holes (ø5,5); the upper mobile plate with 120 needles (ø5, 20<sup>o</sup> conical sharp, W1.4571) disposed in the same shape and reciprocity distance as into the industrial brine injection equipment (Roşca et al. 2014, UTEFIL Data Base 2005 - 2017). The meat's simultaneous compression and piercing is realized between the two plates of the *Multi - needle compression and piercing device* that is actuated by the crosshead of universal testing machines LBG 10 with maximum force 2000 N and constant speed 200 mm/min.

In actual massaging vacuum processing equipment (tumbler) the vacuum level do not exceed - 0,7 bar. Recent American and European research papers con-cerning in meat tenderizing recommend increasing the vacuum level up to - 0,95 bar (Maddock 2008, Xargayó et al. 2011).

This paper proposes the simultaneous curing and massaging of the tenderized meat by using *intensive cyclic vacuum* processing. In principle, the experimental equipment for *Intensive Cyclic Vacuum Processing (ICVP)* consists in vacuum pump and vacuum processing vessel (Figure 2). The main characteristics of the vacuum pump (HYVAC type): maximum flow rate up to 40 l/min; absolute pressure up to 50 mbar.

The vacuum processing vessel (stainless steel W1.4571; welding coef. 1) permits vacuum experiments for absolute pressure up to 0,5 mtorr. A rotative mixing device (mounted into the vacuum; stainless steel W1.4571) can be put in operation for rotational motion (1 - 30 rot / min) by a special electromechanical transmission speed

variator. To observe the inlet vessel during vacuum process, one of the flanges is made in transparent visor (high resistance polycarbonate) (UTEFIL Data Base 2005 - 2017).





Figure 1. Multi - needle compression Figure 2. Experimental equipment for ICVP and piercing device

The experimental equipment for *ICVP* and *Multi - needle compression and piercing device*, too, were designed and made by Unconventional Technologies and Equipment for Food Industry Laboratory - *UTEFIL* within Faculty of Agriculture and Horticulture, in collaboration with *EIL*.

The intensive cyclic vacuum process (*ICVP*) consists in the following steps:

- The compressed and pierced meat (each piece tenderized after pressing / compression step at 3500 N, followed by simultaneous compression and piercing as above was presented) is introduced into the rotative mixing device of the vacuum processing vessel that contain only 3% salted concentration brine (proportion 2:1 for brine and meat, respectively); 10 rot / min rotational speed was set.

- Each intensive vacuum cycle lasts 40 min consists in 20 consecutive steps, each step lasting 2 min: slow vacuuming up to - 0,85 bar (during 1 min), followed by maintaining for 1 min at this vacuum level, followed by fast de-vacuuming up to the ambient atmosphere (Figure 3).

- After finishing each 40 min intensive cyclic vacuum process, the used brine was replaced by a fresh cold one. The used brine replace lasts 3...4 min, and during this period the meat is relaxed after it was "squeezed" by the vacuuming process.

This paper presents the results obtained by using 3 types of intensive vacuum cycles:

- 40 min intensive vacuum cycle (consisting in 20 consecutive steps, each step lasting 2 min): *ICVP 3500-40*;

- 80 min intensive vacuum cycle (consisting in 40 consecutive steps, each step lasting 2 min): *ICVP 3500-80*;

- 120 min intensive vacuum cycle (consisting in 60 consecutive steps, each step lasting 2 min): *ICVP 3500-120*.

All the four meat pieces (1 pieces no tenderization - TRAD, brined in 12 % salt concentration, during 10 days; each of 3 pieces tenderized by compression, simultaneous compressed and pierced at 3500 N, followed by intensive cyclic vacuum process during 40 min, 80 min, 120 min, respectively) were ripened in cold ventilation for 6 hours, then cold smoked 22 -  $25^{\circ}$ C / 6 hours and hot smoked 80 -  $85^{\circ}$ C / 4 hours.

#### Tenderness evaluation by using Warner - Bratzler method

The most relevant and utilized methods to estimate meat's tenderness are compression test, and Warner - Bratzler shear test. During Warner - Bratzler test the shear blade acts simultaneously compression and slicing / shearing of the product (Wheeler et al. 2001, Xargayó et al. 2011, Xianzhong & Shaofang 2011).



Figure 3. Intensive cyclic vacuum process diagram

To perform inter-disciplinary researches concerning tenderness analysis, universal testing machine Lloyd Instruments LRXPlus 5 (within *UTEFIL*) was used.

Due to collaboration between *UTEFIL* and *EIL*, a Warner - Bratzler experi-mental equipment was made. In principle, this equipment consists in a special rigid frame that permits operative / fast fit and sliding of interchangeable Warner - Bratzler shear blades (UTEFIL Data Base 2005 - 2017). The experimental research performed for this paper, 100 mm/min cutting speed was set.

Mathematical model for compression strength to tenderize the meat

The compression strength behavior is represented by the internal stress within the meat when certain compression force determines the decrease of the sample height's amount (deformation during compression force action) (Graiver et al. 2009).

In this paper, a mathematical method based on MathCAD software was proposed to study the compression strength' behavior.

Therefore, to determine the deformation's variation diagrams when a certain force is applied, experimental data were used to describe the compression force matrix (Roşca et al. 2014):

$$\mathbf{M} := \begin{pmatrix} \mathbf{F}_{1}^{5} & \mathbf{F}_{1}^{4} & \mathbf{F}_{1}^{3} & \mathbf{F}_{1}^{2} & \mathbf{F}_{1} \\ \mathbf{F}_{2}^{5} & \mathbf{F}_{2}^{4} & \mathbf{F}_{2}^{3} & \mathbf{F}_{2}^{2} & \mathbf{F}_{2} \\ \mathbf{F}_{3}^{5} & \mathbf{F}_{3}^{4} & \mathbf{F}_{3}^{3} & \mathbf{F}_{3}^{2} & \mathbf{F}_{3} \\ \mathbf{F}_{4}^{5} & \mathbf{F}_{4}^{4} & \mathbf{F}_{4}^{3} & \mathbf{F}_{4}^{2} & \mathbf{F}_{4} \\ \mathbf{F}_{5}^{5} & \mathbf{F}_{5}^{4} & \mathbf{F}_{5}^{3} & \mathbf{F}_{5}^{2} & \mathbf{F}_{5} \end{pmatrix}$$

(1)

and the vectors that define the each deformation determined by each compression force

$$\mathbf{v} := \begin{pmatrix} \mathbf{d}_{I} \\ \mathbf{d}_{2} \\ \mathbf{d}_{3} \\ \mathbf{d}_{4} \\ \mathbf{d}_{5} \end{pmatrix}, \tag{2}$$

where each d := k, k + m...l, (k :=0; m := 0,5; l := 10).

After several preliminary analytical calculi, it was observed that the nearest function which approximates the experimental force - deformation diagram, is the fifth degree polynomial function defined by the relation (Roşca et al. 2014)

$$F(d) = \alpha \cdot d^5 + \beta \cdot d^4 + \gamma \cdot d^3 + \delta \cdot d^2 + \varepsilon \cdot d$$
(3)

(4)

where  $\alpha,..., \epsilon$  represent influence coefficients that have to be determined solving the relation (Roşca et al. 2014)

soln := Isolve (M, v)

The mechanical work when compression force is applied, has to be obtained solving the equation (Rosca et al. 2014)

$$W_{mec} = \int_{d_1}^{d_2} F(d) \cdot d(d)$$
(5)

#### **RESULTS AND DISCUSSIONS**

In order to determine the influence of compression process on meat's compression strength and the final product tenderness' too, the meat was stressed with 3500 N compression force; the experimental diagram is presented in Figure 4a. Using rel. (1), rel. (2) and rel. (4), for 3500 N compression force, were determined

	$(0,173^{5})$	$0,173^4$	$0,173^3$	$0,173^2$	0,173		(-1,472)
	2,175 <sup>5</sup>	2,175 <sup>4</sup>	2,175	2,175 <sup>2</sup>	2,175		0,033
M :=	4,877 <sup>5</sup>	4,877 <sup>4</sup>	4,877 <sup>3</sup>	4,877 <sup>2</sup>	4,877	soln =	0,417
	7,321 <sup>5</sup>	7,321 <sup>4</sup>	7,321 <sup>3</sup>	7,321 <sup>2</sup>	7,321		-5,212
	$9,752^{5}$	$9,752^{4}$	9,752 <sup>3</sup>	9,752 <sup>2</sup>	9,752		68.40

and after solving rel. (3), it was obtained the particular polynomial function that best describes the diagram's evolution of meat's deformation when compression force is applied

$$F(d) = -1,472 \cdot d^5 + 0,033 \cdot d^4 + 0,417 \cdot d^3 - 5,212 \cdot d^2 + 68,40 \cdot d \tag{6}$$

The force - deformation diagrams plotted by using rel. (6) for 3500 N, is presented in Figure 4b. The comparison between diagrams in Figure 4a and Figure 4b when 3500 N compression force is applied, confirms the correctness of the proposed mathematical model.

Table 1 presents the experimental mechanical work and the theoretical mechanical work obtained by using the mathematical model, when compression force 3500 N is applied.

The differences between the mechanical work determined by using experimental method and the mathematical model are smaller than 7,8%, that confirms the correctness of the proposed mathematical model. These small differences could be explained by viscoelastical deformation of the tissues' structure when meat is stressed during compression force acts.

#### Mechanical work for 3500 N compression force

Method	Mechanical work, Nmm		
Experimental	23840		
Mathematical Model	21990		





#### Table 1

To evaluate the efficiency of the 3 types of the proposed method (*ICVP 3500-40*, *ICVP 3500-80*; *ICVP 3500-120*) on the tenderness of cured - hot smoked "*Ceafa Hituita*" final product, the Warner - Bratzler shear force experimentally deter-mined for these types processing method was compared with the shear force amounts obtained for "*Ceafa Hituita*" made using traditional method (*TRAD*).

In Table 2 are presented the maximum shear force amount and the shear force average for each "*Ceafa Hituita*" type. Table 2 presents too, the decrease of percentage average of shear force, in comparison with traditional homemade "*Ceafa Hituita*"s tenderness, that demonstrates the final product tenderness' increasing.

Table 2 presents the influence of compression / pressing process, followed by simultaneous compression and piercing, and finally followed by intensive cyclic vacuum process on the tenderness of cured - hot smoked on "*Ceafa Hituita*" final tenderness': the final product tenderness' improving with 35,04% for *ICVP 3500-40*, with 41,76% for *ICVP 3500-80* and with 47,18% for *ICVP 3500-120*, respectively.

Table 2

Sample	Maximum shear force	Shear force Decrease of she	
code	min…max amount, N	average, N	force average, %
TRAD	163,40208,93	184,64	-
ICVP 3500-40	109,48139,57	119,46	35,04
ICVP 3500-80	92,11122,62	107,53	41,76
ICVP 3500-120	86,73106,49	97,52	47,18

Warner - Bratzler shear force for "Ceafa Hituita"

#### CONCLUSIONS

In order to improve the cured - hot smoked "*Ceafa Hituita*" tenderness', the proposed tenderizing method consists in compression / pressing process, followed by simultaneous compression and piercing, and finally followed by intensive cyclic vacuum process.

All these mechanical tenderization steps causes pressing and relaxation of the muscle structure and breaking up of the cells, making the membranes more permeable and increasing mobilization of the proteins up toward the surface of the muscle when the intensive cyclic vacuum process is applied. The degree to which the muscle structure is opened will determine the final quantity of proteins present in the exudates.

Due to much intensive osmosis phenomena when simultaneous compre-ssion and piercing followed by cyclic vacuum process is applied, meat's compression strength decreasing deter-mines faster brine's infusion into the meat' tissues.

The salt percentage in all the final product "*Ceafa Hituita*" made by using any one of the three types of the proposed method (*ICVP 3500-40*; *ICVP 3500-80*; *ICVP 3500-120*) was 2,6...3% (according to the analyses performed by Sanitary and Veterinary Direction, Dolj).

As one of the most recommended analyze method, the Warner - Bratzler shear force test offered objective results concerning the influence of compression / pressing process, followed by simultaneous compression and piercing, and finally followed by intensive cyclic vacuum process on final products tenderness'.

In main, the method described in this paper has two important advantages:

- Reducing the wet curing phase from 2 weeks to only 120 min (for the best results).

- Improving the tenderness of Romanian traditional cured - hot smoked pro-duct "*Ceafa Hituita*" (35,04...47,18%), in comparison with the traditional product "*Ceafa Perperlita*" made with no tenderized meat.

- Low salt content (up to 3 %) and uniform distribution in the product volume.

Each mechanical tenderizing step determines significant changes of specific strain within the meat's tissues, which determines the tenderness' improvement. Considering only physically issues, these mechanical tenderizing steps reduce the meat's mechanical characteristics amounts of the final product.

The main conclusion drawn in this paper refers the correctness of the mathematical model, proved both by the diagrams' configuration and by the intermediary or maximum amounts similarities' between the numerical tenderizing diagrams, and the experimentally diagrams.

Further general and specific conclusions could be draw after this mathe-matical model will be applied for other types of meat, before and after the same or other mechanical tenderizing methods. The data presented in this paper can be important for all the specialists interested in decreasing the wet curing period and in tenderness improvement too, of the traditional meat products.

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# DETERMINING THE HEALTH STATUS OF PLATANUS X ACERIFOLIA TREES BY MEANS OF TOMOGRAPHY

Sima Sanda (Petredeanu)<sup>1<sup>\*</sup></sup>, Baciu Aurelian Adrian<sup>1</sup>) Faculty of Horticulture, University of Craiova, 13 A.I. Cuza Street, 200585, Craiova, Romania \*) corresponding author, e-mail: sanda.petredeanu@bnro.ro

Key words: regeneration, London Plan Tree, trunk health,

#### ABSTRACT

Urban green areas, faced with increasing levels of real estate and social pressure, are suffering a decrease in quality and value. This fact draws our attention to elements that play the role of memory benchmarks, landscape values, which can define and characterize the evolution of a society. Centennial trees or trees of an esthetic, historical, memorial, and social value are also cultural landmarks that require a great deal of attention considering their value, correlated with a lack of vision in terms of protecting and preserving their historical significance and importance for the landscape. In the current context, the public or private green areas of Bucharest host a number of 110 protected trees, declared monuments of nature -- according to the List of protected trees, maintained by the Romanian Academy.

#### INTRODUCTION

The decrease in the number and size of green area accentuates environmental risks present in the urban environment, and have an immediate negative impact on the quality of life and general health of the population. This is why it is necessary to create new green areas, especially in the most crowded urban centers, or, where they already exist, to manage them properly (Chiriac et al., 2009).

Law 24/2007 on the regulation and administration of green areas in urban environments stipulates that "the State recognizes every person's right to a healthy environment, free recreational access to public green areas, the right to contribute to the setup and care of green areas, to the creation of tree and bush lines, with the observance of the current provisions of the law." The same law defines urban green areas as a mosaic-like network or a system of semi-natural ecosystems, characterized by vegetation (woods, trees, bushes, flowers, herbs), which include parks, squares, planted street linings or clear, unproductive plots within the built area.

The national program for improving the quality of the environment by setting up green areas in municipalities allowed the funding of projects for the creation of new parks, or for the rehabilitation and expansion of existing parks (Toti, 2008).

The locations and plots assigned to green areas in European municipalities depend on current management as well as the existing traditions in various cities. Considering the pressure that green areas are exposed to, their size as well as the changes they face become an important indicator in the assessment of policies planning the "urban green." But, in addition to ensuring an optimal area of greenery, European municipalities must also consider the requirements of various groups of users, the demands for the variety of forms of recreation, the improvement of the urban environment.

The most important are parks, because of their area, facilities and functions they meet (Stănescu, 2008).

A green area of this type in Bucharest includes an arboreal vegetation comprised of sycamores, maple trees, poplars, carob trees, chestnut trees, linden trees and firs. Because the area is populated by interesting specimens of centennial sycamores, belonging to the *Platanus x acerifolia* species, it is known as "Sycamore Park" (Parcul cu Platani). The land underwent several transformations, and it currently belongs to the National Bank of Romania.

The study herein aims to analyze the specimens of *Platanus x acerifolia* existent in the Sycamore Park from the point of view of their heritage value, in order to perform a complex analysis of the characteristics of each specimen and determine its health status, with the end goal of preserving and protecting this heritage.

All the on-site determinations and analyses will inform the decision-making process regarding measures of regeneration and preservation of highly valuable specimens at the Sycamore Park.

#### MATERIAL AND METHODS

The study was carried out on a pool of 47 *Platanus x acerifolia* specimens, this species lending its name to the place - Sycamore Park - with 31 of them being centennial and having various health statuses. These 31 specimens are featured on the list of protected trees, managed by the Romanian Academy.

Each specimen was analyzed individually, according to a scheme created as per the principles of Sustainable management, having an individual analysis sheet assigned, containing aspects regarding the morphology of the tree (basic anatomic elements: trunk, crown), the internal structure of the trunk (assessed in accordance with the tomographic analyses carried out), the overall health and appearance of the tree.

Before the trees were analyzed, they were coded, taking into account the acronym of the Latin name of the genus and species (Pa – *Platanus x acerifolia*) as well as the tree identification number (01, 02 etc.).

Tomographic profiles were carried out with the help of the ARBOTOM system, containing highly sensitive sensors for measuring the duration of transmission of the operator-generated impulses, so as to obtain a high-resolution diagram of the transversal arrangement of the areas affected by wood decomposition.

The number of sensors installed was established according to trunk shape and diameter. Sensor 1 was always placed towards north, so that the affected areas within the trunk could be established later. The nails used for anchoring sensors are inserted into the wood (the ritidom/bark must be fully pierced), and in order to intercept the shape of the trunk as faithfully as possible in its transversal section, the nails must be anchored in points of minimum and maximum radius to the tree axis (fig. 1). In order to obtain 2D diagrams, safety nails are installed on the same plane. The average height at which the tomographic profiles were carried out is approximately 20cm. In the case of visibly damaged specimens, two tomography sessions were carried out: one at the basis of the trunk, and one at a higher level, in order to ascertain whether the degradation is advanced or in remission.



Fig. 1. Location of sensors (user's handbook)

According to the degree of degradation, the analyzed sycamores were classified in four categories:



c – medium damage d – advanced damage Fig. 2 – Categories of degradation of the analyzed trees

#### **RESULTS AND DISCUSSION**

The analysis of the existing relations between the monitored features is of great importance, as it allows the selection of genotypes that present an association of several useful features. The value of the correlation coefficient may vary between  $\pm$  1. Values close to +1 indicate a strong positive correlation; those close to -1 indicate a strong negative correlation, whereas values close to 0 indicate the fact that there is no correlation between the two features.



Fig.3. The correlation between trunk diameter and age, for the sycamores at the Sycamore Park - NBR.

The correlation between trunk diameter (cm) and calculated age (years) shows that there is a close dependency between the two, with a highly significant correlation coefficient (r = 0.9995); between the two determinations there is a direct positive relation (fig. 3).

This proves the fact that the more a tree ages, the higher the quantity of biomass it accumulates, including the one stored in the trunk.

Yet if we study the correlation between the circumference of the trunk and its degree of degradation, we find that this correlation is positive, but with a value of r = 0.32599, meaning the degree of degradation is less influenced by trunk size (fig. 4).

A value r = 0.326874 of the correlation between the degradation of sycamore trunks and their calculated age indicates the fact that, in addition to age, there are several other elements influencing their health status (wounds -- cavities, periodic pruning methods etc).



Fig.4. The correlation between trunk degradation and trunk circumference, for the sycamores at the Sycamore Park - NBR.





Out of the total 47 specimens of sycamores analyzed, it was concluded that 8 trees (17.02%) are healthy, 18 trees (38.30%) are slightly damaged, 10 trees (21.28%) suffer medium degradation, while 11 sycamores (23.40%) suffer from advanced degradation of the trunk (table 1).

In public parks and gardens, in the green areas of boulevards and avenues as well as in green areas managed by the Public Administration, tree cutting (shearing, pruning) techniques are carried out by qualified staff, in unity and harmony with the other elements of the existing landscape architecture.

This study was carried out as it is compulsory to perform a periodical (5-10 years) identification of the physiological status of trees for the establishment and enforcement of the required works - pruning, cutting down, maintenance and regeneration - in order to render the administration and protection of the city's dendrologic fund more efficient.

#### CONCLUSIONS

1. For determining the physiological state of trees in order to establish and perform maintenance works it is recommended to perform health check-ups every 5-10 years.

2. Trees' health status can be determined by using the ARBOTOM system, which carries out tomographic profiling.

3. According to the degree of damage, established by tomography, the sycamores were grouped into four categories: healthy, slight damage, medium damage and advanced damage.

4. By analyzing the correlations between various parameters, we find various degrees of correlation.

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Table 1.

Crt No	Tree code	No. tomography sessions	Degree of degradation (%)	Trunk circumference (cm)	Trunk diameter (cm)	Calculated age years (Ø x 6,956)
1	Pa 01	2	50/40	330	105	151
2	Pa 02	1	40	395	126	181
3	Pa 03	1	31	316	101	145
4	Pa 04	2	51/35	404	129	185
5	Pa 05	1	0	312	99	142
6	Pa 17	1	35	312	99	142
7	Pa 18	1	35	267	85	122
8	Pa 19	1	0	288	92	132
9	Pa 20	1	5	253	81	116
10	Pa 21	2	29/23	218	69	99
11	Pa 22	1	16	278	88	127
12	Pa 23	1	16	2/5	78	112
12	Pa 24	1	0	305	07	130
14	Pa 24	2	22/45	303	97	110
14	Pa 20	2	23/43	202	102	147
10	Fa 30		37/40	320	67	06
10	Pa 43	1	20	210	112	90
10	F a 44	1	20	211	00	142
10	Pa 40	1	22	201	99	142
19	Pa 40	1	0	291	93	104
20	Pa 47	1	0	303	110	107
21	Pa 46	1	0/14	341	109	107
22	Pa 50	<u> </u>	9/14	316	101	145
23	Pa 51	1	3	280	91	131
24	Pa 52	1	22	270	00	127
20	Pa 53	1	10	270	00	124
20	Pa 54	<u> </u>	14/0	293	93	134
27	Pa 55	1	1	182	58	83
28	Pa 56	1	11	264	84	121
29	Pa 57	1	0	243	70	111
30	Pa 59	1	28	248	79	114
31	Pa 60	1	12	264	84	121
32		1	23	207	60 75	127
33	Pa 62	1	3	237	C1	108
34	Pa 63	1		300	04	100
35	Pa 64	1	6	264	84	121
30	Pa 65	1	23	399	127	183
37	Pa 66	1	1	258	82	118
38	Pa 67	1	2	2/3	8/	125
39	Pa 68	1	0	240	/6	109
40	Pa 69	2	54/36	393	125	180
41	Pa 73	1	13	332	106	152
42	Pa 74	1	1	3/2	118	1/0
43	Pa 75	2	35/17	342	109	157
44	Pa /6	2	19/6	255	81	116
45	Pa //	1	0	310	99	142
46	Pa 126	1	0	335	107	154
47	Pa 127	1	12	378	120	173

Summary of sycamores analyzed by tomography

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# BRIEF AGROBIOLOGICAL AND TECHNOLOGICAL CHARACTERISTICS OF NEW TABLE GRAPES CANDIDATE-VARIETIES

Simeonov Iliyan<sup>1\*</sup>, Ivanov Miroslav<sup>1</sup>, Nakov Zdravko<sup>1</sup>, Iliev Anatoly<sup>1</sup>

\* Institute of Viticulture and Enology, Pleven, Bulgaria, e-mail: iliannsimeonov@gmail.com

Key words: vine, table grapes candidate-varieties, agrobiological and technological characteristics

#### ABSTRACT

An ampelographic study on the newly-selected Vit, Nayden and Miro table grapes candidate varieties at the Insitute of Viticulture and Enology, Pleven was carried out. The study found that, according to the time of the consumer's maturity of the grapes, the Vit and Miro varieties belong to the group of early maturing, while Nayden to the group of medium-ripening varieties of vines. The vines of newly selected table grapes candidate varieties are characterized by medium to strong growth, good habits, high actual fertility and productivity, which are relatively constant in years. By mechanical composition of the grapes, the candidate varieties studied are typically dessert, with specific characteristics of the grapes and grains, suitable for fresh consumption. Candidate varieties Vit and Nayden obtained by intra-hybridization are characterized by high sensitivity to low winter temperatures. They can be grown in all winegrowing regions of the country where the risk of freezing of vines is less. The candidate variety Miro, obtained through the interspecific hybridization, has a genetically determined increased resistance to low winter temperatures, mana and oidium and can be grown in all wine-growing regions of the country. As regards of the appearance and organoleptic profile of the grapes, it closely matches to the best table grapes varieties of Vitis vinifera L.

#### INTRODUCTION

As a result of mankind development, the selection of vine varieties from their natural habitats and their propagation and distribution, a large number of varieties have been grown nowadays, as their main set is specific for each country. These varieties differ in their morphological signs, agrobiological features and technological gualities, and their grapes could be used for various purposes (Ivanov, 2011). The soil and weather conditions in Bulgaria are extremely favourable for planting and growing vineyards with table grapes varieties. That is one of the reasons for the growing interest of the viticultural science in the country to expand the available gene pool of table grapes varieties through comprehensive selection-genetic research. Grapes quality and the products derived from it has been directly dependent on the variety as a means of production with its specific ampelographic features and the complex of factors of the environment and the production conditions by which the soil and climatic resources of nature had been used in the most rational way (Katerov et al., 2005). The combination of the natural conditions and the potential of the varieties was a prerequisite for grapes production of individual guality, superior appearance and excellent organoleptic qualities (Todorov, 1998).

Recent studies have shown that, on the background of the constantly changing market situation of grapes and wine, the increasing agrotechnical level and the dynamical climate changes, the variety structure in Bulgaria has not been flexible enough with prevailing varieties whose grapes ripen from mid-August to mid-September (Bachvarova et al., 2007, Krumov, 2014). That revealed the need of creating and introducing new vine varieties with valuable biological and economic qualities, increasing the share of early and late ripening seeded and seedless varieties with big berries. The experience of the global viticulture science has demonstrated that even today, gender hybridization has been the most current and efficient selection method for obtaining new diverse grapevine varieties (Valchev, 1978, 1990; Stoev, 1984; Alleweldt & Possingham 1988). As a result of the achievements in the field of vine genetics and selection, a number of new valuable table grapes and wine varieties have been created in Bulgaria in the recent decades (Lazarov et al., 2004).

The objective of this study was to make a brief economic characteristic of the newly-selected at IVE – Pleven table grapes candidate-varieties.

#### MATERIAL AND METHODS

The candidate-varieties Vit, Nayden (intra-species varieties) and Miro (interspecific variety) has been object of the study. The ampelographic investigation was carried out during the period 2014-2016 in vineyard (hybrid section) at the Experimental base of IVE-Pleven. The vines were grown on improved Guyot training system, at planting distance 2.20/1.30 m, grafted to Berlandieri x Riparia SO4 rootstock. For the ampelographic investigation of the variety the methodology described in Bulgarian Ampelography volume 1 (Katerov et al., 1990) was used. It included the following indicators:

- Botanical description of a cluster and a berry;

- Agrobiological characteristics – Phenological observations – budding, flowering, interspersing, consumption maturity; Actual fertility – developed eyes ratio, fruit shoots ratio, number of clusters, actual fertility rate; average yield per vine, kg.

- Mechanical analysis of a cluster and a berry – mass and size of the cluster and the berry, ratio of rachis, seeds, skin, mesocarp, theoretical yield, tearing and pressure;

- Technological characteristics – Chemical composition – sugars content (%), and titratable acids (g/dm<sup>3</sup>).

#### **RESULTS AND DISCUSSIONS**

# Candidate-variety V 6-18 (Vit)

Candidate-variety V 6-18 (Vit) was obtained by intra-species hybridization from the crossing of Dunav x Kondarev 10 varieties at the Experimental base of the Institute of Viticulture and Enology - Pleven by M. Ivanov, Z. Nakov and I. Simeonov.

#### **Botanical description**

**Cluster.** The cluster is large (26.7/16.5 cm), conical, loose to semi-compact. The stem is medium long, medium thick. The rachis is green. The average mass per cluster is 785.5 g (Fig. 1).

**Berry.** The berry is large (28.46/18.96 mm), egg-shaped. The skin is dark blue, thin and fragile, with wax coating. The texture is fleshy-juicy. The taste is harmonious, neutral. The average mass per 100 berries is 633.3 g.

#### Agrobiological characteristics

Candidate- variety V 6-18 (Vit) is an early ripening red seedless table grapes variety. Its grapes ripen at the end of August. The vegetation period duration is about 150 days. The vines of candidate-variety Vit have medium to intense growth, good

habitus and abundantly covered with foliage. At ground and stem training systems the candidate-variety has good fertility and yield, relatively constant per years. Vit is not liable to putting forth catkins and milerandage and it is distinguished for very good pollination and fertilization. The candidate-variety Vit is obtained by intra-species hybridization and it is susceptible to low winter temperatures, powdery mildew, downy mildew and gray rot. At ground and stem cultivation it could be applied improved Guyot training with cane length of 14-16 eyes. For grapes quality improvement green pruning treatments should be done – suckering and removal of part of the leaves around the clusters.



Figura 1. Candidate-variety V 6-18 (Vit)

# **Technological characteristics**

According to the grapes mechanical composition the candidate-variety is a typical tables grape variety. The berries ratio in the cluster is 97.50%, of the rachis – 2.50%. The skins in the berries are 3.41% and mesocarp – 96.59. At consumption maturity the grapes contain 15-17% sugars and 5-6 g/dm<sup>3</sup> titratable acidity. The grapes are suitable for fresh consumption and meeting the market demand for quality seedless table grapes during the second half of August.

# Candidate-variety V 8-2 (Nayden)

Candidate-variety V 8-2 (Nayden) was obtained by intra-species hybridization from the crossing of Dunav x Rusalka 3 varieties at the Experimental base of the Institute of Viticulture and Enology - Pleven by M. Ivanov, Z. Nakov and I. Simeonov.

# **Botanical description**

**Cluster.** The cluster is large (19.2/17.2 cm), conical, loose to semi-compact. The stem is medium long, medium thick. The rachis is green. The average mass per cluster is 817.7 g (Fig. 2).



Figura 2. Candidate-variety V 8-2 (Nayden)

**Berry.** The berry is large to very large (38.69/18.10 mm), drop-like (specific). The skin is deep purple, thin and delicate. The texture is fleshy, crisp, and the taste – neutral, harmonious. The berry stem is medium, with middle bed. The rachis is medium long, pale green. The average mass per 100 berries-873.3 g.

# Agrobiological characteristics

Candidate-variety V 8-2 (Nayden) is medium ripening red seedless table grapes variety. In the Pleven region its grapes ripen early in September. The vegetation period duration is about 170 days. The vines of candidate-variety Nayden have medium to intense growth, good habitus and plenty of foliage. At ground and stem training systems the candidate-variety is characterized with good fertility and yield, constant per years. Nayden is not liable to putting forth catkins and milerandage and it is distinguished for very good pollination and fertilization. The candidate-variety is susceptible to low winter temperatures and downy mildew but it is relatively resistant to powdery mildew and gray rot. V 8-2 (Nayden) might be grown at ground and stem improved Guyot training system with cane length of 14 to 16 eyes. For grapes quality improvement green pruning treatments should be done during the vegetation – suckering and removal of part of the leaves around the clusters.

# **Technological characteristics**

According to the grapes mechanical composition candidate-variety Nayden is a typical tables grape variety. The berries ratio in the cluster is 97.06%, of the rachis – 2.94%. The skins in the berries are 4.08%, the seeds 1.38%, and mesocarp – 94.54%. At consumption maturity its grapes contain 14-16% sugars and 5.5-6.5 g/dm<sup>3</sup> titratable acidity. The grapes of the candidate-variety are suitable for fresh consumption and storage. It has very good transportability. After storage it keeps its appearance and taste qualities.

# Candidate-variety V 1-40 (Miro)

Candidate-variety V 1-40 (Miro) was obtained by interspecific hybridization from the crossing of Plevenski Favorit x Nadezhda varieties at the Experimental base

of the Institute of Viticulture and Enology - Pleven by M. Ivanov, Z. Nakov and I. Simeonov.

# **Botanical description**

**Cluster.** The cluster is very large (23.0/16.5 cm), cylindrical-conical, semicompact. The stem is medium long, thick, woody at the base. The rachis is green. The berry stem is medium long, thick with average large cone-like bed. The average mass per cluster is 862.8 g (Fig. 3).

**Berry.** The berry is very large (32.07/23.15 mm), oval. The skin is greenishyellow in colour, thin and delicate, with wax coating. Mesocarp texture is fleshy-juicy. The taste is harmonious and neutral. The average mass per 100 berries is 955.0 g.

#### Agrobiological characteristics

Candidate-variety V 1-40 (Miro) is early ripening white table grapes variety. Its grapes ripen in late August. The vegetation period duration is about 150-160 days. The vines of candidate-variety Miro have medium to intense growth and plenty of foliage. At ground and stem training systems it is distinguished with very good fertility and yield, constant per years. The candidate-variety Miro is not liable to putting forth catkins and milerandage and it is characterized by very good pollination and fertilization. The candidate-variety Miro has higher resistance (1-2 scores) to fungal diseases compared to the control varieties belonging to *Vitis vinifera L*. It has high resistance to low winter temperatures and downy mildew and very good restoring capacity after frost. At ground and stem cultivation it could be applied improved Guyot training system with cane length of 14 to 16 eyes. For grapes quality improvement green pruning treatments should be done during the vegetation – suckering, pinching off and removal of part of the leaves around the clusters.



Figura 3. Candidate-variety V 1-40 (Miro)

# **Technological characteristics**

According to the grapes mechanical composition candidate-variety V 1-40 (Miro) is a typical tables grape variety. The berries ratio is 97.28%, of the rachis – 2.72%. The skins from the structure of the berries are 3.54%, the seeds 1.31%, and

mesocarp – 95.02%. At consumption maturity its grapes contain 15-16% sugars and 5.5-6.5 g/dm<sup>3</sup> titratable acidity. The grapes of the candidate-variety Miro are suitable for fresh consumption and storage. It has very good transportability. After storage it keeps its appearance and taste qualities.

#### CONCLUSIONS

According to the term of grapes consumption maturity, the candidate-varieties Vit and Miro belong to the group of early ripening while Nayden to the medium ripening grapevine varieties.

The vines of the newly selected table grapes candidate-varieties are characterized by medium to intense growth, good habits, high actual fertility and productivity, relatively constant per years. In grapes mechanical composition the candidate-varieties are typically table grapes, with specific characteristics of the cluster and the berries, suitable for fresh consumption.

The candidate-varieties Vit and Nayden, obtained by intra-species hybridization are characterized by high susceptibility to low winter temperatures. They can be grown in all vine-growing regions of the country where the risk of frost is smaller.

The candidate-variety Miro, obtained by interspecific hybridization, has a genetically determined increased resistance to low winter temperatures, downy mildew and powdery mildew and can be grown in all the vine-growing regions of the country. Regarding the appearance and organoleptic profile the grapes closely rivals the best table grapes varieties of Vitis vinifera L.

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#### CULTIVAR INFLUENCE UPON LETTUCE PRODUCTION, CULTIVATED IN AUTUMN IN AN ECOLOGICAL SYSTEM

Ştef Adrian Valeriu<sup>1</sup>, Apahidean Alexandru Silviu<sup>1</sup>, Cărbunar Mihai<sup>2</sup>, Bei Mariana<sup>2</sup>, Apahidean Alexandru Ioan<sup>1\*</sup>, Domocoş Daniela<sup>1</sup>

<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, 3-5 Mănăştur Street, Cluj-Napoca, 400372, Romania, <sup>2</sup>University of Oradea, Faculty of Environmental Protection, B-dul Gen.Magheru 26,.Oradea, Romania Corresponding author. E-mail: \*alexandru.apahidean@usamvcluj.ro

Key words: garden lettuce, cultivar, planting age, production

#### ABSTRACT

Garden lettuce is grown for the head and leaves, which are mainly consumed in green salads, simple or combined with other green vegetables. It is important in nutrition due to its high content in vitamins and mineral salts. It is cultivated in the field, early spring and autumn, as well as in greenhouses and polyethylene tunnels, to obtain production during winter-spring or autumn-winter period. Being a species with a short period of vegetation is cultivated in the system of successive crops, before or after a basic crop, as well as in associated crops. In temperate climate, salad culture is not practiced during the summer because in long day conditions associated with high temperatures, plants emit floral stems in most varieties. The experience was carried out in 2016, in the western part of Romania, in Husasău de Tinca, county of Bihor, where 14 salad varieties were grown in an ecological system.

#### INTRODUCTION

Garden lettuce is grown for leaves and heads that are used in fresh or prepared form. It is demanded by consumers throughout the year, being cultivated both in the field and protected areas in successive or associated crops.

Lettuce heads and leaves contain 4-8% dry substance, 2-3.5% carbohydrates, 1-1.6% protides, vitamins B1, B2, C (5-20 mg) P and E, carotene (1-3 mg) and mineral salts: potassium-260 mg, iron 1.2-1.7 mg, calcium, phosphorus - 40 mg, magnesium - 24 mg, per 100 g of fresh product. Leaves also contain latex, which prints the bitter taste (Gherghi et al., 2002). It is a lightly digestible, low caloric intake (16-20 calories/100 g), corresponding to a dietary regimen. Lettuce that gets through winter in open field has a higher content in vitamins than in forced or spring crops (Apahidean & Apahidean, 2016). Consumed fresh, lettuce contributes to the revitalization of muscle tissue, brain and nerves, maintains the fluidity of the blood, is a good diuretic, stimulates appetite, etc. (Soare & Duţă, 2008). Lettuce consumption reduces the risk of heart disease, cancer and cataracts. It is rich in fibres that help reduce cholesterol (Lagunovschi-Luchian & Vânătoru, 2016).

Lettuce because it has a high nitrate value, over 2500 mg  $NO_3/kg$ , is included in the group of high nitrate vegetables. This level is influenced by both genetic and

ecotoxic factors, so measures can be taken to obtain productions with lower nitrate and nitrite content (Soare & Duță, 2008).

Lettuce is a long day plant, conditions in which it has a short period of vegetation and forms floral stems before forming the head. Sensitivity to the length of the day occurs between the end of May and the first half of June, during which the length of the day is over 14 hours (Still, 2007). Plant growth can be influenced by various physiological treatments (Okuda et al., 2017). Under short conditions, it forms a rich foliage and large head, which do not pass into the floral stem phase (Ciofu et al., 2003; Posta, 2008).

Optimal temperature for leaf growth and head formation is around 16°C, and for the formation of floral stems and flowers, 20-22°C (Stan et al., 2003).

Lettuce production is influenced by the cultivar used, the culture period and the culture system (Stoleru, 2013). At a protected crop during the spring, with different cultivars, Szabo et al., 2010, obtained plants with an average weight of 353.0 to 474.0 g/plant. The average yield was 46.0 t/ha and the maximum yield of 54.77 t/ha was achieved by the Limax cultivar.

#### MATERIALS AND METHODS

Experience was carried out in 2016, in the western part of Romania, in Husasău de Tinca, county of Bihor where a lettuce culture was carried out in an ecological system.

Village is located in the Miersig Plain, which consists of a higher stretch of flats with a slight slope and terraces located below the Hidişelului (Taşadului) Hills and another lower, in the west, close to Criş river channel. Northern and southern boundaries are given by Crişului Repede and the Crişului Negru Meadow. South-western boundaries are between Colector-Culiser and Crisul Negru, where a low-lying area, extending at 90-105 m, which extends to the low plain of Crişul Negru, the approximate limit passing south of the Tica-Tulca.

Biological material consisted of 14 salad varieties of several types, recommended for the organic farming system:

- *Roman Lettuce* – represented by the following varieties: Dark Green, Blonde Maraichere, Blonde Lente a Monter;

- Letuce type Batavia – represented by the following varieties: Long Stading Batavian, Jester;

- Leaf lettuce – was represented by Lola Rosa and Lola Bionda;

- *Head forming lettuce* - Anueme, Gloire de Mantes, Merveille de 4 Saison, Grass Blonde Peresuble, Laituie Silvesta and Laituie Appia;

*Dark Green* - roman salad that forms a lax head of light green to dark green. Leaves are fleshy with a clear, crisp, and very savory main nervure.

*Blond Maraichere* - roman salad, which forms a tall, elongated but rather large head. Outside leaves are light green.

Blonde lente a Monter - roman salad that forms a compact head. Leaves have shape of a spatula, long petiolate pale green. It has a good headdress even in the warm summer conditions.

Long Standing Batavian - Batavian salad with a bulky and high head. Leaves are very thick in light green color. It is one of the most resistant batavian salads in the issue of floral stems.

*Jester -* is a Batavian salad with a very beautiful look. It forms a large rosette of very voluminous leaves. Leaves are large, half-embossed, toothed edges, bright green pigmented with reddish spots. leafs are crisp and tasty.

Anuenue - is a salad that has the ability to grow up in warm soil. Leaves are bright green. It is adapted to high temperatures.

*Kwiek* - is a vigorous variety with green leaves with a slight reddish tinge. Forms a medium-sized head.

*Gloire De Nantes* - forms a thick head with slightly light-colored leaves, light green. It resists well at high temperatures.

*Merveille of 4 Saisons* - is a fast growing and vigorous variety. It forms an elongated colored ruby head to light red. It can be cultivated in all seasons, but it prefers more spring and summer.

*Lollo Rossa* - is a salad with brown green leaves and the red hot tip. It's very tasty. It resists the heat.

Lollo Bionda - forms a light curly green leaf rosette.

*Grosse Blonde Paresseuse* - forms a large flattened head. Leaves are light green and the interior leaves are pale green. It resists the heat.

*Laituie Sylvesta-* is a bright green rustic head salad. Leaves are crisp and very tasty.

*Laituie Appia* - is a kind of salad of green head, with finely crispy, glossy, very tasty leaves. Resistant to the issue of floral stems.

Culture was set up in autumn in two different epochs and in two different culture systems (protected and unprotected). For the first epoch, planting took place on 12.09.2016, in the field, and the second epoch was set up in a polyethyelene tunnel, planting the seedlings on 15.10.2016. Culture was established by seedling produced in alveolar trays with 104 cells per tray. The soil mix for sowing was made of peat and peat (with neutral pH). Planting was done 35 days after sowing. The planting distances were 30 cm both between the rows and between the plants per row. 15 days after planting a nettle macerate treatment was performed. Harvesting took place between October and November, staggered according to variety and place of culture.

#### **RESULTS AND DISCUSSIONS**

Field crop production in autumn crops was between 2.70 kg/m<sup>2</sup> for Anuenue variety and 3.73 kg/m<sup>2</sup> for Dark Green variety (Table 1). Compared with experience average, the varieties of Anuenue, Kwiek, Lollo Rossa, Lollo Bionda, Gloire de Nantes, Merveille des 4 Saisons, Grosse Blonde Paresseuse and Laitue Silvesta produced lower yields. Dark Green, Blonde Maraichère, Blonde Lente à Monter, Long Standing Batavian, and Laitue Pomme Appia exceeded the average with 5.28-23.10%.

Dark Green variety produced a yield of  $3.73 \text{ kg/m}^2$ , the difference in production compared to the control being very significant and in Blonde Lente à Monter the production was  $3.56 \text{ kg/m}^2$ , with a distinctly significant production difference (Table 1).

Polyethylene tunnel crop production ranged from 3.23 kg/m<sup>2</sup> at Kwiek and 4.32 kg/m<sup>2</sup> in Dark Green variety (Table 2). In comparison with the average of the experience, the varieties Anuenue, Kwiek, Lollo Rossa, Lollo Bionda, Gloire de Nantes, Merveille des 4 Saisons, Grosse Blonde Paresseuse and Laitue Silvesta produced lower yields. Dark Green, Blonde Maraichère, Blonde Lente à Monter, Long Standing Batavian, and Laitue Pomme Appia exceeded the average with 5.11-16.13%.

Dark Green variety obtained a production of 4.32 kg/m<sup>2</sup>, the difference in production compared to control being very significant, and at Blonde Lente à Monter variety, the production was 4.25 kg/m<sup>2</sup>, with a distinct difference in production (Table 2). A significant difference in production was also recorded in at Laitue Pomme Appia variety.

#### Table 1

Variant	Average r	Average production Difference to		Significance
	ka/m <sup>2</sup>	%	culture average	of the
	Ng/III	70	(kg/m <sup>2</sup> )	difference
Dark Green	3.73	123.10	0.70	***
Blonde Maraichère	3.42	112.87	0.39	*
Blonde Lente à Monter	3.56	117.49	0.53	**
Long Standing Batavian	3.19	105.28	0.16	-
Jester	2.90	95.70	-0.13	-
Anuenue	2.70	89.10	-0.33	0
Kwiek	2.73	90.09	-0.30	0
Lollo Rossa	2.75	90.75	-0.28	0
Lollo Bionda	2.77	91.42	-0.26	0
Gloire de Nantes	2.74	90.43	0.29	0
Merveille des 4 Saisons	2.81	92.74	0.22	-
Grosse Blonde	2.87	94.72	0.16	-
Paresseuse				
Laitue Silvesta	2.90	95.70	0.13	-
Laitue Pomme Appia	3.40	112.21	0.37	*
Average	3.03	100.00	-	-
	Variant Dark Green Blonde Maraichère Blonde Lente à Monter Long Standing Batavian Jester Anuenue Kwiek Lollo Rossa Lollo Bionda Gloire de Nantes Merveille des 4 Saisons Grosse Blonde Paresseuse Laitue Silvesta Laitue Pomme Appia Average	VariantAverage p kg/m²Dark Green3.73Blonde Maraichère3.42Blonde Lente à Monter3.56Long Standing Batavian3.19Jester2.90Anuenue2.70Kwiek2.73Lollo Rossa2.75Lollo Bionda2.77Gloire de Nantes2.74Merveille des 4 Saisons2.81Grosse Blonde2.87Paresseuse2.90Laitue Silvesta2.90Laitue Pomme Appia3.40Average3.03	VariantAverage production kg/m²Dark Green3.73123.10Blonde Maraichère3.42112.87Blonde Lente à Monter3.56117.49Long Standing Batavian3.19105.28Jester2.9095.70Anuenue2.7390.09Lollo Rossa2.7590.75Lollo Bionda2.7791.42Gloire de Nantes2.8192.74Grosse Blonde2.8794.72Paresseuse	VariantAverage production kg/m²Difference to culture average (kg/m²)Dark Green3.73123.100.70Blonde Maraichère3.42112.870.39Blonde Lente à Monter3.56117.490.53Long Standing Batavian3.19105.280.16Jester2.9095.70-0.13Anuenue2.7089.10-0.33Kwiek2.7390.09-0.30Lollo Rossa2.7590.75-0.28Lollo Bionda2.7791.42-0.26Gloire de Nantes2.8192.740.22Grosse Blonde2.8794.720.16ParesseuseLaitue Silvesta2.9095.700.13Laitue Pomme Appia3.40112.210.37Average3.03100.00-

Cultivar influence on the production of lettuce cultivated in the autumn in the field

DL P5% 0,25; DL P1% 0,47; DL P 0,1% 0,62

Table 2

Cultivar influence upon production of lettuce cultivated in autumn in polyethylene tunnel

Variant	Average p	roduction	Difference to	Significance of
	kg/m²	%	culture average	the difference
	-		(kg/m <sup>2</sup> )	
Dark Green	4.32	116.13	0.60	***
Blonde Maraichère	3.91	105.11	0.19	-
Blonde Lente à Monter	3.97	106.72	0.25	*
Long Standing Batavian	4.25	114.24	0.53	**
Jester	3.73	100.26	0.01	-
Anuenue	3.51	94.35	-0.21	-
Kwiek	3.23	86.82	-0.49	00
Lollo Rossa	3.58	96.73	-0.14	-
Lollo Bionda	3.45	92.74 -0.27		0
Gloire de Nantes	e Nantes 3.26 87.63		-0.46	00
Merveille des 4 Saisons	3.57	95.96	-0.15	-
Grosse Blonde Paresseuse	3.58	96.23	-0.14	-
Laitue Silvesta	3.65	98.11	-0.07	-
Laitue Pomme Appia	4.13	111.02	0.41	**
Average	3.72	100.00	-	-
DL P5% 0,24; D	LP1% 0,39	; DL P 0,19	% 0,55	

Table 3

Variant	Average prod	uction	Difference to	% field production
	Polyethylene	Open	culture	compared to
	tunnel	Field	average	polyethyelene
			(kg/m²)	tunnel
Dark Green	4.32	3.73	0.59	86.34
Blonde Maraichère	3.91	3.42	0.49	87.46
Blonde Lente à Monter	3.97	3.56	0.41	89.67
Long Standing	4.25	3.19	1.06	75.05
Batavian				
Jester	3.73	2.90	0.83	77.74
Anuenue	3.51	2.70	0.81	76.92
Kwiek	3.23	2.73	0.50	84.52
Lollo Rossa	3.58	2.75	0.83	76.81
Lollo Bionda	3.45	2.77	0.68	80.28
Gloire de Nantes	3.26	2.74	0.52	84.04
Merveille des 4	3.57	2.81	0.76	78.71
Saisons				
Grosse Blonde	3.58	2.87	0.71	80.16
Paresseuse				
Laitue Silvesta	3.65	2.90	0.75	79.45
Laitue Pomme Appia	4.13	3.40	0.73	82.32

Cultivar influence on the production of lettuce cultivated in autumn in open field and polyethylene tunnel

Analyzing the behavior of the 14 cultivars in the two crop systems (protected and unprotected), it was found that in the protected crop system, yields were higher with  $0.5-1.6 \text{ kg/m}^2$  compared to the unprotected crop system (Table 3).

Dark Green, Blonde Maraichère, Blonde Lente à Monter, Kwiek, Lollo Bionda, Gloire de Nantes, Grosse Blonde Paresseuse and Laitue Pomme Appia produced at least 80% unprotected crop production compared to the protected system.

#### CONCLUSIONS

Based on the results obtained from the research on lettuce grown in open field and in polyethylene tunnel, in the specific conditions in the western part of Romania, in ecological system, using 14 cultivation, the following conclusions were drawn:

- crop production in the field, in autumn culture, was between 2.70 kg/m<sup>2</sup> for the Anuenue variety and 3.73 kg/m<sup>2</sup> for the Dark Green variety;

- production of autumn lettuce in polyethylene tunnel ranged from 3.23 kg/m<sup>2</sup> at Kwiek and 4.32 kg/m<sup>2</sup> in Dark Green variety;

- In protected crop system, yields were higher by 0.5-1.6 kg/m<sup>2</sup> compared to the unprotected crop system, depending on the cultivar;

- Dark Green, Blonde Maraichère, Blonde Lente à Monter, Kwiek, Lollo Bionda, Gloire de Nantes, Grosse Blonde Paresseuse and Laitue Pomme Appia have obtained a yield in unprotected crop system of at least 80% compared to the protected culture system.

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#### ✓ Environmental engineering

#### ON VINEYARD CULTIVATION IN ECOLOGICAL SYSTEM IN DEALUL BUJORULUI VINEYARD UNDER THE CLIMATIC CONDITIONS OF 2016

Tabaranu Gabriel<sup>1</sup>, Viorica Enache<sup>1</sup>, Donici Alina<sup>1</sup>

<sup>1\*</sup> Research Station and Development for Vineyard and Vinification Bujoru, România, e-mail: gabitabaranu@yahoo.com

Key words: grapevine, pathogens, vineyard

#### ABSTRACT

The research was carried out at the Bujoru Viticulture and Wine Growing Research and Development Stage between 2015-2016. The paper presents the results of the research on vine cultivation in ecological system. In the technology of vine cultivation, phytosanitary protection is an important technological link for obtaining superior quantitative and qualitative productions. The researches focused on the analysis of the disturbing factors in the ecological cultivation in the ecological viticultural ecosystem. Pathogens were monitored by field observations and notations to determine the frequency (F), intensity (I) and attack rate (G.A%). The recorded data were correlated with the climatic conditions specific to the Dealul Bujorului vineyard.

#### INTRODUCTION

After entry of pests and diseases in the Americas growing countries in Europe, growers, both practitioners and researchers alike have sought ways and means to prevent and fight as effectively. Achieve high yields and good quality viticulture involves the correct and optimal timing of all technological links, where it occupies an important place against pathogens and pests.

The emergence and evolution of the main pathogens and pests of wine grapes in the vineyard area Dealu Bujorului is influenced by direct and indirect effects of technological and ecological factors specific area, affecting the quality and quantity of grape production (Mirică I. *et at*, 1976; Tabaranu G. *et al*, 2005, 2007,2016; Teodorescu Georgeta *et at*, 2003).

#### MATERIAL AND METHODS

The researches were conducted at the Research and Development Station for Viticulture and Winemaking Bujoru in a vineyard cultivated with Merlot and Feteasca regala aged 37 years.

Location experiment was conducted on land with a slope of 3-5% chernozem soil type with a humus content between 1.14 to 1.86% in the A horizon and a weak alkaline reaction (pH 7,44 to 8,30) and sandy loam; exhibition eastern land, about 140-170 m altitude, orientation north-south rows, planting distance of 2,1 m x 1,2 m, density provided 3968 vines / hectare Berlandieri x Riparia rootstock selection Telecky Openheim SO4-4.

The organization experience of comprised the following varieties:

- Merlot ecological technology ;
- Feteasca regala ecological technology;
- Merlot conventional technology;
- Feteasca regala conventional technology

For the determine the gravity of the attack produced by the main vine diseases of (manna, powdery mildew, gray rot) have been commented on the intensity (I) the frequency (F) and degree of attack (AD/GA%) of their leaves and grapevine.

#### **RESULTS AND DISCUSSIONS**

Research has focused on technology experience of growing vines in the ecological system, analyze quality grape production obtained and tested for efficacy using various techniques and products to control pathogens and pests in organic vineyards. To characterize the specific vineyard microclimate conditions Dealu Bujorului were used weather data recorded at the meteorological station at Research Station and Development for Vineyard and Vinification Bujoru.

From the analysis of the main factors climate during the growing season (dezmugurit - defoliation) compared to multi-media mode (thermal, fluid, air humidity, insolation), show that the condition of their vegetative development, production, its quality and aggression of pathogens. Average temperatures in most months of the vegetation period were close to normal, with the exception of April when average temperatures were recorded higher than normal values (Table 1).

Table 1

The monthy	onthy		Amount degrees of temperature					
Months,	aver tempe	age rature	Global		Acti	ve	Effective	
year	the norm al	IV-IX 2016	the normal	IV-IX 2016	the normal	IV-IX 2016	the normal	IV-IX 2016
IV. 2016	11.7	13.0	349.5	389.8	278.8	338.3	78.06	98.3
V. 2016	18.1	15.3	560.4	473.4	555,9	473,4	257,5	16,4
VI. 2016	21,8	21,3	654,1	639,3	654,1	639,3	353,9	339,3
VII. 2016	24,1	22,9	744,2	709,2	744,2	709,2	435,0	399,2
VIII. 2016	23,1	22,3	698,6	690,5	716,7	690,5	406,6	380,5
IX. 2016	17,5	17,6	525,6	528,3	523,7	519,1	226,2	229,1

Thermal regime during April – September 2016

Highest temperature was recorded in August and 35.8°C in air. It is also worth noting that in these months, there was a large number of days with maximum temperatures above 30°C (75 days) as follows: 14 days in June, 24 days July 23 days in August and 14 days in September. September was warmer than normal, the average temperature was 17.6°C to 17.5°C annual average. The amounts degrees of global temperature (global  $\Sigma^{\circ}$ C), active (active  $\Sigma^{\circ}$ C) and output ( $\Sigma$  useful °C) during the growing season were much lower than the multi-values (Table 1). Thus, the entire vegetation period of the overall heat balance was 3430.5°C to 3532.3°C multi-value, the active heat balance was 3369.8°C to 3473.4°C, and useful thermal balance, was of 1462.8°C to 1757.26°C. Rainfall recorded during the growing season have been very unevenly distributed, so were months when there have been very small amounts, well below the normal value, such as July and August (Table 2) and Monday the s-recorded greater amounts than normal: April, May (63.4 mm to 48.0 mm) and

June. The amount of rainfall during the growing season (April-September) was 319.0 mm against the normal 256.0 mm SCDVV Bujoru (Table 2).

Months,	Precipitations (mm)		The number of days with rain	Hygroso (%	Hygroscopicity (%)		Heatstroke (hours)	
year	the	IV-IX	> 10	the	IV-IX	the	IV-IX	
	normal	2016	>10	normal	2016	normal	2016	
IV. 2016	36.5	66.2	3	67.3	68.4	162.4	181.1	
V. 2016	48.0	63.4	3	64.3	70.2	232.7	226.6	
VI. 2016	72.1	74.2	2	64.6	70.0	236.0	238.1	
VII. 2016	50.5	12.2	1	62.4	57.7	266.2	333.0	
VIII. 2016	48.6	38.0	1	63.0	58.9	246.0	274.6	
IX. 2016	40.3	65.0	2	69.3	63.1	172.4	196.1	

Weather data from the period april - september 2016

Table 2

Under these conditions and relative air humidity values were much higher than the annual average 68.4% in April, 70.2% in May and 70.0% in June (Table 2). Heatstroke measured by the number of hours of sunshine was higher than normal in all months during the growing season, with a total of 1449.5 hours of 1315.7 hours SCDVV Bujoru normal value. Regarding risk factors during the growing season, we can say that there was a total of 75 days with maximum temperatures above 30°C, the combined amount of low rainfall in July and August led installing phenomenon drought soil and air.Achieving high yields and good quality in viticulture, requires proper and timely implementation of all links technology, in which an important place occupied by combating pathogens and pests. In developing regimen for green variants took into account the sensitivity of the two varieties studied, the economic damage threshold, and restrictions imposed by legislation (Table 3).

In the climatic conditions of 2016 have been alerted and made seven treatments to combat mildew of the vine, 6 treatments to combat and vine downy mildew traps 3-6 / synthetic sex pheromone generation type for the control of grape moth Atrabot (Figure 1).



Figure 1. Traps with pheromones type Atrabot

**The vines manna** (*Plasmopara viticola* - Berk. Et Curt.): favorable weather conditions (precipitation frequency, high air humidity, etc.) have the appearance and development of the pathogen to the compaction of the bloom clusters. In this case, fungicides used were protected vines, limiting the degree of attack (GA%). Thus, Merlot and Fetească regală - the degree of attack organic solution was slightly higher compared to conventional alternatives, both on the leaves and grapes (Table 5).

Table 3

Date of treatment	Phenological stage	Pathogens and pests controlled	Plant protection product use	Dose Kg., lt./ha
19 april	Shoots 2-6 cm	Oidium Eudemis G a I-a	Sulfomat 80 PU Traps with pheromones type Atrabot	4,00 3 buc.
11 may	Shoots 30-50 cm	Manna Oidium	Champ 77 WG Sulfomat 93 P	3,00 10,00
31 may	Before blooming	Manna Oidium	Champ 77 WG Sulfomat 93 P	3,00 10,00
17 june	Immediately after flowering	Manna Oidium Eudemis G a-II-a	Bouille bordelaise Sulfomat 93 P Traps with pheromones type Atrabot	5,00 12,00 6,0 buc.
27 june	Growing shoots, grains as peas	Manna Oidium	Bouille bordelaise Sulfomat 93 P	5,00 12,00
10 july	Berries growing	Manna Oidium	Bouille bordelaise Sulfomat 93 P	5,00 15,00
25 july	Compaction of grape bunches	Manna Oidium Eudemis G a-III-a	Bouille bordelaise Sulfomat 93 P Traps with pheromones type Atrabot	5,00 15,00 6,0 buc.

Phytosanitary treatments applied in ecological technology - 2016

Table 4

Phytosanitary treatments applied in conventional technology - 2016

Date of treatment	Phenological stage	Pathogens and pests controlled	Plant protection product use	Dose Kg., lt./ha
19 april	Shoots 2-6 cm	Oidium	Sulfomat 80 PU	4,00
11 may	Shoots 30-50 cm	Manna Oidium	Dithane M 45 Sulfomat 93 P	2,00 10,00
31 may	Before blooming	Manna Oidium	Mikal Flash Sulfomat 93 P	3,00 10,00
17 june	Immediately after flowering	Manna Oidium	Verita Bumper 250 EC	2,50 0,20
27 june	Growing shoots, grains as peas	Manna Oidium	Cupertine Super Falcon 460 EC Sulfomat 93 P	4,00 0,30 10,00
10 july	Berries growing	Manna Oidium	Champ 77WG Sulfomat 93 P	3,00 15,00
25 july	Compaction of grape bunches	Manna Oidium	Bouille bordelaise Sulfomat 93 P	5,00 15,00

**Grapevine powdery mildew** (*Uncinula necator* - Schw. Burr.): powdery mildew of vines degree of attack (GA%) amounts similar in all experimental versions embodiment except for the green - royal Fetească which records a 13.97% AD% on grapes (Table 5).

**Gray rot of grapes** (*Botrytis cinerea -* Pers.): Due to adverse climatic conditions in 2016 (precipitations low from summer period, low atmospheric humidity) symptoms pathogen attacks not been signaled in experimental groups.

Table 5

#### Evolution of the main diseases in the year 2016

	The degree of attack (%)						
Varieties, technology	Mar	nna	Oio	Gray rot			
	leaves	grapes	leaves	grapes	grapes		
Merlot - ecological technology							
	3.17	16.64	0.46	3.04	0.0		
Feteasca regala - ecological							
technology	2.08	4.26	4.01	13.97	0.0		
Merlot - conventional							
technology	0.33	3.57	0.46	5.67	0.0		
Feteasca regala - conventional							
technology	0.94	0.94	3.87	3.30	0.59		

Table 6

#### Evolution of grape moth (Lobesia botrana) in the year 2016

	The degree of attack (%)						
Varieties, technology	The generation	The generation	The generation				
	a I-a	a-II-a	a-III-a				
Merlot - ecological	0.33	0.0	3.66				
technology							
Feteasca regala -	0.33	4.00	7.00				
ecological technology							
Merlot - conventional	2.33	4.00	8.00				
technology							
Feteasca regala -	0.66	5.00	11.66				
conventional technology							

**Grape moth (***Lobesia botrana***-Den et Schiff):** in the period analyzed the behavior and potential vine pest attack was highly influenced by climatic conditions (temperature, humidity etc.).Temperature has a major influence on the development of grape moth, such as: high temperature >  $34-36^{\circ}$ C are lethal for the eggs and adults, eggs and larvae resistant to temperatures between  $0^{\circ}$ C and  $10^{\circ}$ C and to  $23^{\circ}$ C crisalidele. Following the observations made on the varieties in the experimental lots, the grape moth recorded a lower degree of attack (G.A%) in all three generations compared to conventional technology (Table 6).

#### CONCLUSIONS

- Analysis of the climatic elements of 2016 highlights the fact that in the period from May to June were favorable conditions for the development of developing and hands, and during July-August for mildew.
- Phytosanitary treatments applied to ecological technology to combat pathogens and pests vines in specific climatic conditions of 2016, highlights that plant protection products applied at the recommended dose and the optimal time protects the vines.

#### ACKNOWLEDGMENT

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#### RESEARCH ON OBTAINING FRESH CHEESE FROM GOAT'S MILK WITH TARRAGON

Tiţa Mihaela-Adriana<sup>1\*</sup>, Popovici Cristina<sup>2</sup>

<sup>1</sup>"Lucian Blaga" University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection <sup>2</sup>Technical University of Moldova, Faculty of Food Technology, Chisinau, Republic of Moldova

\*Correspondence author. E-mail: mihaela.tita@ulbsibiu.ro

Key words: cheese, tarragon, innovative, bioactive components

#### ABSTRACT

This study aims at developing an innovative dairy product – cheeses, which besides the benefit of the existing native ingredients in the raw material – goat's milk, it is enriched with natural products of vegetable origin with valuable bioactive principles. The natural product of vegetable origin - tarragon, comes from the local flora, being rich in volatile oils, aromatic compounds, antioxidants, minerals, vitamins. Have been realized three samples of cheese that contain different quantities of tarragon and have been analyzed over a period of 6 days from a sensory and physicochemical point of view.

#### INTRODUCTION

In recent years, many cheeses (C.Banu şi Vizireanu, 1998) have responded to consumer demands of various varieties by adding flavor ingredients, herbal supplements such as parsley or chives, garlic, pepper or cumin, black pepper. Aromatic plants are a source of biologically active compounds which, introduced into food, can give them primarily the flavor of the volatile oils they contain, secondly they can improve their color through anthocyanins and flavonoids, and last but not least increases the antioxidant capacity of foods through their own vitamins, polyphenols or flavonoids (Acharya Sn, Thomas Je, Basu Sk. ,2007). A strong aromatic plant that draws its origins from Mongolia and Siberia, the tarragon (Arthemisia Dracunculus) was introduced in Europe in the 16th century. In the chemical composition of tarragon are volatile oils, aromatic compounds, antioxidants, minerals, vitamins, tannins, bitter substances, iodine, mucilages, substances giving the plant carminative, diuretic, expectorant, calming, digestive, aperitive, antiparasitic, antispasmodic, antiviral, decongestant, antiinfectious, disinfectant, anti-inflammatory and flavoring proprieties. The tarragon is a plant rich in vitamins, such as vitamin C, vitamin A, B complex vitamins, folic acid, pyridoxine, niacin, riboflavin, etc., which function as antioxidants as well as co-factors of metabolism. The tarragon is especially an excellent source of minerals, such as calcium, manganese, iron, magnesium, copper, potassium and zinc. The tarragon is widely used in cooking and flavor, but little of us know that it is also effective in medicine. It is a key plant in French cuisine (it is an essential ingredient in Bearnaise sauce) and works very well with egg, cheese. In our country, it is known as essential spice in the Ardeal cuisine and is usually used in dry form, although the taste of fresh tarragon is much more interesting and more armatically than dry.

#### MATERIAL AND METHODS

In this research we used goat's milk from a private farm, which has a corresponding microbiological quality, being processed into fresh cheese according to a traditional recipe. After the squeeze process, we made three samples of fresh cheese (Costin Gh. M., 2003) with a different addition of tarragon, which was previously washed, finely chopped and mixed with a little salt. The samples contain 200 grams of cheese with tarragon and was noted like this: PB1 - containing an addition of 10 grams of tarragon, PB2 - 15 grams of tarragon, PB3 - 20 grams of tarragon. These samples were analyzed over a period of six days in sensory and physicochemical terms (Tita M, 2002). Sensory analysis - using the method of comparison with unit scoring scales, which consists of assessing each sensory characteristic and obtaining a medium score given by 3 tasters. The score for assessing the sensory characteristics was as follows: 5 points - very good; 4 points - good; 3 points - satisfactory; 2 points - unsatisfactory; 1 point - inappropriate: 0 points - altered: Determination of dry substance - Moisture analyzer AND ML-50 is based on the principle of thermogravimetric analysis, drying of the samples using the halogen lamp and obtaining the moisture content in %; Determination of lactic acid - Semiautomatic Clinical Biochemistry Analyzer State Fax 1904, expressed in grams/100 grams product; Determination of water activity using the "Novasina" apparatus.

#### **RESULTS AND DISCUSSIONS**

a. Sensory analysis: The tasters analyzed the sensory characteristics of cheese with tarragon for a period of 14 days, by comparing with scoring ranges from 0 to 5 points, established in sensory analysis standards. The scores and also the ratings given by the tasters for this type of cheese are shown in table no.1.

Table 1

	Censory endracementes of encese with tarragen								
Samples	Day 1		Da	ay 3	Day 6				
analyzed	Scores	Qualifying	Scores	Qualifying	Scores	Qualifying			
Sample 1	4	В	3	S	2	NS			
Sample 2	5	FB	4	В	4	В			
Sample 3	3	S	2	NS	2	NS			

#### Sensory characteristics of cheese with tarragon

Note: FB - very good; B - good; S - satisfactorily; NS - unsatisfactory; N - improperly; A - adulterated

From the results obtained, we can see that the PB2 sample obtained the best scores and qualifiers, which denotes the content of tarragon in the cheese is optimal, compared to the other samples.

b. Determination of dry substance at thermobalance: The results obtained in determining the dry substance are shown in figure no.1.

From the results presented, it is observed that the three samples subjected to the analysis show slight increases of the dry substance over the analyzed period, and the different content of the dry substance of the three samples is given by the amount of tarragon that differs.



c. Determination of lactic acid: The results obtained in determination of lactic acid are shown in figure no. 2



Figure no. 2. Evolution of lactic acid

It is noted that the lactic acid content increases as a result of lactic acid activity on lactose throughout the analyzed period. The lactic acid content is also influenced by the amount of tarragon, so sample PB1 has the highest amount of lactic acid and PB3 has a lower amount of lactic acid. For this reason, the shelf life of PB3 is higher than that of PB2.

d. Determination of water activity: The result obtained in determination of water activity are shown in figure no. 3

Water activity is the result of processes that take place during the analyzed period and is seen to increase due to the fact that a balance is achieved between the components of the products, and from the data presented, it is observed that the water activity does not show any significant differences during the analyzed period, which represents a safety of tarragon cheeses samples from a microbiological point of view.



Figure no. 3. Evolution of water activity

#### CONCLUSIONS

Following the analysis of the three samples of fresh tarragon cheese during the analyzed period, the following conclusions can be drawn:

- PB2 on sensory analysis has received the highest scores and ratings, which means it has the optimal content of tarragon;
- The content of dry substance and lactic acid is influenced by the amount of tarragon added;
- The activity of water does not show significant differences during the analyzed period, which is a safety of tarragon cheeses samples from a microbiological point of view.

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#### THERMAL ANALYSIS OF CURD OBTAINED BY DIFFERENT METHODS

Tiţa Mihaela-Adriana<sup>1\*</sup>, Popovici Cristina<sup>2</sup>

<sup>1\*</sup>"Lucian Blaga" University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection <sup>2</sup>Technical University of Moldova, Faculty of Food Technology, Chisinau, Republic of Moldova \*Correspondence author. E-mail: mihaela.tita@ulbsibiu.ro

**Key words**: coagulation process, quality, maturation, differential scanning calorymetry

#### ABSTRACT

To obtain high-quality cheese is dependent of the coagulation mode, which can be about acids or enzymes. A coagulation process that respects the technological parameters afford the obtaining of a good quality curd. In our study we used goat milk which was coagulated with enzymes using the rennet and the coagulated with acid using citric acid, acetic acid. The resulting curd was analyzed over a period of 14 days using differential scanning calorimetry (DSC). Following the analysis performed by differential scanning calorimetry were determined melting temperatures of the three types of curd, taking into account the time in which melting occurs. The results obtained showed that the highest temperatures melting occurred at the curd obtained by adding rennet, which has a specific consistency and allows to obtain a high quality cheese, both in terms of sensory and physico-chemical.

#### INTRODUCTION

Improved coagulation properties of milk from individual goats (C. Banu, et.al., 2007) were shown to be associated with a higher content of total and colloidal calcium to some extent, low casein micelles. Cold storage has been shown to induce RCT longer for both types of milk after 24 hours, where the gel strength was reduced primarily in good coagulation of milk samples during storage. Milk, cold storage for up to 14 hours did not affect the rheological, although RCT tended to decrease after pasteurization HTST. Further studies are needed to understand how milk processing steps affecting the distribution of casein and colloidalmineral phases between individual serum and milk coagulation with different properties. In addition, pasteurization HTST currently applied has no significant effect on coagulation properties, even if RCT was reduced by about 7 min. This is in contrast to earlier studies, where pasteurization of the cheese making properties improved by partially reverse micelle dissociation of Ca, P inorganic casein occur during cold storage (Ali AE, et.al.1980). Differences in developing coagulation properties could be attributed to changes affecting the second stage coagulation. In this way, more investigation is needed to characterize the relationship between the second stage of coagulation induced chymosin and casein micelles changes occurring during storage in cold milk coagulation distinct individual skills. Technological intervention milk through the production line cheese (ex: cold storage, standardization fat, pasteurising, CaCl<sub>2</sub> addition and acidification) can have a significant impact on the physico-chemical in milk and therefore on its properties and the rheological characteristics of the cheese.(De la Fuente M,1988).

It is known that the pH affects the rate of renneting, curd firmness and syneresis and the recovery of fat, the retention of calcium and, therefore, final cheese texture.(Watkinson P,et.al.,2001). However, the stored cold pasteurization of milk is known to recover its coagulation properties in a certain extent by reversing the abovementioned changes in the structure of the casein micelle. (Frederiksen PD, et.al.,2011). Chymosin induced coagulation is a two-step process. The first step is  $\kappa$ -CN chymosin cleavage in para- $\kappa$ -CN and casein macropeptides (CMP). To about 85% of  $\kappa$ -CN it was cleaved to promote the formation of aggregates. An increase in viscosity is observed followed by branching of the aggregates, as characterized the second stage. (Dalgleish DGD, Corredig M, 2012). It was questioned whether molecules of  $\kappa$ -CN milk non coagulants are actually split in the same way as the  $\kappa$ -CN good milk coagulant chymosin, but in a previous study, it was observed that the first phase held at comparable as far as good and non coagulating. (Frederiksen PD, et.al.,2011).

#### MATERIAL AND METHODS

The milk used to obtain different types of clot is the goat originated from a farm in the county of Sibiu. Milk has undergone technological operations reception, pasteurization and cooling (.

#### Differential scanning calorimetry (DSC)

Thermal analysis (TA) is a group of techniques in which changes of physical or chemical properties of the sample are monitored against time or temperature, while the temperature of the sample is programmed. The temperature program may involve heating or cooling at a fixed rate, holding the temperature constant (isothermal), or any sequence of these. Determines: melting - crystallization; polymorphism/phase diagram; glass transition; degree of crystallinity of semi-crystalline material; degree of curing/cross linking; specific heat capacity (J/gK;) decomposition onset; reaction kinetics, enthalpy; oxygen resistance (oxidative stability, OIT); purity determination. The characteristics thermal analysis: samples can be analyzed in a wide range of temperatures, using various temperature programs, dynamic and isothermal conditions; samples can be in any physical form (solid, liquid, gel) in different forms (powder, films, granules, fibers, fabrics, plate, etc.); it is necessary a small amount of sample (0.1 mg-10 mg); the time required for analysis varies from a few minutes to several hours; atmosphere around the sample can be defined (air/inert gas); measurement techniques and sample handling is not difficult; there are a variety of tools AT commercially available, and the price is moderate.

Differential scanning calorimetry (DSC) (G. Bruylants, et.al, 2005) is a technique of recording energy required to maintain a zero temperature difference between the sample cup and the cup is the reference, both of which are simultaneously heated or cooled at a controlled rate. If the sample undergoes a transition, the endo- or exothermic process it or reference cup will receive an equivalent energy so as to maintain the temperature equal to two cups (reference sample respectively).

#### **RESULTS AND DISCUSSIONS**

Slow heating rate leads to a balance between the reactions taking place. At first the temperature is high, the sample undergoes a glass transition (glass), then at a certain temperature and produce heat crystallized sample is an exothermic process taking place, so at the lowest recorded temperature crystallization. Heating the samples in turn heats the crystalline areas is a process of melting, i.e. an endothermic

process, so that the highest peak indicates the melting temperature of the sample. For each sample analyzed at T0, T7 and T14 the two thermogram were obtained in the first are the melting temperature of the test sample, and in the second time at which the melting of the sample. The results obtained are presented in the following figures:



Fig.1. Analysis curds obtained by adding rennet. Melting temperature



Fig.2. Analysis curds obtained by adding rennet. Melting time.

The melting temperature for the sample T0 is  $110^{\circ}$ C to 8 minutes at  $100^{\circ}$ C T<sub>7</sub> is 7 minutes at  $105^{\circ}$ C T14 is 7.5 minutes. Thus the highest melting temperature was recorded at T<sub>0</sub>, and the lowest at T<sub>7</sub>.



Fig.3. Analysis curds obtained by adding acetic acid. Melting temperature.



Fig.4. Analysis curds obtained by adding acetic acid. Melting time

The melting temperature for the sample T0 is  $97^{\circ}C$  6.8 minutes at  $105^{\circ}C$  T7 is 7.5 minutes at  $102^{\circ}C$  T14 is 7.3 minutes. Thus the highest melting temperature was recorded at T7, and the lowest at T0.



Fig.5. Analysis curds obtained by adding citric acid. Melting temperature.



Fig.6. Analysis curds obtained by adding citric acid. Melting time.

The melting temperature for the sample T0 is  $103^{\circ}$ C, 7.3 minutes at  $96^{\circ}$ C T7 is 6.6 minutes at  $100^{\circ}$ C T14 is 7.0 minutes. Thus the highest melting temperature was recorded at T0, and the lowest at T7.

#### CONCLUSIONS

This study showed that the analyzes performed were determined by DSC melting temperatures of the four types of curd, as was revealed that the highest melting temperatures were recorded at curd obtained by adding rennet, which has a specific consistency and allows to obtain a high quality cheese, both in terms of sensory and physico-chemical.

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# USING THE BIOACTIVE POTENTIAL OF PLANTS BY GETTING TONIC DRINKS

Tița Ovidiu<sup>1\*</sup>

<sup>1</sup>"Lucian Blaga" University of Sibiu, Victoriei Boulevard, no. 10, Sibiu, Romania Corresponding author, e-mail: ovidiu.tita@ulbsibiu.ro

Key words: potentially bioactive, tonifying, plants, extract, wine

#### ABSTRACT

The paper presents important issues regarding the possibility of using plants with bioactive potential recognized in the production of tonic drinks with a beneficial role in human health. Bioactive plants are currently used both for medical purposes and for the production of alcoholic beverages, extracts or tinctures. We can use plants that grow very well in our country and are the subject of important crops for export. Diversifying their use can have beneficial effects. The use of these in everyday life in various forms ensures the natural resistance of the organism to some contamination factors such as bacteria or fungi.

#### INTRODUCTION

The beneficial effects of plant extracts in healing or ameliorating diseases have long been used in folk medicine (Tabata et al., 1988). The properties of these plants are based on their rich composition of bioactive compounds such as essential oils, tannins, flavones or polyphenols. Terpene, monoterpene, diterpene, sesquiterpene, these have antibacterial, antiseptic, antiinflammatory or antiviral properties (Wallace, 2004). Antimicrobial activity is due to compounds such as carvacol, camphor or thymol, the activity of which is beneficial to the human body, having uses in the pharmaceutical or food industry (Bhaskara et al., 1998; *Ali-Shtayeh et al. 2008; Hadizadeh et al. 2009*). Plant aromas lead to an olfactory perception, based on higher alcohols, terpenic compounds, bitter substances, aldehydes and ketones, esters, and quantitative and qualitative quantification is achieved by modern methods such as gas chromatography (Târdea, 2010). Polyphenols are natural compounds found in herbal foods, such as fruits, vegetables, whole grains, tea, wine, cocoa, etc. (Pandey & Rizvi, 2009).

#### MATERIAL AND METHODS

The plants with potentially bioactive potential used were: aphid (leaves and fruits) (*Vaccinium myrtillus L.*), rosehip (*Rosa canina L.*), cranberry (*Vaccinium vitisidaea L.*), blackberries (*Rubus fruticosus L.*), wormwood (*Artemisia absinthium L.*), zmeur (leaves and fruits) (*Rubus idaeus L.*) which have been dried and milled. The obtained powder was homogenized in 50% alcoholic solution in order to extract the bioactive principles in a ratio of 1:10 for 24 hours. The samples were centrifuged and the supernatant was used in the assays. Evaluation of aromatic compounds in plants was performed using the GC / FID system (gas chromatograph coupled with a flame ionization detector. Maceration of plants to obtain plant maceration was performed at

40-450  $^\circ$  C with a 45-50% vol. Hydroalcoholic solution for 18 hours. The plant / solvent ratio was selected to 1/10.

The ratio of the weight of the 6 plants used to obtain the maceration is confidential. Specialty studies on red wines show that their composition and properties can be a basis for the production of tonic beverages. Wines of the Fetească Neagră variety are indigenous wines with physicochemical properties and special sensors, well appreciated on the domestic and foreign market, and due to these attributes it is also suitable for use in various recipe for toning drinks. A wine of this variety from the 2015 harvest year was used in the Dealul Mare Vineyard with the following characteristics: reducing sugar 3.7 g / L, pH 3.8, total acidity of 4.1 g / L tartaric acid and alcoholic strength of 12.5% v / v.

#### **RESULTS AND DISCUSSIONS**

In the realization of the herbal tonic drink, the specific stages of raw materials analysis, wine, sugar, refined food alcohols, aromatic plants, conditioning of the used raw material, preparation of sugar syrup and aromatic herbs macerate were covered. We then proceeded to the actual realization of the tonifying drink that remained in the twinning of the components for 30 days of 15-18<sup>o</sup>C before carrying out physico-chemical and sensory analyzes.

They have been identified as particularly valuable wine compounds: 1431.257 mg / L of total polyphenols, of which 488.137 mg / I tannin, 369.027 mg / L anthocyanins and 101 271 mg / L of phenolic acids.

A series of phenolic compounds were also identified by HPLC methods: quercetin 0.5215 mg / 100 mL vin, gallic acid 8.8147 mg / 100 mL vin, acaric syrup with 1.9957 mg / 100 mL vin, caffeic acid with 4.17975 mg / 100 mL of wine. Resveratrol was identified in the amount of 0.9173 mg / 100 mL of wine.



Figure 1. Sensory analysis for Black Fetească wine



Figure 2. Sensory Analysis 1 for Tonic drink



Figure 3. Sensory Analysis 2 for Tonic drink

The not very high acidity of the wine, its aroma and its softness cover the astringent of the plant extract used to make the toning drink.

As it results from the sensory analysis of the Black Feteasca wine, it is distinguished by its typical character, it has a pronounced floral character, the mineral character being more discreet. It has an excellent taste balance and a very good persistence of taste. It has a pleasant astringency rated with a score that can print harmony and ensures a pleasant tonic drink.

A first sensory analysis of the tonic drink obtained is presented as a product with a strong taste of wormwood, but also of dried flowers and fruits, keeping very well the basic material from which the product originates. It has a complex, pleasant, harmonious taste. The parameters analyzed for the second sensory analysis attest to the achievement of a harmonious, good-tasting product, a persistent pleasant, refreshing taste without a astringency that would lower its value.

#### CONCLUSIONS

The tonic beverages have a small caloric intake, they help digestion, contain plant compounds with high antioxidant potential and are recommended for various diseases or as digestive and toning drinks. Polyphenols from these herbal drinks based on red vines have a pronounced antiviral, antibacterial and protective role of the body against atherosclerosis, facilitating biochemical reactions that help protect the cardiovascular system. In addition to the antioxidant effect, it promotes vitamin C action, helps reduce cholesterol, and resveratrol has the role of stopping the spread of cancer cells. The content of polyphenols, tannins, flavones, vitamins, minerals or phenolic acids give rise to specific flavors, which ultimately contribute to achieving the particularly pleasant sensory aspect of the toning product obtained.

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#### INFLUENCE OF FRUIT LOAD ON THE QUALITY OF GRAPE PRODUCTION AND EVOLUTION OF PHENOLIC COMPOUNDS DURING THE FERMENTATION MACERATION OF WINES IN DEALU MARE VINEYARD

Tudor Georgeta<sup>1</sup>\*, Pîrcălabu Liliana

<sup>1\*</sup>Research Institute for Viticulture and Enology Valea Calugareasca \* Correspondence author. E-mail: andreea\_tga@yahoo.es

Key words: polyphenols, alcohol content, maceration-fermentation

#### ABSTRACT

The varieties taken into study were Feteasca neagra and Cabernet Sauvignon, part of the basic assortment for the production of quality red wines, typically for Dealu Mare vineyard, which are very valuable in view of the oenological aspect.

At harvest (19 September 2016) the grapes has sugar accumulations of 222 g/l (Cabernet Sauvignon) and 226 g/l (Feteasca neagra). The total anthocyanin concentration was 1346 mg/l in case of Cabernet Sauvignon variety and 1267 mg/l at Feteasca neagra, values closed to those of grapes rich in anthocyanins which have more than 1200 mg/l (Ribereau-Gayon P., 1964, 1972). The extractibility of anthocyans (62% at Cabernet Sauvignon and 57% at Feteasca neagra) and the maturity of the seeds (72% at Cabernet Sauvignon and 69% at Feteasca neagra) were very good.

#### INTRODUCTION

The factors determining the alcohol content of wines are: varieties of vines; the soil on which the vine is grown (calcareous soils generally giving the most alcohol-rich wines); the climate of the vineyard and the way climatic factors evolve during the year; technological conditions and especially fermentation of must (TARDEA, 2007; PATIC, 2006).

The evolution of phenolic compounds during the maturing of grapes for red wines was determined and the amount of polyphenols in the skin of the berry grape is 10 to 100 times higher than in the must, increasing in the order of Feteasca neagra and Cabernet Sauvignon, with large differences between varieties (KONTEK Adriana, 1975, 1983). The total amount of polyphenols in the skins is increasing after the grape harvest, up to technological maturity and found in seeds, 150 times more than in the must, and the flavonoly tannins are found in large quantities in the seeds (BOURZEIX M., 1975, 1976).

#### MATERIAL AND METHODS

The research was carried out in two vineyards grown with Feteasca neagra and Cabernet Sauvignon. The study was performed during 2016 harvest, with climatic conditions favorable for the over-maturing of grapes. The cutting system practiced was Guyot Multiple with 3 loadings: 20 eyes/vine (T1), 28 eyes/vine (Mt) and 36 eyes/vine (T2), with the attribution of a cutting load of 8-10 eyes per vine and two eyes taps. nder the conditions of microvinification, three experimental variants were made for each selected vinifera variety, the differentiation being made according to the time of separation of the marc, respectively 8 and 16 days.

#### **RESULTS AND DISCUSSIONS**

The experimental results obtained showed that under the ecoclimatic conditions specific to 2016 year, characterized by a moderate heliothermic regime on the background of rich water resources, the experimental technological variants had a different impact on the quality of the grape production and wine. The lowest minimum temperature was recorded in September (14.1°C) and the highest in July (18.3°C), the lowest maximum temperature was in September (25.6°C) and the higher in July (29.6°C), (Table 1).

Table 1

		1			
Luna	Average temperature (°C)	Average minimum temperature (°C)	Average minimum temperature (°C)	Rainfalls (mm)	Huglin Index
June	22.1	17.1	27.4	81.8	442.5
July	23.7	18.3	29.6	70.2	516.2
August	22.7	17.5	29.2	78.2	494.5
September	19.0	14.1	25.6	66.0	381.3

The thermal and pluviometric regime during June-September

During the ripening and full maturation rainfall data indicate precipitation amounts with a minimum of 66 mm in September and a maximum of 81.8 in June.

At harvest (19 September 2016) the grapes has sugar accumulations of 222 g/l (Cabernet Sauvignon) and 226 g/l (Feteasca neagra). The total anthocyanin concentration was 1346 mg/l in case of Cabernet Sauvignon variety and 1267 mg/l at Feteasca neagra, values closed to those of grapes rich in anthocyanins which have more than 1200 mg/l (Ribereau-Gayon P., 2012). The extractibility of anthocyans (62% at Cabernet Sauvignon and 57% at Feteasca neagra) and the maturity of the seeds (72% at Cabernet Sauvignon and 69% at Feteasca neagra) were very good.

The maceration-fermentation process began on 22 September 2016 and ended on 7 October 2016. The dynamics of polyphenols extraction was continued until the separation of the wine from marc. The amount of anthocyanins increased progressively until day 7, and then decreased continuously until day 15, due to their involvement in polyphenolic stabilization and absorption on the yeast.

The dynamics of anthocyanins during fermentation maceration is continued until the time of separation of the marc (8th day of fermentation maceration), respectively 436 mg/L in case of Feteasca neagra 20 eyes/vine and 317 mg/L to Feteasca neagra 36 eyes/vine. The content polyphenols shows variations between 1809 mg/L (Feteasca neagra 20 eyes/vine) and 1605 mg/L (Feteasca neagra 36 eyes/vine), (Table 2).

#### Table 2

# The content of anthocyanins and polyphenols of the separated fractions at 8 and 16 days

an otoacoa noagra								
The maceration-	Anthocyanyns (mg/L)			Total polyphenols (mg/L)				
fermentation period	36 eyes/ vines	eyes/ 28 eyes/ 20 eyes/ ines vines vines	36 eyes/ vines	28 eyes/ vines	20 eyes/ vines			
8 days	317	375	436	1605	1655	1809		
16 days	285	306	339	1587	1610	1649		

#### a.Feteasca neagra

#### b. Cabernet Sauvignon

The maceration- fermentation period	Anthocyanyns (mg/L)			Total polyphenols (mg/L)			
	36 eyes/ vines	28 eyes/ vines	20 eyes/ vines	36 eyes/ vines	28 eyes/ vines	20 eyes/ vines	
8 days	364	364	424	2081	2213	2567	
16 days	343	350	404	2053	2334	2526	

When separating the fractions to 16 days the content of anthocyanins is continuously decreasing, respectively in the Feteasca neagra variety from 339 mg/L (20 eyes/vine) to 285 mg/L 936 eyes/vine), and in the case of the Cabernet Sauvignon variety from 404 mg/L (20 eyes/vine) to 343 mg/L (36 eyes/vine).

Extending the maceration-fermentation time from 8 to 16 days after the end of alcoholic fermentation leads to important changes in the red wine structure: decreases the total anthocyanin content and the color intensity of the wine; increases the content in total polyphenols, modifying the indices characterizing the phenolic complex in wine, namely:

The color intensity for Feteasca neagra variety (20 eyes/vine) from 8,350 (8 days) to 7,610 (16 days), 28 eyes/vine from 6,990 (8 days) to 6,370 (16 days), 36 eyes/vine from 6,970 (8 days) to 6,260 (16 days). The same variation is found for Cabernet Sauvignon 20 eyes/vine from 11,350 (8 days) to 9,350 (16 days), 28 eyes/vine from 10,920 (8 days) to 8,850 (16 days) to 36 eyes/vine from 10,030 (8 days) to 8,740 (16 days).

The extension of the maceration-fermentation period induces an increase in the total polyphenols content, as follows: in Feteasca neagra variety in case of 20 eyes/vine from 1585 (8 days) to 1783 (16 days) at 28 eyes/vine at 1580 (8 days) at 1612 (16 days) and at 36 eyes/vine from 1527 (8 days) to 1569 (16 days). And in the Cabernet Sauvignon variety, increases in total polyphenols in 20 eyes/vine from 2441 (8 days) to 2500 (16 days), 28 eyes/vine from 2165 (8 days) to 2282 (16 days), and at 36 eyes from 1968 (8 days) to 2011 (16 days), (Table 3).

The sensory profile of the wine was obtained on the basis of a tasting score using the red wine specific descriptors. The quality level of aromatic flavor components was average for Feteasca neagra wine, harvest 2016, with the exception of the caramel note that had 4 points for 20 eyes/vine -8 days. The amount of tannins was average, perceived with a slight astringency in 20 eyes/vine and 36 eyes/vine (Figure 1 and Figure 2).

	Feteasca neagra						
Variant/Parameters	28 eyes/vine		20 eyes/vine		36 eyes/vine		
	8 days	16 days	8 days	16 days	8 days	16 days	
Alcoholic concentration (% vol)	13.0	13.2	13.2	13.4	12.8	13.0	
Sugars reducing (g/l)	4.85	4.96	4.03	4.07	5.20	5.30	
Total acidity (g/l tartric acid)	5.79	5.45	5.49	5.27	6.09	6.17	
Volatile acidity (g/l acetic acid)	0.63	0.67	0.62	0.64	0.65	0.67	
Total extract (g/l)	23.7	24.2	22.7	22.9	24.8	28.7	
Nereducator extract (g/l)	18.85	19.24	18.67	18.83	19.6	23.4	
Color intensity (Ic)	6.990	6.370	8.350	7.610	6.970	6.260	
Anthocyanins (520 nm)	347	298	425	334	309	278	
Total polyphenols	1580	1612	1585	1783	1527	1569	

Physico-chemical characteristics of Feteasca neagra wines

Physico-chemical characteristics of Cabernet Sauvignon wines

	Cabernet Sauvignon						
Variant/Parameters	28 eyes/vine		20 eyes/vine		36 eyes/vine		
	8	16	8	16	8	16	
	days	days	days	days	days	days	
Alcoholic concentration (% vol)	12.5	12.8	12.7	12.8	12.0	12.4	
Sugars reducing (g/l)	5.56	5.68	4.95	4.98	5.86	5.94	
Total acidity (g/l tartric acid)	5.79	5.64	5.72	5.49	6.55	5.94	
Volatile acidity (g/l acetic acid)	0.75	0.76	0.74	0.75	0.77	0.79	
Total extract (g/l)	25.3	25.8	23.7	23.9	25.5	26.0	
Nereducator extract (g/l)	19.74	20.12	18.75	18.92	19.64	20.06	
Color intensity (Ic)	10.92	8.850	11.35	9.350	10.03	8.740	
Anthocyanins (520 nm)	347	336	415	394	340	326	
Total polyphenols	2165	2282	2441	2500	1968	2011	

The Cabernet Sauvignon wine has a reddish color with violet shades and a gusto-olfactory herbaceous character. The dominant feature is blackcurrant and bitter chocolate (20 eyes/vine - 8 days) and the amount of tannin was maximum in 20 eyes/vine - 16 days.



Figure 1. The sensory profile of the Feteasca neagra wines



Figure 2. The sensory profile of the Cabernet Sauvignon wines

#### CONCLUSIONS

Extending the maceration-fermentation time from 8 to 16 days after the end of alcoholic fermentation leads to important changes in the structure of red wines: decreases the total anthocyanin content and the color intensity of the wine; increases the content in total polyphenols, modifying the indices that characterize the phenolic complex in wine.

Red wines obtained by maceration-fermentation for 8 and 16 days recorded increasing values of the anthocyanin content.

The extension of the maceration-fermentation period induces an increase in the total polyphenols content

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#### THE BEHAVIOUR OF SOME GRAPEVINE GENOTYPES CREATED AT INCOBH STEFANESTI ARGES, *INITIAL* MATERIAL G0, IN DEPOSITARY GREENHOUSE CONDITIONS

Diana Elena Vizitiu<sup>1</sup>, Gina Nedelea<sup>1</sup>, Carmen Bejan<sup>1</sup>, Emilia Vișoiu

<sup>1</sup>National Research and Development Institute for Biotechnology in Horticulture Ștefănești Argeș, Romania \* Correspondence author. E-mail: vizitiud@yahoo.com

Key words: starch, glucides, Auriu de Ştefăneşti, Perlette

#### ABSTRACT

This paper presents the behavior of the propagation Initial material belonging to grapevine genotypes created at NRDIBH Stefanesti Arges. The obtained results after carrying out the biochemical tests regarding the maturation of annual elements used in the multiplication process of biological material shows a good ability of studied genotypes to adapt at the culture conditions from the depositary greenhouse, fact due, largely, to the grapevine plasticity in the situation of equilibrated supply with nutrients and water, as well as a good phytosanitary protection throughout the entire vegetation period. In the case of studied genotypes the accumulation of the reserve substances in woody tissue (soluble glucides and starch) was positively correlated with the total length of shoots and with the matured chord portion.

#### INTRODUCTION

The grapevine culture occupies an important place in Romania and it would not be possible without a high quality propagation material. In the strategy of viticultural sector development, the *Initial* G0 vine propagation material is the first step in multiplying the valuable genotypes for romanian viticulture, having in perspective a particular importance in the grapevines genetic amelioration. Therefore, the depositary with *Initial* G0 vine propagation material, from NRDIBH Stefanesti Arges must be continuously enriched and studied, at the same time.

By maintaining the grapevine propagation *Initial* material (G0) in the depositary greenhouse are fulfilled two actual aspects of Romanian viticulture: firstly it avoids the natural or accidental disappearance of valuable autochthonous grapevines varieties and, secondly, it protects and promotes an biological material with a very good status by point of view sanitary and physiologically. Over the years, the NRDIBH Stefanesti Arges was delivered to the vine research units G0 *Initia* propagation material as scion and rootstock eye and rooted cuttings in pots in order to create the *Base* mother plantation (Şerdinescu, 2011).

#### MATERIAL AND METHODS

The effectuated studies had as primary objective the appreciation of the adaptation capacity of some grapevines genotypes (Auriu de Stefanesti, Perlette 10 St., Fetească alba 97 St., Muscat Ottonel 16 St., Merlot 202 St. and Burgund mare 86 St.) at the growing conditions from the depositary greenhouse by quantitative and

qualitative evaluation of woody biological material obtained annually during 5 years of study, between 2009-2013.

Maturation degree evalution of strings was performed using biochemical tests (for determining the wood content in water, soluble carbohydrates and starch), after the period with the lowest temperatures (20.01.2009, 01.02.2010, 07.02.2011, 21.02.2012 01.31.2013). The analyzed sample was made up from internods fragments (comprising nodes and internodes) from the entire length of string. The string portions of the base, middle and top were analyzed separately. the final result being the mean of three determinations. As working methods, were used the standard ones, used in specialized laboratories. The free water content determination from the woody material was assumed the drying of the material at 40°C, up to constant weight, and the determination of total water content it was carried out at 105°C. The determination of soluble carbohidrates and starch from woody material was effectuated by the colorimetric method, with anthrone reagent (Scott and Melvin, 1953).

#### **RESULTS AND DISCUSSIONS**

The wood maturation is a phisiological process influenced by environmental factors (Burzo I. et al., 1999). During the periods when the ambient temperature decrease at values between 10°C and 0°C it takes place the intensification of soluble carbohydrates translocation process from leaves to perennial organs, and, their water content gradually decreasing.

In the course of the study it was found that the plants from the depositary greenhouse have completed all phenophases approximately with one month earlier in all years studied (2009, 2010, 2011, 2012, 2013), compared to grapevines plants from field.

The periodic registrations effectuated at the grapevine genotipes from depositary greenhouse, showed that the conduct of the phenological phases was closely correlated with the climatic variations specific to the greenhouse, so that, in the study years, between 15.03 -04.04, took place: flowering 28.04-15.05, firstfruits 01.07-31.07 full maturation: 20.07-15.08, strings maturation and leaf fall: 25.08-30.11; also the studies have indicated, depending on the genotyp, a vegetation period of 185-250 days.

At the end of the vegetation period of each year, in depositary greenhouse, the vegetative growths reached lengths of over 250 cm, from which a average of 204 cm was maturated wood. The string diameter and the distance between the knots constituted indicators in the quantitative assessment of the number of buds from the maturated wood portion. Thus, the number of total buds was supperior for the studied genotypes (210-250 eyes / 4 plants), where the distance between nodes was between 12-15 cm and the strings diameter 5-8 mm.

In this study, the temperature decrease in greenhouse occurred after November 15, when it registered positive values of 3-4°C in a longer period of time (10-12 days). Thus, before leaf fall, took place the protein and carbohydrates (poliglucide) hydrolysis from leaves and their translocation as amino acids, respectively, soluble carbohydrates.



Figure 1. The grapevine plants in the depositary greenhouse

The total water content obtained from the genotypes analyzed from the depository greenhouse registered medium multiannual values between 47 and 50% (Fig. 2d). The material derived from the clones for the production of white wines (Feteasca alba 97 St. and Muscat Ottonel 16 St) registered an average content in total water of 47.9 - 48.6% and those for the production of red wines (Merlot 202 St and Burgund 86 St) it was 47.2 % (Fig. 2 b and c). At grapevine genotypes for table grapes (Auriu de Stefanesti and Perlette) the multiannual averages were situated around 50% (Fig. 2).



Figure 2. The total water content from the woody material obtained from grapevine genotypes tested between 2009-2013 (a, b, c) and multiannual average (d) In this study the bound water content from grapevine strings registred appropriated multiannual average values (2.3 - 2.9%), observating its decrease at all genotypes tested in 2010 and 2012 (Fig. 3 a, b, c). Quantitatively, the bound water recorded higher values at Perlette 10 St. clone (fig. 3 a).



Figure 3. The bound water content of woody material between 2009-2013 (a, b, c)

The glucides quantification showed a good maturation of the woody material at all tested genotypes. At the table varieties, soluble glucides content was situated between 10.3% dry substance at Auriu de Stefanesti and 12.7% at Perlette 10 St.clone (multiannual average - Fig. 4 d). In the viticultural year 2012-2013 both genotypes have accumulated significant amounts of glucides in wood (12.3% at Auriu de Stefanesti varieties; 13.9% at Perlette 10 St. clone). At the grapevine genotypes intended for quality red and white wines, the soluble glucides acumulation were between 10.1% - Burgund 86 St. and 11.8% - Muscat Ottonel 16 St., accepted limits for a biological material found in the rest period, corresponding to December and January, when the soluble glucides content reaches a maximum of 10-12% glucides (Fig. 4 a, b and c).



Figure 4. The soluble glucides content between 2009-2013 (a, b, c) and the multiannual average of soluble glucides and starch (d)

Regarding the starch content from canes, this recorded values close to the normal in January and February, framing between 3.8 - 6.1% (multiannual average). Higher starch accumulation were reported also in the viticultural year 2012-2013 at
clones for quality red wines (Merlot 202 Şt. - 7.3 %, Burgund 86 Şt. - 7.4 %). The Perlette 10 St clone registred minimal value of starch in all studied years (Fig. 5 a, b and c).



Figure 5. The starch content of woody between 2009-2013 (a, b, c) The studied genotypes have accumulated in the maturity stage of wood the important quantities of sugars due to the optimal nutritional and health status of hubs, but also due to the mild conditions from greenhouse where the minimum temperature recorded values up to -10°C, when, in the field conditions, absolute minimum temperature of air was -22°C (last decade of January 2010). The content evolution of the total glucides from woody material belonging the clones and the varieties studied between 2009-2013 is shown in Figure 6 a, b and c.



Figure 6. The total glucides content of woody material between 2009-2013 (a, b, c) and the multiannual average (d)

The multiannual average indicated accumulation of total sugars between 14.7 - 17.6%, which led at obtaining of superior primary buds viability, namely 97.2 - 99.6% (Fig. 6 d). Meanwhile, the buds viability at annual woody elements harvested from the studied genotypes was positively correlated with accumulated amount of total glucides.

Similar results for the obtaining of the quality viticultural planting material in protected spaces has been achieved at Feteasca neagra 6 St., Pinot noir 3 St., Cabernet Sauvignon 131 St., Fetească regală 72 St. (Bejan şi colab, 2013) grapevine clones.

The results obtained after biochemical determinations on the biological material, reveals that the soluble glucides and starch acumulation in woody tissue was correlated with total length of shoots and with the matured chord portion.

#### CONCLUSIONS

At the studied genotypes the contents of the woody material in bound water was recorded multiannual average values between 2.3 - 2.9%, observing a decrease of this indes at all tested genotypes between 2010 – 2012. quantitatively bound water showed higher values at Perlette 10 St. The total water content of material obtained from the analyzed genotypes recorded multiannual average values between 47 and 50%, values characteristic of a mature wood. In 2012-2013 years were recorded significant amounts of soluble glucides at the table grapes genotypes and higher starch accumulation at varieties clones for quality red wines.

The acumulation of soluble glucides and starch was fluctuated depending on the genotype and on the specific climate of the year in which the evaluation was conducted, but was within the limits of a biological material found during the rest period.

The obtained results show a very good capacity for adaptation of studied genotypes at depositary greenhouse culture conditions, due the grapevine plasticity in the conditions of a equilibrated supply with nutrients and water, as well as a good phytosanitary protection throughout the entire vegetation period.

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# THE LABEL. THE EUROPEAN WINE "PASSPORT"

Voinea-Mic Cătălin<sup>1</sup>, Giugea Nicolae<sup>1\*</sup>, Berta Corina<sup>1</sup>, Mărăcineanu Liviu<sup>1</sup>

<sup>1\*</sup>University of Craiova, Faculty of Horticulture \* Correspondence author. E-mail: giugeanicolae@gmail.com

Key words: label, geographical indications, controlled designation of origin, specific traditional mentions, consumer

#### ABSTRACT

Starting from the European desideratum to protect the legitimate interests of wine consumers as important as the producers' interest in capitalizing their production depending on the quality of the supplied products, the European Community has established higher standards than the national legislation, designed to regulate how to present the wine once it is commercialized.

#### INTRODUCTION

The label is a genuine wine identity document, a passport in international trade relations, along with the fiscal and commercial documents accompanying the product, the true business card of the bottled product. By referring strictly to the common clients, the label is, in fact, the major milestone in the wine selection process, starting from the chromatic aspect and ending with the strict specialty inscriptions on it.

#### MATERIAL AND METHODS

This paper was based on the European regulations and European jurisprudence in the field, using the classical methods of legal interpretation of legislation and legal analysis on the text of parts of the procedural documents in some cases solved by the CJEU.

#### **RESULTS AND DISCUSSIONS**

According to European standards (Notice Regulation no. 1493/1999 on the common organization of the wine market, Annex no. VII<sup>1</sup>, the label is defined as "a set of descriptions and other mentions, insignia, illustrations or trademarks that characterize the product, placed on the same container, including on the closure devices, or on the label attached to the container".

From the legal definition, several conclusions can be drawn:

- the label may contain illustrations, insignia or distinguishing marks - trademarks;

- the notion of "label" also includes closing devices, as far as they are fit to be inscribed, illustrated;

- in addition to the classic attachment formula or sticking on the container, the label may also be attached to the container (usually with a string, ribbon, etc.); there should be mentioned that the packaging of the product does not represent the labeling; the packaging is done with paper, straws of any kind, cardboard, cassettes, etc. and it

is intended exclusively for the transport of one or more containers and/or their presentation for sale to the final consumer.

Beyond the manufacturer's desire for product presentation through the label, the European standards establish that, from the date of putting into circulation, any container with a nominal capacity of not more than 60 liters must be labeled. It is obvious that each wine producer is tempted to produce a label with a maximum impact on consumers. The right to ownership of the wine, of the container, and implicitly of the label, is not equivalent to the trader's absolute freedom to insert the specifications on the product presentation label. The consumer protection and, implicitly, the protection of the interests of traders of similar products require that the description and presentation of the products through the presentation label are not misleading or likely to cause confusion to consumers. The limits of the right to present the wine product are set at European level by Regulation no. 1493/1999 (In force since 14.07.1999, published in JOCE no. 179 of 14.07.1999) on the common organization of the wine market, an European act containing rules obliging the insertion of certain mentions, authorization rules for certain mentions, the protection and control of mentions, the use of geographical indications and traditional mentions, rules on imported product labeling (to the extent that the regulation permits these operations), on the authorization of the use of consumer information, etc. The label must necessarily accompany the wine categories specifically specified by the Frame Regulation, the content of which is formed of mandatory and optional mentions. Thus, in the categories of mandatory mentions referring to wine categories and sales denominations, we distinguish:

a) labeling of table wines with a geographical indication and wines with a controlled designation of origin (DOC); the label will contain the name of the bottling company and the name of the locality and the Member State of which it is part; in the case of bottling (Operation of the product introduction in a container of less than 60 liters for the purpose of sale) in containers of more than 60 liters, it will be compulsory to includ the name of the sender;

b) the labeling of wines coming from third party countries other than liqueur wines, petillant wine, whether or not sparkling, as listed in Annex I to the framework Regulation; the name of the importer will be placed on the label and if the imported wine has been bottled in the EU, the name of the bottler will be also included;

c) the labeling of liqueur wines, sparkling or non-sparkling petillant wines as referred to in Annex I to the framework Regulation, including those of third party country origin; in the case of these wines, the mention of the sales name of the product, the nominal volume, the alcoholic strength gained in volume and the lot number must be included (According to Council Directive EEC 89/396 of 14 July 1989 on indications or marks identifying the lot to which a food product belongs<sup>1</sup>;

d) the sales name, which is composed of the following terms, depending on the characteristics of the product, as follows:

i. for <u>table wines</u> - the mention "table wine" accompanied by the name of the EU country where the grapes were produced and vinified when the export is made to another Member State; the mention "table wine" accompanied by the phrase "blend of wines from different European Community countries" when wines are the result of grapes processing from several EU countries; the mention 'table wine' accompanied by the words 'wine obtained in ... from grapes harvested from ...' when the wines are harvested in a Member State and are being vinified in another Member State;

ii. for <u>table wines with geographical indication</u> - the mention "table wine" followed by the name of the geographical unit; in the case when there have been established known names in order to determine the specific geographical indication of

a country at European level (eg "regional wine"), the reference "table wine" is no longer mandatory, and the geographical area followed by this mention is inserted;

iii, for <u>wines with a controlled designation of origin</u> (VDOC), the term "VDOC" (or "wine with a controlled designation of origin"), the mention "LWDOC." (or "liqueur wine with a controlled designation of origin"), the mention "PWDOC" (or "petillant wine with a controlled designation of origin"), a specific traditional mention, as we will discuss below;

v. for <u>imported wines</u> - the word "wine" accompanied by the name of the country of origin and the name of the relevant geographical area (if applicable);

vi. for liqueur wines - the term "liqueur wine";

vii. for petillant wines - the mention "petillant wine";

viii. for sparkling petillant wines - the term "sparkling petillant wine";

The optional mentions, called by the European legislator "optional indications", allow manufacturers /traders to add on the label the following indications designed to better characterize the product, namely: the name, address and function of one of the persons who participated in the trading, type of product, specific colour, harvest year, name of one or more vine varieties, a prize, a medal or a contest (Notice the comment below on the medals and distinctions inserted on the labels<sup>)</sup>, indications on the method of obtaining and production, the name of the winemaking company, additional traditional mentions, etc.

As part of the optional indications, the European legislator has also inserted additional traditional mentions which, along with other information, can provide a clear picture of the product. Thus, the mandatory mentions on the label, supplemented with regulated optional indications, may be supplemented with other indications, provided that the additional text does not create a risk of confusion among addressees as regards mandatory mentions and optional indications. According to art. 23 of Regulation no. 753/2002 laying down certain detailed rules for the application of Council Regulation (EC) No. 1493/1999 as regards the designation, nomination, presentation and protection of certain wine products, through the words "Additional traditional mention" shall be used to indicate the method of production, preparation, maturation or the quality, colour, type of place or a special event related to the history of the wine in question. Both theoretically and practically, this comprehensive definition grants the Member States the right to designate the nationally produced wines that meet the criteria mentioned in the definition established at European level.

If, in the case of mandatory and optional mentions expressly regulated, the things cannot be interpreted, the problems arise in the case of optional mentions that may be subject to violations of rights already authorized. In this respect, the European legislation restricts the use of additional information other than the mandatory ones, establishing as a barrier the situation in which there may be a risk of confusion among consumers regarding the regulated mandatory and optional mentions.

It should be noted that this risk of confusion must be assessed by an average consumer, not addressed to a wine specialist. The CJEU has stated that "... it must be demonstrated that the use of the mark is indeed likely to mislead the targeted consumers and thereby influence their economic behavior. In this regard, the national court must take into account the presumed expectations regarding these indications of an <u>average consumer</u>, <u>normally informed</u>, sufficiently attentive and competent. " (Regulation of 28 January 1999, Sektkellerei Kessler (C-303/97, ECR I-513, paragraph 32) (risk of confusion / labeling of German sparkling wine - Sekt)

In order to avoid confusion with other similar products, the European legislation requires that on the label applied to the container the following elements should be necessarily inserted: the identity and quality of natural and legal persons or

associates who have been or are involved in the production or commercial trade of wine, composition, alcohol level by volume, colour, origin or provenance, quality, vine varieties, harvest year or nominal volume of the containers. The mandatory indications on the description, designation, presentation and protection of certain wine products (Except for sparkling wines) must be presented in a clustered, clearly visible, clear and distinct manner from the other inscriptions on the label. The importer mentions and batch number may be inserted separately from the other mandatory information. The ink or printing method must be resistant to external factors, so as to avoid the risk of deleting this information during the transport, handling process etc.

Regarding the alcoholic strength by volume, this mention must be given on labels with a height of at least 5 millimeters of characters if the nominal volume is more than 100 centilitres, of at least 3 millimeters if the volume is lower or equal to 100 centilitres and bigger than 20 centilitres and at least 2 millimeters if the volume is less than or equal to 20 centilitres. The obtained distinctions or medals can be mentioned on the label of the table wines with geographical indication or wines QWPSR only if these distinctions have been awarded to the batch of wine in question in competitions authorized by EU Member States or third party States to the extent that they were organized under advertising conditions designed to remove any suspicion of doubt. Thus, the mention of "wine awarded at ..." inserted on a label applied to a batch of wine which differs from the legal batch equates to an infringement of European rules on consumer protection and market competitors.

Regarding the language used for the mentions on the label, the European legislation requires to be one or more of the official languages of the European Community, so that the information of the consumer to be easier understood. Given their specificity, the mentions referring to the name of the region of production/name of another geographical unit, the traditional mentions and additional traditional mentions (By "additional traditional mention", it is indicated the method of production, elaboration, maturation or quality, colour, type of place or a special event linked to the history of the wine in question), the name of the winemaking company, the mentions about bottling can be expressed in the official language(s) of the member state territory on which that product was produced. Also in the countries where other languages are spoken besides the official language, these specific mentions may also be expressed in the language traditionally used in that area if it is closely related to that product.

#### CONCLUSIONS

The label represents, together with other accompanying fiscal and customs documents, the way in which wine entries into consumer consciousness, contributing greatly to building quality standards both in terms of protecting the final recipient and in establishing an economic order based on the principles which governs loyal competition between manufacturers. Editing legal requirements requiring mandatory mentions insertions on the labels attached to bottled products does not constitute an infringement of the right to promote, but double guarantees in favor of both consumers and honest traders in the vast field of wine production and trading.

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# WINE DESCRIPTION. THE ADDITIONAL TRADITIONAL MENTION

Voinea-Mic Cătălin<sup>1</sup>, Giugea Nicolae<sup>1\*</sup>, Berta Corina<sup>1</sup>, Mărăcineanu Liviu<sup>1</sup>

<sup>1\*</sup>University of Craiova, Faculty of Horticulture \* Correspondence author. E-mail: giugeanicolae@gmail.com

Key words: additional traditional mention, labels, optional mentions, misleading, average consumer

#### ABSTRACT

The description of wine products by means of additional traditional mentions is not limited to the regulations expressly provided by the Community or national legislator, and it can take various forms of expression as long as there is no concrete misleading of the consumer.

#### INTRODUCTION

Given the very wide appreciation left by the European legislator for additional traditional mentions, referring to the established principle of legislation liberalizing on product names marketed in the European Union, this article aims to analyze the extent to which the trading of wine products under strictly unregulated denominations by prior authorization, is permitted. More specifically, it is interested in the extent to which additional mentions may be inserted on labels insofar as they are not part of the mandatory and optional mentions expressly regulated by the legislator.

#### MATERIAL AND METHODS

This article was based on European regulations and European jurisprudence in the field, using the classical methods of legal interpretation of legislation and legal analysis of the text of parts of the procedural documents in some cases solved by the CJEU.

#### **RESULTS AND DISCUSSIONS**

According to art. 23 of Regulation no. 753/2002 laying down certain detailed rules for the application of Council Regulation (EC) No 1493/1999 (Concerning the common organization of the wine market, in force since 4 May 2002, published in JOCE no. 118 of May 4, 2002) (hereinafter referred to as the "*Implementing Regulation*"), the term "*Additional traditional mention*" means the term used to indicate the method of production, preparation, maturation or quality, colour, type of place or special event related to the history of the wine in question. This comprehensive definition leaves the Member States the option of designating the wines produced at national level on the basis of the criteria set out in the definition established at European level.

Given the very wide margin of appreciation left by the European legislator regarding the additional traditional mentions, referring to the established principle of the legislation liberalization of denomination, this article aims to analyze to what extent the trading of wine products under unregulated denominations strictly subject to prior authorization is permitted. More specifically, it is interested in the extent to which additional traditional mentions can be inserted on labels insofar as they are not part of the mandatory and optional mentions expressly regulated by the legislator.

If the range of these mentions is quite wide, given the very permissive definition, we make it clear that there are regulations that expressly refer to the necessity and / or the possibility to insert the additional traditional mention.

Thus, as an example, we introduced the provisions of art. 14 par. (1) lit. (c) of the "Implementing Regulation" for grape must in fermentation intended for direct human consumption or for wines obtained in the Community from over-maturated grapes, where it is expressly stated that the name under which these products may be put up for sale may indicate, besides the name of the geographical unit of production and the product category (Grape must in fermentation" or "wines obtained from overmaturated grapes") which are mandatory, also a specific traditional mention, to the extent that it does not become repetitive to the name under which the product is marketed.

Besides the punctual regulations, the limits of the use of these mentions, or rather the condition that limits the use of this type of description, is given by art. 6 par. (1) of the "Implementing Regulation" according to which the labeling of the concerned products may be "supplemented with other indications, provided that the additional text does not create a risk of confusion for the persons to whom it is addressed", in particular regarding the mandatory and optional mentions.

It should be noted that, for an effective analysis of the permissiveness of an additional traditional mention, we must refer, on the one hand, to the list of mandatory mentions and optional mentions stipulated in section A.1. and section B.1. of Annex VII to Regulation (EC) No. No 1493/1999 on the common organization of the wine market and on the other hand to the risk of confusion, misleading, which this mention may bring to consumers.

If, regarding the mandatory mentions, the situation is clear in the sense that an additional traditional mention does not have to create confusion with a mandatory mention, problems arise when referring to an optional mention, regulated by the legislator. More specifically, in practice, the question of the lawfulness of the presentation of a wine product with an additional traditional mention which does not appear to be regulated *expressis verbis* by the legislator has been raised.

Starting from the regulation of mandatory and optional indications, we can say that any indication referring to the method of production, maturation elaboration and wine quality can be authorized not only as an optional indication regulated under point B.1. (b) of the fifth indent of Annex VII to Regulation No. 1493/1999 because the labeling of the products within the community can be supplemented with additional traditional mentions, according to the rules provided by the producer state. In this respect, Art. 23 of the "Implementing Regulation" provides that Member States shall lay down the framework and conditions for the use of indications concerning the method of production or the methods for the preparation of table wines with a geographical indication or of wines QWPSR (Quality wines produced in specified regions) for the wines produced on their territory.

In support of the conclusion that traders may use additional traditional mentions, unregulated, the European rules provided in point B.3 of Annex VII to Regulation No. 1493/1999 that, in the case of table wines, table wines with a geographical indication, wines with a controlled designation of origin (VDOC), liqueur wines, petillant wines and sparkling petillant wines (Mentioned in Annex I of the Regulation no. 1493/1999 on the common organization of the wine market, in force

since 14.07.1999, published in JOCE no. 179 of 14.07.1999), including those coming from third party countries of the European Union, the labeling may contain other information than the mandatory and optional mentions expressly referred to in the European regulation framework.

Basically, from the point of view of the regulation, we consider that the only obstacle to the use of an additional traditional mention, other than those expressly regulated, is the existence of an express provision prohibiting the use of that mention at Community or national level. In other words, authorizing the use of an additional traditional mention should not be inflexible in relation to the existence or not of the regulation but must be analyzed on a case-by-case basis whether or not there is a risk of misleading the consumers.

Thus, the second condition to be met with regard to specific traditional mentions, is that there is no risk of misleading the consumer. In other words, each case must be analyzed in concrete terms to determine whether a particular name is misleading or not. "*The purely abstract danger of creating the appearance that it is a protected designation cannot lead to the prohibition of the use of an unregulated indication, as long as there is no actual misleading of the consumer*" (Conclusions of the Advocate General VERIKA TRSTENJAK delivered on 25 October 2007 in Case C-285/06 Heinrich Stefan Schneider vs Land Rheinland - Pfalz, published at eur-lex.europa.eu on 25 October 2007).

Regarding the consumer, or the danger of misleading the consumer, we notice that always this risk of confusion must be appreciated by an average consumer, not reported to a wine specialist. The CJEU (Court of Justice of the European Union) has stated that "... it must be demonstrated that the use of the mark is indeed likely to mislead the target consumers and thereby influence their economic behavior. In this regard, the national court must take into account the presumed expectations regarding these indications of an <u>average consumer</u>, normally informed, sufficiently attentive and competent" (Resolution on 28 January 1999, Sektkellerei Kessler in Case C-303/97 ECR, p. I-513, paragraph 32; (risk of confusion / labeling of German sparkling wine - 'Sekt').

It is well-known that this consumer will not know the European regulations regarding names, traditional or other additional regulated mentions, the appreciation of the risk of confusion being reported by the ability of the unregulated mention to mislead this consumer always by reference to mandatory or optional mentions already existing in the market. Always an increased attention in the assessment of the risk of misleading should be based on the stipulation of art. 24 paragraph (2) of the "Implementation Regulation", according to which an existing traditional mention is protected against "any illicit, imitation or use of citations, even if the mention in question is accompanied by an expression as follows <<kind>>, <<type>>, <<imitation>>, <<br/>timitation>>, <<br/>

In this regard, as an example, we demonstrate that there is no imitation or citation unless the language used is the same as that in which the already protected traditional mentions are indicated. Thus, the legislator limited the scope of protecting traditional mentions to their own language. The use of the German expression "Reserve" for German wines as a specific traditional mention contravenes Annex no. III to the "Implementing Regulation" which recorded this mention for Austrian wines and which would mislead the consumers of the Austrian wines holding this mention. However, derivative mentions such as "Privat-Reserve" may be used instead, without contravening the European standards, the legislator even allowing translations of the mentions, but without allowing the copying of already used and legally protected names (it is forbidden to have the already used names in the language of origin).

#### CONCLUSIONS

In conclusion we can specify that specific traditional mentions can be used without barriers as long as they do not contravene the European or national norms defining these categories of mentions, the legality of their use being censored on a case by case basis in terms of avoiding the mislead of the consumer, in relation to already existing and protected names at Community level.

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### EFFECT OF RESIDUE GREEN ROBUSTA COFFEE, OBTAINED AFTER THE REMOVAL OF HOT AQUEOUS EXTRACT, ON SOIL CHEMICAL PROPERTIES

Wogiatzi Eleni<sup>1</sup>, Gougoulias Nikolaos<sup>1</sup>\*, Giannoulis Kyriakos<sup>1</sup>, Miliakidi Eleni-Maria<sup>1</sup>

<sup>1</sup> Department of Agricultural Technology, Technological Educational Institute of Thessaly, 41110 Larissa, Greece \*Correspondence author. email: ngougoulias@teilar.gr

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## ABSTRACT

The effects of the green coffee residue, obtained after from the removal of the extract with hot water, on soil chemical properties for an incubation experiment, were studied. The air dried residue incorporated at four different rates (0, 0.3, 0.6, and 0.9g per 50g of soil mixture with 9,84g of manure) resulted in increase in organic carbon mineralization, at the two higher rates by 17.4 and 24.5%, respectively, at compared to the control. The addition of the residue Robusta in all treatments increased available forms of Mn, Fe and organic P, while at the two highest rates increased the K-exchangeable and N-NO<sub>3</sub>, and at the highest dose increased the N-NH<sub>4</sub><sup>+</sup>, and available Cu. Available forms of P and Zn was not significantly affected by the residue addition. The results of this study indicated that residue coffee could be applied to the soil without any extremely negative effect on the soil chemical properties.

#### INTRODUCTION

In biological agriculture is mandatory to replace chemicals with natural additives. Between them, various organic residues for their role in the soil fertility, the plant essential oils for their role as pesticides against on the soil pathogens (Gougoulias et al. 2010, Gravanis et al. 2004, Hartemink and Hutting 2005).

Green coffee beans contain a tremendous number of phenolic compounds, such as chlorogenic acids, ferulic acid, tannins, proanthocyanidins, lignans, and tocopherols, which exert strong antioxidant effect (Borelli et al. 2002, Farah and Donangelo 2006).

Literature on coffee waste as a soil amendment is limited, however studies have shown that coffee waste is a valuable organic fertilizer, particularly for highly poor soils (Zake et al. 2000). In addition, coffee waste can be used as a fertilizer for sandy soils and an improvement of the soil physico-chemical properties (Kasongo et al. 2011).

The objectives of this research were to study, in the laboratory, the effect of the application of the green coffee solid residue obtained after the removal of the extract with hot water on soil organic matter and on soil chemical properties.

#### MATERIAL AND METHODS

Commercial green Robusta coffee beans from the local market in Larisa were dried in a dark place at room temperature, finely ground, shaken at 150 rpm for 30 min using boiling water. The extraction of the green Robusta coffee with boiling water solvent results in the extraction of phenol compounds equal to16.86 mg (GAE)/g DW and antiradical activity (DPPH•) 93.6 (µmol Trolox /g) DW. Green Robusta coffee beans are a source of bioactive components and the extracts can find applications as food additives, either in the form of powder or as extracts highly concentrated at polyphenols and antioxidant activity. The green coffee solid residue, which remained after the removal of the extract with hot water, was air dried and was used for the incubation experiment.

**Incubation experiment**: In this study, 9.84 g of a sheep manure containing 2.5 g of organic matter (Table 1), obtained from the farming establishments of TEI of Thessaly, and was added to 50 g of air-dried soil that was poor in organic matter, derived from the same region (Table 1). Into 50 g of this soil plus 9.84 g of manure, 0, 0.3, 0.6 and 0.9 g of air-dried and well milled green Robusta coffee residue, were added. Thus an experimental unit is constituted by 50 g of soil, 9.84 g of manure and a variable amount of residue green Robusta coffee. In the incubator, the treatments were prepared in four replicates and kept at 28 °C for a period of 15 weeks. During the first three weeks of the incubation period, the moisture was maintained at two-thirds of field capacity, but for the next three weeks the soils were left to dry. This process was repeated until the end of the incubation period according to (Wu and Brookes 2005) they reported that the alternation of drying and rewetting soil samples enhances mineralization of both soil biomass organic matter and non-biomass organic matter. Soil aeration and regulation of moisture enhance the growth and metabolic activity of aerobic soil microorganism (Gordon et al. 2008). At the end of the incubation period, soil samples were analyzed.

**Methods of analyses:** Samples were analyzed using the following methods which are referred by (Page et al. 1982).

Organic matter was analyzed by chemical oxidation with 1 mol/l  $K_2Cr_2O_7$  and titration of the remaining reagent with 0.5 mol/l FeSO<sub>4</sub>.

Both ammonium and nitrate nitrogen were extracted with 0.5 mol/l CaCl<sub>2</sub> and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) was extracted with 0.5 mol/l NaHCO<sub>3</sub> and measured by spectroscopy. Exchangeable forms of potassium and sodium ware extracted with 1 mol/l CH<sub>3</sub>COONH<sub>4</sub> and measured by flame photometer.

Organic phosphorus was measured after mineralization by combustion of the sample and subtraction of the mineral phosphorus amounts, which had previously been estimated in the laboratory. The mineral amounts were extracted with 1 mol/l  $H_2SO_4$  and all forms were measured by spectroscopy.

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid  $0.005 \text{ mol/l} + \text{CaCl}_2 0.01 \text{ mol/l} + \text{triethanolamine } 0.1 \text{ mol/l})$  and measured by atomic absorption.

For the determination of total metals Mn, Cu and Zn , 1 g of material, digestion at  $350^{\circ}C + 10 \text{ ml HNO}_3 + 5 \text{ ml HCLO}_4$ . According to the method described by (Varian 1989), the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus), with the use of flame and air-acetylene mixture.

**Statistical analysis**: Data analysis was made using the MINITAB (Ryan et al. 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

#### Table 1

Chemical properties of soil samples, manure and residue green Robusta coffee used in the experiment

Property	Soil	*Manure	Residue green
			Robusta coffee
Texture	Sandy Loam		
рН	7.94 ± 0.4	8.85 ± 0.6	
EC, dS/m	0.46 ± 0.05	2.33 ± 0.3	
Organic matter (%)	0.85 ± 0.05	25.41 ± 1.3	
CaCO <sub>3</sub> (%)	8.68 ± 0.6	7.95 ± 0.6	
N -Total (g/kg)	1.26 ± 0.10	9.36 ± 0.6	20.79 ± 0.9
N-NH4 <sup>+</sup> (mg/kg)	47.2 ± 9.3		
N-NO <sub>3</sub> <sup>-</sup> (mg/kg)	113.2 ± 14.2		
K-exchangeable (mg/kg)	223.7 ± 7.6		
K-Total (g/kg)	4.73 ± 0.24	5.30 ± 0.5	0.43 ±0.02
Na-exchangeable (mg/kg)	222.3 ±9.6		
Na-Total (g/kg)	0.68 ± 0.04	0.47 ± 0.02	0.17 ±0.01
CEC (cmol/kg)	19.8 ± 1.2		
P -Olsen (mg/kg)	14.2 ± 3.3		
P -Total (g/kg)	0.32 ± 0.04	4.37 ± 0.2	1.51± 0.09
Cu –DTPA (mg/kg)	0.83 ± 0.05		
Zn -DTPA (mg/kg)	1.31 ± 0.09		
Mn -DTPA (mg/kg)	2.33 ± 0.13		
Cu –Total (mg/kg)		27.5 ± 2.7	3.97 ± 0.22
Zn -Total (mg/kg)		271.4 ± 15.2	0.12 ± 0.01
Mn -Total (mg/kg)		643.5 ± 36.4	2.02 ± 0.14
Fe-Total (g/kg)		8.14 ± 0.3	0.099 ± 0.002
Mg-Total (g/kg)		14.58 ± 0.9	1.35 ± 0.08

\*Digested sheep manure four months; Electrical conductivity, (EC) and soil pH is determined in (1:5) soil/water extract; Data represent average means and SE deviation. (n)=4.

#### **RESULTS AND DISCUSSIONS**

Decomposition of organic matter was increased with the increase of residue Robusta concentration in soil, as compared with the control (soil plus manure). In particular, the decomposition of organic matter in soil where the two higher rates of the residue Robusta were incorporated were 17.4 and 24.5 % higher, respectively, compared to the control (Table 2). However, was not significantly affected by the smaller quantity addition of the residue Robusta.

The analysis of variance performed for the electrical conductivity data indicated that the electrical conductivity was slightly increased by the added of the residue Robusta. In particular, the highest residue Robusta concentration, increased electrical conductivity by 24.5%, without any risk of salinity (Table 2). In contrast, the soil pH was reduced by the residue Robusta addition in all treatments at comparison with control (soil plus manure).

Ammonium was increased by the addition of the highest rate of the residue Robusta and was not significantly affected by the two lower quantities of the residue Robusta. Also, nitrate nitrogen was increased by the addition of the residue Robusta of the two upper rates, and it was not significantly affected by the smaller quantity of the residue Robusta (Table 2). In particular, the addition of residue Robusta at the highest dose increased ammonium and nitrate nitrogen by 202% and 43.5% respectively in comparison with the control (soil plus manure).

The analysis of variance performed for the organic phosphorus and available P data indicated that the organic phosphorus was increased by the residue Robusta added. The greatest increase was caused by the greatest dose of residue Robusta (Table 2). However, available P was not significantly affected by the residue Robusta addition.

Table 2

Properties	Treatments			
	С	R1	R2	R3
C-organic mineralization	257.4b	238.0b	302.1a	320.4a
(g/kg initial organic carbon)				
EC (dS/m)	1.06b	1.12b	1.15b	1.32a
рН	7.01a	6.88b	6.87b	6.86b
N-NH4 <sup>+</sup> (mg/kg)	44.0c	54.7c	47.25c	133.0a
N-NO <sub>3</sub> (mg/kg)	543.2c	528.35c	654.5b	779.8a
P-Olsen (mg/kg)	216.8a	212.3a	207.8a	202.1a
P-Organic (mg/kg)	620c	749.5b	1507.3a	1333.2a
K- exchangeable (mg/kg)	613.1c	624.1c	690.43b	817.46a
Na- exchangeable (meq/100 g)	0.511a	0.474a	0.486a	0.524a
CEC (mmol/kg)	16.6c	16c	17.1b	18.9a
Cu-DTPA (mg/kg)	0.87b	0.68b	0.87b	1.26a
Zn-DTPA (mg/kg)	6.36a	6.30a	6.40a	6.80a
Mn-DTPA (mg/kg)	2.10c	2.76b	3.32a	3.43a
Fe-DTPA (mg/kg)	0.48b	1.09a	1.38a	1.19a

Chemical properties of soil mixtures at the end of the incubation period

Electrical conductivity, (EC) and pH measured in (1:5) soil/water extract. For each chemical property of soil mixtures, lines of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). C, control (50g soil plus 9.84g manure); R1, R2 or R3, control and residue Robusta 0.3, 0.6 or 0.9g, respectively.

The exchangeable form of potassium was significantly affected at the end of the incubation period by the residue Robusta addition. In particular, the exchangeable form of potassium was increased by the two highest rates of the residue Robusta by 12.6% and 33.3% respectively compared to the control. However, exchangeable Na was not significantly affected by the residue Robusta addition (Table 2).

The available copper was increased by the addition of the highest rate of the residue Robusta and was not significantly affected by the two lower quantities of the residue Robusta. The addition of the residue Robusta at the highest dose increased available copper by 43.8% at comparison to the control. However, available zinc was

not significantly affected by the residue Robusta addition. The available manganese was increased with increasing residues Robusta concentration by 31.4, 58.1 and 63.3% respectively compared to the control. Also, available iron was increased by the residue Robusta addition in all treatments at comparison to the control by 127.1% to 187.5%.

The total forms of potassium and phosphorus was increased at the end of the incubation period by the residue Robusta addition at comparison to the control. In particular, total potassium increased of the highest rate addition of residue Robusta by 6.3% compared to the control, while total phosphorus was increased by the two highest rates of the residue Robusta by 43.9% and 44.5% respectively, at comparison to the control (soil plus manure). However, total forms of sodium, magnesium, copper, zinc, manganese and iron was not significantly affected by the residue Robusta addition at comparison to the control.

Table 3

Properties	Treatments				
	С	R1	R2	R3	
Na-total (g/kg)	0.41a	0.40a	0.45a	0.41a	
K-total (g/kg)	6.23b	6.04b	6.23b	6.62a	
Mg-total (g/kg)	6.46a	6.38a	6.27a	6.28a	
P-Total (g/kg)	1.82b	1.88b	2.62a	2.63b	
Fe-total (g/kg)	22.47a	21.69a	21.89a	21.98a	
Cu-total (mg/kg)	24.15a	19.32a	19.32a	19.32a	
Zn-total (mg/kg)	84.22a	94.28a	84.22a	88.92a	
Mn-total (mg/kg)	828.7a	788.2a	763.0a	828.8a	

Total forms of elements of the soil mixtures at the end of the incubation period

For each chemical property of soil mixtures, lines of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). C, control (50g soil plus 9.84g manure); R1, R2 or R3, control and residue robusta 0.3, 0.6 or 0.9g, respectively.

#### CONCLUSIONS

Three different rates of green coffee residue, obtained after the removal of the extract with hot water to determine the antioxidant activity, were applied into a soil amended with manure. These results, confirm the role of green coffee residue as it could be applied to the soil as an acceleration agent for soil organic matter biodegradation, without any extremely negative effect on the available forms of nutritional mineral elements, while that recycling constitutes a useful practice for environment protection.

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