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#### CALORIMETRIC DETERMINATION OF THE QUANTITATIVE INHIBITORY PARAMETERS OF ETHANOL FOR SOME YEAST STRAINS SELECTED IN VALEA CALUGAREASCA AND PANCIU REGIONS FOR WINE PRODUCTION

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KEY WORDS: ethanol, inhibition parameters, winemaking, microbial calorimetry, yeast selection

#### ABSTRACT

Tolerance to ethanol is a key parameter for any yeast strain that is to be used for winemaking. In this work the quantitative inhibitory parameters regarding ethanol for 9 yeast strains selected in Valea Călugărească and Panciu vineyards and wines were determined by the use of an isothermal microbial calorimetry technique. A group of strains belonging to the Saccharomyces genus, with good tolerance to ethanol, was thus identified. These yeasts, after some further testing for the sensory qualities of the wines they produce, could, in principle, be recommended for the production of wines with a local character. A number of non-Saccharomyces yeast strains were also identified, which belong rather to the category of spoilage veasts. Some of the strains of this last group can also be, in principle, recommended for wine production, but before that they should be tested further on wines for the evaluation of the chemical and sensory profile.

#### **INTRODUCTION**

Most quality wines are obtained nowadays by controlled fermentations in the presence of specially selected yeasts which confer good and stable oenological characteristics (Rosini, 1984; Goldfarb, 1994; Rainieri and Pretorius, 2000). As a result, many yeasts are at present selected (Heard, 1999) and commercialized by several companies from abroad, each yeast strain having certain traits worth to be taken into consideration for a certain style of wine. For many years the most important wine producing countries changed their traditional winemaking practices by introducing the inoculation of grape must with starter (Kunkee and Amerine, 1970), especially form S. cerevisiae species. However, most often these strains selected in another geographical location, from another grape variety or even from other sources (Rainieri et al., 1998; Castellari et al., 1994), can significantly change the sensory profile of a local wine, wiping away the sensory characteristics that account for their typicality. Finding an yeast strain

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possessing an ideal combination of technological and qualitative traits is not an easy task. Therefore, there are still many winemakers who favour spontaneous fermentation and, in 1999 was estimated that in the world around 80% of the wines were made without the addition of a starter culture (Heard, 1999). A way to avoid losing regional typicality is the selection of a yeasts from local vineyards (Romano *et al.*, 2002), able to ferment the musts in good conditions, without confering off-flavours or causing sluggish or stuck fermentations. One of the most important characteristics of a selected yeast is the tolerance to the end-product of fermentation, that is ethanol (Zambonelli, 2003). Therefore, after the isolation of the yeasts from local vineyards, in order to select the most suitable for wine fermentation, the inhibition parameters of ethanol should be determined (Jimenez and Benitez, 1986). In this work a calorimetric method is applied for the determination of growth inhibitory parameters in the presence of ethanol for nine yeast strains selected in Romanian vineyards.

#### MATERIAL AND METHODS

The yeasts evaluated in this work were isolated and selected in two Romanian vineyards, Valea Călugărească and Panciu, either in conventional monovarietal plantations, or in ecological plantations. The yeast strains isolated were identified as belonging to *Saccharomyces* genus, but also to some other genera. The isolation location and grape variety, codification and the species are included in Table 1.

For the determination of the ethanol tolerance, the yeast growth in the presence of ethanol in various concentrations was determined by monitoring the heat evolved during culture growth in a microbial isothermal calorimeter that can record the signals of 24 cultures in a single experiment. Each yeast was inocculated in vials containing 5 ml liquid medium with 20 g/l of glucose. Aside of the 4 control vials, that contained no added ethanol, in the rest of the 20 vials there ethanol was added in increasing concentrations (v./v.): 1.75% (3 vials); 2.62% (3 vials); 3.49% (2 vials); 4.36% (2 vials); 5.24% (2 vials); 6.11%; 6.98% (2 vials); 7.85% (2 vials); 8.73% (2 vials).

For each strain the quantitative inhibition parameters for ethanol were calculated taking into consideration both the reduction of growth activity and the retardation of growth (Antoce *et al.*, 1996a). Both methods provide reliable quantitative parameters, the first method being the most used, while the second one is referred to only in the case of a bactericidal type of inhibition, when the growth rate remains constant and the method based on reduction of growth activity cannot be applied.

The quantitative parameters that are derived by processing the signals recorded by the calorimeter's computer during yeast growth are the following:

- the specific growth activity  $\mu_i/\mu_m$  where  $\mu_i$  represents the growth rate constant of a culture in the presence of ethanol in concentration *i* and  $\mu_m$  is the growth rate constant reached in the absence of ethanol (maximum growth rate constant);

- the specific growth retardation  $t_{\alpha}(0)/t_{\alpha}(i)$  where  $t_{\alpha}(0)$  represents the time required for a culture to reach the growth level  $\alpha$  in the absence of ethanol (minimum time) and  $t_{\alpha}(i)$  is the time required for a culture to reach the same  $\alpha$  level of growth in the presence of ethanol in concentration *i*;

- the concentration of inhibitor at which the growth activity of the yeast cells is inhibited by 50%,  $K_{\mu}$ , the value derived form the  $\mu_i/\mu_m$  drug potency curve (variation of  $\mu_i/\mu_m$  with the inhibitor concentration), after fitting with the drug potency equation:  $\mu_i/\mu_m = 1/(1+i^{m_\mu}/K_u)$ 

- the concentration of the drug which doubles the time required for the culture to reach a certain level of growth,  $K_{\theta}$ , the value derived form the  $t_{\alpha}(0)/t_{\alpha}(i)$  drug potency curve (variation of  $t_{\alpha}(0)/t_{\alpha}(i)$  with the inhibitor concentration) after fitting with the drug potency equation:  $t_{\alpha}(0)/t_{\alpha}(i) = 1/(1+i^{m_{\theta}}/K_{\theta})$ 

- the minimum inhibitory concentration MIC<sub>µ</sub>, representing the concentration at which the growth activity of the microorganisms is completely inhibited, determined by fitting the  $\mu_i/\mu_m$  drug potency curve with the MIC equation:  $MIC_u = (1/k_1)^{1/m_i}$ 

- the minimum inhibitory concentration MIC<sub>0</sub>, also representing the concentration of inhibitor at which the growth activity of the microorganisms is completely inhibited, but determined by fitting the  $t_{\alpha}(0)/t_{\alpha}(i)$  drug potency curve with the MIC equation:  $\text{MIC}_{\theta} = (1/k_2)^{1/m_2}$ 

- the cooperativity degree  $m_{\mu}$  and  $m_{\theta}$ , resulting also during the regression analysis applied for the deduction of  $K_{\mu}$  and  $K_{\theta}$ ; they are derived from the same drug potency equations and are related to the steepness of the drug potency curves and represent a measure of the cooperativity in the drug-cell interaction.

A complete description of these parameters and the theoretical background are given extensively elsewhere (Antoce, 1998).

#### **RESULTS AND DISCUSSION**

In this work the calorimetric technique was applied for a rapid determination of the quantitative inhibitory parameters of ethanol, in order to select from the isolated yeasts the most resistant and hence more suitable ones for the local wine production. In the same time calorimetry allowed for the evaluation of the growth rate, a parameter related to the adaptability of a yeast to a growth medium and its capability of growing rapidly and competing with other strains, as it is the case of the grape must.

For each strain the ethanol inhibition was followed in triplicate and the calculated inhibitory parameters are reported in Table 2 as mean values and standard deviations.

The drug potency curves that were fitted with the drug potency equations and MIC equations are presented for all the evaluated yeast strains, the first five in Fig. 1 and the other five in Fig. 2. In each figure, on the left side there are the drug potency curves derived from the specific growth activity  $\mu_i/\mu_m$  and in the right side the curves obtained on the basis of the specific growth retardation,  $t_{\alpha}(0)/t_{\alpha}(i)$ . Each set of data points was fitted first with the drug potency equation (continuous line) in order to derive the 50% inhibitory concentration,  $K_{\mu}$  and  $K_{\theta}$ , respectively, then with the MIC equation (dotted line), in order to obtain the minimum inhibitory concentration, MIC<sub>µ</sub> or MIC<sub>θ</sub>, at the interception of the line with the ethanol concentration axis.

As stated before (Antoce *et al.*, 1997a; Antoce *et al.*, 1997b) the most used parameters for the evaluation of the effect of an inhibitor are those derived from the specific growth activity,  $\mu_i/\mu_m$ , that is, the parameters  $K_{\mu}$  and MIC<sub> $\mu$ </sub>. These best describe the effect of an inhibitor that acts based on a bacteriostatic inhibition mechanism. Actually, theoretically speaking, in the case of a pure bacteriostatic action, the calculated parameters do not differ signifficantly from the complementary ones evaluate from the data points of specific growth retardation, that is  $K_{\theta}$  and MIC<sub> $\theta$ </sub>. However, in practice we have situations in which the inhibitor, ethanol in our case, acts as a bactericidal drug, or has a mixed action mechanism. In the case of a pure bactericidal effect, a significant number of cells are killed from the beginning, when they get in contact with the ethanol, but the surviving cells manage afterwards to multipliy with the same growth rate as in the absence of ethanol. This behaviour leads to the impossibility of calculation of the specific growth rate activity,  $\mu_i/\mu_m$ , in this case  $\mu_i$  remaining aproximately constant and equal to  $\mu_m$ . In such conditions, only the specific growth retardation  $t_{\alpha}(0)/t_{\alpha}(i)$  analysis can be performed, offering a working alternative for the calculation of the inhibitory parameters.

In the present group of yeasts, as it can be seen both in Fig. 1 and 2, and also in Table 2, there was no bactericidal effect recorded for the action of ethanol under these conditions. However, mixed or bactericidal effects of ethanol on the yeast growth were observed for other evaluated yeast strains (Antoce and Takahashi, 1996 b; Antoce *et al.*, 1997c). Another parameter that demonstrates that in all these cases the effect of ethanol was mostly bacteriostatic is the cooperativity degree, both  $m_{\mu}$  and  $m_{\theta}$  being in the range of 0.9-1.7. Values of cooperativity degree of 1 signify that the drug has a pure bacteriostatic action. The drug potency curves plotted in Fig. 1 and 2 also show a normal steepness, characteristic to a mainly bacteriostatic action.

Of all the isolated and tested yeast strains the lowest resistance to the presence of ethanol in the growth medium was observed for PN5 B-B-6, the maximum concentration at which it is estimated this yeast would still survive, with a reduced growth rate just above zero is  $8.904 \pm 0.692 \%$  v./v. ethanol (MIC<sub>µ</sub>). For this yeast, at a concentration of ethanol of  $3.895 \pm 0.167 \%$  v./v. the growth rate is already reduced by 50% compared to the growth rate in the absence of ethanol or by the ethanol released in the fermentation must by other competitive yeasts. It is, therefore, a yeast not recommended as a wine yeast and, to confirm this result, this strain was later on identified as belonging to the *Kloeckera* genus.

Other non-Saccharomyces yeast strains, identified as Candida utilis and Rhodotorula glutinis, CSEC B-2 and CSEC B-3, respectively, proved to be resistant enough to survive in a medium with ethanol in concentration normally occuring in wine or even higher. Thus, the strain of Candida utilis had an MIC<sub>µ</sub> of 14.092  $\pm$  1.039 % v./v. ethanol, while the strain of Rhodotorula glutinis reached an impressive MIC<sub>µ</sub> of 24.064  $\pm$  4.339 % v./v. ethanol, with a 50% reduction of the growth rate at an ethanol concentration usually obtained in wines, of 12.253  $\pm$  1.375 % v./v. Although the non-Saccharomyces yeasts are not normally used for winemaking, in recent years there is a trend of also trying strains of other genera, hoping to obtain products with special flavours for certain consumer market segments. However, most of the non-Saccharomyces yeast strains tested so far proved to introduce in wines off-flavours and if this last yeast strain is of this type it could be a source of wine spoilage rather then a selected yeast for quality wines.

The other 6 selected yeasts are all classified as *Saccharomyces cerevisiae*, even though they do not form a compact group as far as the ethanol resistance is concerned. They are all, however, more resistant than the control yeast, the comercially available EC 1118, which has good parameters for wine production, 50% inhibitory concentration  $K_{\mu} = 8.696 \pm 0.629$  and 100% inhibitory concentration MIC<sub> $\mu$ </sub> = 15.319 ± 1.818 % v./v. ethanol. Compared to the control yeast, the most resistant was the yeast strain that was selected from Cabernet Sauvignon must at the end of fermentation, CS SF-8, followed by PN5 IV-18 selected in a conventional plantation of Pinot noir, both strains originating in the Valea Călugărească regia conventional plantation, also displayed very good tolerance to ethanol, but slightly lower than those selected in Valea Călugărească, their minimum inhibitory concentration for ethanol being in the range of 18-25% v./v.

So far, there was no corelation found between the type of plantation (conventional or ecological) and the ethanol tolerance of the selected *Saccharomyces* yeasts.

#### CONCLUSION

The calorimetric determination of the ethanol growth inhibition parameters for 10 selected yeast strains isolated in Valea Călugărească and Panciu vineyards lead to the identification of a group of yeasts of *Saccharomyces* genus with very good resistance to this fermentation end product. The strains of this group can be, in principle, recommended for wine production, but before that they should be tested further on wines for the evaluation of the chemical and sensory profile.

The isolated non-*Saccharomyces* strains should be regarded with caution, one being not resistant enough to sustain a complete fermentation (*Kloeckera*) and the other two (*Candida utilis* and *Rhodotorula glutinis*) being rather spoilage yeasts than recommendable yeasts. It is however reported in the literature (Jolly et al., 2006) that some non-*Saccharomyces* yeasts could be interesting for wine production, and this aspect should be further investigated.

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# Table 1.

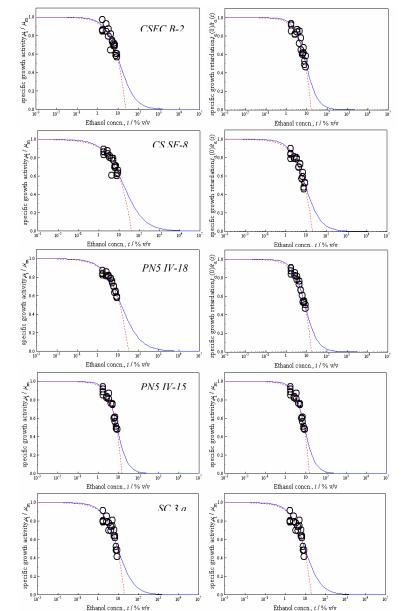
Yeast strains isolated from Valea Călugărească and Panciu vineyards from conventional and ecological plantations

••	Strain	a .	Isolation				
No.	codification	Species	location	Grape variety	Type of plantation		
1	PN5 B-B-6	Kloeckera spp	Valea Calugareasca	Pinot noir	conventional		
2	CSEC B-2	Candida utilis	Valea Calugareasca	Cabernet Sauvignon	ecological		
3	CSEC B-3	Rhodotorula glutinis	Valea Calugareasca	Cabernet Sauvignon	ecological		
4	CS SF - 8	Saccharomyces cerevisiae	Valea Calugareasca	Cabernet Sauvignon	ecological (at the end of must fermentation)		
5	PN5 IV-15	S. cerevisiae	Valea Calugareasca	Pinot noir	conventional		
6	PN5 IV-18	S. cerevisiae	Valea Calugareasca	Pinot noir	conventional		
7	SC 3 a	S. cerevisiae	Panciu	Feteasca regala	conventional		
8	SC 6 a	S. cerevisiae	Panciu	Feteasca regala	conventional		
9	SC 6 b	S. cerevisiae	Panciu	Feteasca regala	conventional		
10	EC 1118	S. cerevisiae	Epernay- Champagne	Commercial yeast			

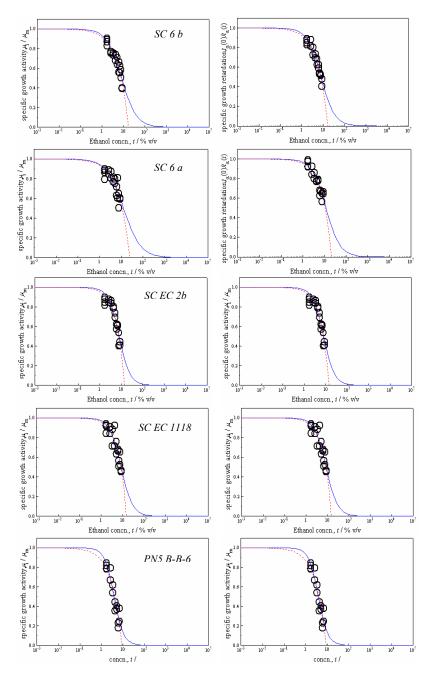
			Table 2.
Quantitative inhibitory parameters d	escribing the ethanol t	olerance of the yeast strains	s selected in
Dealu Mare vineyard	ds (YPG broth, incuba	tion temperature 30°C)	

	Dealu Mare vineyards (YPG broth, incubation temperature 30°C)							
Yeast strain	т	K MIC ethanol % v./v.		$m_{ heta}$	$K_{\theta}$ MIC <sub><math>\theta</math></sub> ethanol % v./v.			
PN5 B-B-6	$1.963 \pm 0.200$	3.895 ± 0.167 8.9	$904 \pm 0.692$	$1.902 \pm 0.107$	$3.390 \pm 0.085$	$8.304 \pm 0.258$		
CSEC B-2	$1.733 \pm 0.194$	$8.075 \pm 0.417$	$\begin{array}{r} 14.092 \ \pm \\ 1.039 \end{array}$	$1.561 \pm 0.079$	$6.730 \pm 0.156$	$\begin{array}{r} 13.927 \pm \\ 0.528 \end{array}$		
CSEC B-3	$1.159 \pm 0.151$	$\begin{array}{rrr} 12.253 \pm \\ 1.375 \end{array} 24$	.064 ± 4.339	$1.513 \pm 0.179$	$9.127\pm0.613$	$16.648 \pm 1.884$		
CS SF-8	$\begin{array}{c} 0.858 \pm \\ 0.131 \end{array}$	16.934 ± 3.165	$40.666 \pm 12.403$	$1.145 \pm 0.123$	$9.176 \pm 0.677$	$\begin{array}{c} 20.750 \pm \\ 2.719 \end{array}$		
PN5 IV-15	$\begin{array}{c} 1.616 \pm \\ 0.131 \end{array}$	8.492 ± 0.350 15	.246 ± 0.957	$1.591 \pm 0.097$	$6.733 \pm 0.185$	$13.658 \pm 0.529$		
PN5 IV-18	$\begin{array}{c} 0.922 \pm \\ 0.080 \end{array}$	$\begin{array}{rrr} 14.531 \pm \\ 1.354 \end{array} 32$	.824 ± 4.412	$\begin{array}{c} 1.400 \pm \\ 0.072 \end{array}$	$8.496 \pm 0.247$	$\begin{array}{c} 17.078 \pm \\ 0.888 \end{array}$		
SC 3 a	$\begin{array}{c} 1.107 \pm \\ 0.171 \end{array}$	9.724 ± 1.096 20	.653 ± 3.595	$1.381 \pm 0.092$	$6.671 \pm 0.221$	15.510 ± 1.144		
SC 6 a	$1.255 \pm 0.129$	8.271 ± 0.507 17	$7.712 \pm 1.805$	$1.368 \pm 0.094$	$8.038\pm0.305$	16.701 ± 1.055		

SC 6 b	$\begin{array}{c} 1.107 \pm \\ 0.187 \end{array}$	12.892 ± 1.996	$25.314 \pm 6.010$	$1.341 \pm 0.147$	$11.840 \pm 1.005$	$21.595 \pm 3.236$
EC 1118	1.634 ± 0.230	8.696 ± 0.629	15.319 ± 1.818	1.496 ± 0.228	$8.260 \pm 0.667$	$15.892 \pm 2.262$



**Fig. 1.** Drug potency curves (continuous line) and MIC curves (dotted lines) fitted by regression analysis for the determination of the 50% inhibitory concentration and 100% inhibitory concentration (MIC) of ethanol for 5 selected yeasts. On the left side the data sets for specific growth activity  $\mu_i/\mu_m$  and on the right side the curves obtained on the basis of the specific growth retardation,  $t_{\alpha}(0)/t_{\alpha}(i)$ .



**Fig. 2.** Drug potency curves (continuous line) and MIC curves (dotted lines) fitted by regression analysis for the determination of the 50% inhibitory concentration and 100% inhibitory concentration (MIC) of ethanol for the other 5 selected yeasts. On the left side the data sets for specific growth activity  $\mu_i/\mu_m$  and on the right side the curves obtained on the basis of the specific growth retardation,  $t_{\alpha}(0)/t_{\alpha}(i)$ .

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#### EVALUATION BY SENSORY ANALYSIS AND ELECTRONIC NOSE OF RED WINES OBTAINED USING SOME YEAST STRAINS SELECTED IN DEALU MARE REGION

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KEY WORDS: ethanol, inhibition parameters, winemaking, microbial calorimetry, yeast selection

#### ABSTRACT

Seven Sacharomyces cerevisiae yeast strains selected for Pinot noir wines and 8 for Cabernet Sauvignon isolated from the fermenting musts in the region of Dealu Mare were tested for the assessment of their oenological traits. Among the selected yeasts there were some that conferred to wines similar sensory traits as the commercial yeast used as control. However, some yeasts were also found which introduced in wines some other sensory particularities, distinguishing these wines and conferring regional specificity. These yeasts, used for the production of CS3, CS6, CS7, CS8, PN3, PN7 sample wines, will be tested further. Due to the poor quality of the PN4 and CS5 wines the yeasts used for their production are not recommended.

#### **INTRODUCTION**

The consumer preferences for wine styles continuously evolves and the market should adapt to any new tendency observed on the market (Swiegers *et al.*, 2006). The sensory properties of the wines depend a great deal on the yeast strain employed for the fermentation, new commercial yeas strains being proposed to wine producers each year (Heard, 1999; Satyanarayana and Kunze, 2009). Yeast strain selection is a very competitive process and tests with newly isolated or even genetically engineered yeasts (Pretorius, 2000; Pretorius *et al.*, 2003) are conducted by many research teams all over the world (Rainieri *et al.*, 2000; Lambrechts and Pretorius, 2000; Palomero *et al.*, 2009).

The yeast selection is aimed at finding yeasts with better technological and oenological properties, the main target being, however, the obtainment of a product with superior sensory properties and aroma profile. Few studies were conducted on Romanian autochthonous yeast starter cultures, but some results regarding the improvement of the characteristics of Romanian wines by using such cultures have been reported previously (Matei-Rădoi *et al.*, 2008).

This work presents the results from the sensory analysis and a comparative evaluation of volatile profile by an electronic nose for wines of Cabernet Sauvignon and

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Pinot noir produced by using *Saccharomyces cerevisiae* yeast strains isolated from the same variety musts obtained in the Valea Calugareasca viticultural center.

#### MATERIAL AND METHODS

The yeast strains were isolated in grape musts in various stages of fermentation, these stages being codified from starting the fermentation (stage I) up to final fermentation phase (stage IV). Several yeast strains were selected in each stage, but only the strains identified as *Saccharomyces cerevisiae* were further applied for winemaking. The strains selected in Cabernet Sauvignon musts were abbreviated as CS and those from Pinot noir as PN, followed by the stage number and finally by the number of the strain. These strains were used in the process of maceration-fermentation of grape musts from the same variety. The wine samples prepared and the codification of the yeast strain used are presented in Table 1.

The wine samples were analyzed 6 months from their production and the classical physico-chemical parameters and quantitative sensory parameters were determined.

The physico-chemical analyses were performed in accordance with the international standards for wine analysis (Recueil des méthodes OIV, 2010), including the determination of total acidity by titrimetry, alcoholic strength and dry content by using a hydrostatic balance Densimat with Alcomat module from Gibertini Elettronica and colour CIELab parameters by using the WinAspect 2.3.0.0 software of the Specord 250 UV-VIS spectrophotometer.

The sensory analysis was performed by a panel of 5 winetasters on the basis of a specially designed evaluation sheet (Antoce and Namolosanu, 2007), the main parameters followed being:

- general sensory parameters: acidity perception, sweetness, astringency, dry content perception, colour intensity and aroma intensity and

- specific sensory parameters: floral note, fruite note, vegetal note, spicy/roasted note, complex aroma, other/off-flavours.

The points awarded for the general sensory parameters varied from 1 to 10, being obtained on continuous scales, while the evaluation for specific sensory parameters was performed on discontinuous scales from 1 to 5 and then normalized to be plotted on the same diagrams as the general parameters. On the basis of this quantitative sensory analysis the sensory profiles for each wine were obtained.

The wines were also differentiated by taking into account the volatile profile detected by a flash dual-column gas-chromatograph with FID detectors working on the principle of an electronic nose. The apparatus is produced by Alpha-MOS, France and the analytical methods and parameter used for wines were developed in our laboratory (Antoce *et al.*, 2008). The data recorded from the two chromatographic columns (a non-polar DB-05 colum and a mid-polar DB 1701 column) were processed with the powerful software for statistical analysis provided by alpha-MOS with the apparatus, the AlphaSoft V11. By using the multivariate analysis the accurate differentiation of the samples based on their volatile profile is possible.

#### **RESULTS AND DISCUSSION**

The wines obtained with the selected yeast strains were analysed 6 months after the completion of the maceration-fermentation process and the result of the main physicochemical analyses, including the CIELab colour parameters, are included in Table 2. As expected, the yeast strain can significantly change the composition of the final product. It can be seen from these parameters only that the PN4 wine sample is clearly different than the rest of the Pinot noir samples, with a lower content of ethanol and dry extract, but with a more intense red colour. The other Pinot noir showed also some differences correlated with the yeast strain applied, but the effect was not so obvious. In Cabernet wine samples CS5 was lowest in alcohol and dry content, the other wines being more uniform from this point of view. Moreover, these two, PN4 and CS5, were the wines that scored lowest in their cathegory from the viewpoint of general sensory evaluation (data not shown).

The electronic nose showed that the yeast strains have an impact on the aromatic profile of wines, but the grape variety has a stronger effect, the tipicality of a varietal wine being first of all dictated by the grape variety. In Fig. 1 the two varieties studied are grouped, Cabernet Sauvignon and Pinot noir, irrespective of the yeast strain used for fermentation, differentiated by use of the Discriminating Factor Analysis (DFA). However, inside of the variety groups, clear differences were found by the electronic nose and human panel, in accordance with the yeast strain applied.

The sensory profiles obtained for the Cabernet Sauvignon wines are presented in Fig. 2 in three diagrames, the wines being grouped taking into consideration the style of the resulted wines. There is a group of yeast strains (Fig. 2.a) which give a complex floral and fruity aroma, leading to wines (CS1, CS2, CS4) sensorially similar to those obtained with the control yeast (CS0). Another group is formed by the CS3, CS6, CS7 and CS8 wines, which are characterized by a simpler aroma, with less floral and fruity note (Fig. 1.b). The strain used in the wine CS5 was totally different than the other, conferring a highly perceptible off-flavour (Fig. 2.c). The acidity perception was similar in all Cabernet wines, as was, more or less, the perception of dry content and astringency, fact that is also correlated with the laboratory results. It was concluded that the yeast strains used for Cabernet mainly modify the specific sensory parameters and not the perception of the general ones. ccc

The electronic nose differentiated all the Cabernet Sauvignon samples by Discriminating Factor Analysis, as can be seen in Fig 3. Moreover, as found by sensory analysis with human panelists, the samples with complex aromatic profile display certain similarities, fact that was also confirmed by the electronic nose. We can see in Fig. 3, in the upper left part of the DFA diagram, that CS0 (control), CS1, CS2 and CS4 are very close positioned.ccc

For Pinot noir, in accordance to the style of the produced wines, we also found three groups of yeast strains (Fig. 4): a group of complex wines (Fig. 4. a), with spicy notes, with good colour and astringency (PN1, PN2, PN5, PN6), sensorially similar to the control wine (PN0), a group of lighter wines (Fig. 4. b), with less astringency and colour and with a perceived vegetal note covering the floral and fruity notes (PN3 and PN7) and finally (Fig. 4. c), the PN4 wine with the highest colour intesity, vegetal and fruity note, but also with an yeasty off-flavour.

The PN4 wine also distinguished itself by the rest of the samples (Fig. 5), being clearly discriminated by the electronic nose and separated from the group of the other yeasts. The rest of the wine samples grouped relatively close, having relatively similar volatile profiles. By eliminating from analysis the PN4 sample that stood out as far as the volatile profile was concerned, we obtain a better discrimination inside of the group (Fig. 6) of the PN wines, showing that each yeast brings some changes in the volatile profile, but no so important as to being clearly differentiated by the human panel.

The outlier PN4 was by far the less complex wine sample, thin and short, being only characterized by a clearly perceived vegetal aroma and a higher acidity as compared to the other Pinot noir samples. Another distinctive characteristic of this sample was the pregnant red colour, also atypical for a wine of this variety.

#### CONCLUSION

The selected yeasts isolated in Dealu Mare region and used as starter culture in two varietal wines showed some interesting oenological traits. For both grape varieties we found yeasts that gave an aromatic profile similar in complexity and quality to that of the commercial yeast used as control, but we also found some yeasts that introduced intro wines some other oenological characteristics, such as a more complex aroma, a higher colour intensity or some more vegetal notes. These yeasts would allow for the production of wines with regional specificity, confering traits that would distinguishe these wines from the wines of other regions. Further tests should be carried on for the oenological and techological characterisation of those strains that induced different sensory and volatile profiles from the control ones, such as those that produced CS3, CS6, CS7, CS8, PN3, PN7. We also identified two yeast strains, the ones that produced the PN4 and CS5 wines, which should be avoided in the future tests, due to the low sensory quality of the resulted wines. Especially the production of H<sub>2</sub>S and volatile acidity should be further investigated, mainly because most *Saccharomyces cerevisiae* strains are high producers of such volatile substances.

#### ACKNOWLEDGMENT

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# Table 1.

Wine	Codification of the yeast strain in	Grape			
sample	accordance to the stage fermentation of	variety			
	must in which was isolated				
CS0	Control yeast FERMACTIVE RC				
CS1	CSEC III – 1 (fermentation stage III)				
CS2	CSEC IV - 1 (fermentation stage IV)				
CS3	CSEC IV - 7 (fermentation stage IV)	Cohormot			
CS4	CSEC IV - 8 (fermentation stage IV)	Cabernet Sauvignon			
CS5					
CS6					
CS7					
CS8	CSEC II - 6 (fermentation stage II)				
PN0	Control yeast FERMACTIVE RC				
PN1	PN III - 5 (fermentation stage III)				
PN2	PN III - 7 (fermentation stage III)				
PN3	PN III - 8 (fermentation stage III)	Dinatnair			
PN4	PN III - 10 (fermentation stage III)	Pinot noir			
PN5					
PN6	PN IV - 4 (fermentation stage IV)	]			
PN7	PN IV-7 (fermentation stage IV)	1			

Saccharomyces cerevisiae yeast strains selected for red wine production from fermentation musts of Cabernet Sauvignon and Pinot noir from Dealu Mare region

### Table 2.

	1	with specific yeast strains isolated in Dealu Mare region					
	Total	Alcohol,	Total		ELab colour par		
	acidity,	% v./v.	dry	Lumi-	Parameter a	Parameter b	
Wine	tartaric		content	nosity,	(colour axis	(colour axis	
sample	acid g/l		g/l	L	red-green)	yellow-blue)	
PN0	6.2042	14.41	30.80	92.1448	5.0619	6.9685	
PN1	6.5691	14.74	37.70	92.5001	4.8793	6.6088	
PN2	6.0582	14.87	31.60	92.5405	5.0438	7.4284	
PN3	6.1312	14.75	29.50	91.4628	5.9397	7.0378	
PN4	6.0582	13.59	28.90	88.7336	10.6912	3.8325	
PN5	6.2042	15.07	32.05	91.4574	6.3651	7.1896	
PN6	4.7444	15.09	33.00	91.9719	5.1117	7.0217	
PN7	5.8392	14.78	33.75	91.9133	5.4456	6.6548	
CS0	7.1530	11.83	26.94	80.9425	22.7635	0.8744	
CS1	7.4450	13.12	29.69	84.0797	16.6554	3.1547	
CS2	7.0800	13.25	27.29	80.1862	21.5525	3.2049	
CS3	6.9341	13.12	25.10	76.1440	27.4987	1.6431	
CS4	6.7151	12.45	25.30	79.3941	23.5005	1.5323	
CS5	6.7151	11.03	20.16	77.4843	27.1159	1.6199	
CS6	6.7881	12.06	24.49	80.0112	23.7568	0.1029	
CS7	7.0800	12.55	26.15	84.0288	17.0007	2.0897	
CS8	6.2042	11.91	25.10	79.2275	24.7614	-0.3951	

Main physico-chemical parameters of the Cabernet Sauvignon and Pinot noir wines produced with specific yeast strains isolated in Dealu Mare region

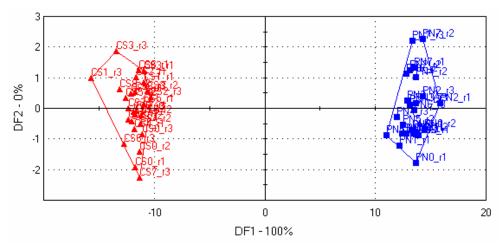


Fig.1. Differentiation by electronic nose of Cabernet Sauvignon and Pinot noir wines, irrespective of the yeast strain used for their production.

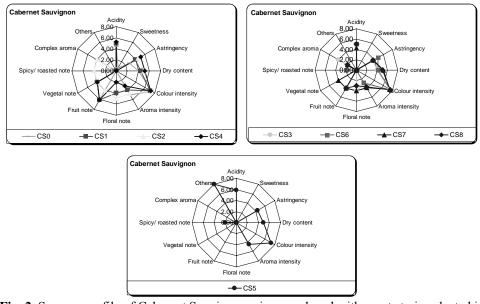


Fig. 2. Sensory profile of Cabernet Sauvignon wines produced with yeast strain selected in Dealu Mare region, grouped in accordance with the similarity of their profiles.

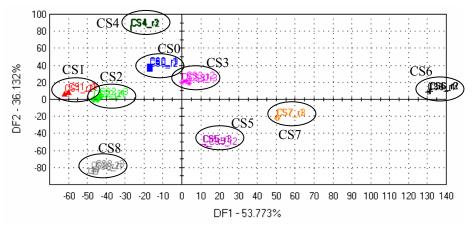


Fig. 3. Factor analysis diagram for Cabernet Sauvignon wines produced with yeast strain selected in Dealu Mare region.

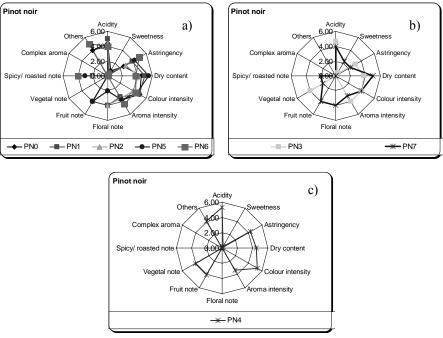


Fig. 4. Sensory profile of Pinot noir wines produced with yeast strain selected in Dealu Mare region, grouped in accordance with the similarity of their profiles.

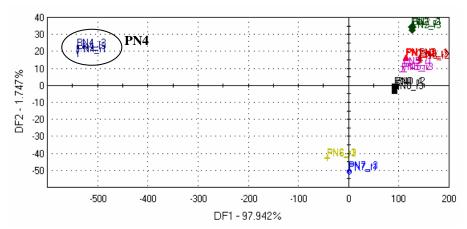
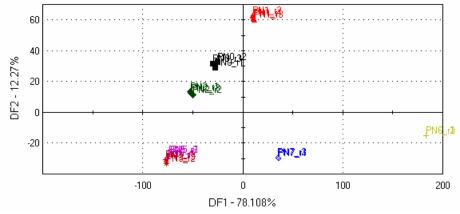


Fig. 5. Factor analysis diagram for all Pinot noir wines produced with yeast strain selected in Dealu Mare region.



**Fig. 6.** Factor analysis diagram for Pinot noir wines displayed in Fig. 5 after the removal of the outlier PN4.

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#### THE ROLE OF THE FOLIAR TREATMENT WITH RAYKAT ON THE SYNTHESIS OF ASCORBIC ACID FOR GENOTYPES OF *CAPSICUM ANNUUM* CULTIVATED IN THE A PROTECTED AREA

Apostol D. F.<sup>1</sup>, Dumitru Mihaela Gabriela<sup>2</sup>, Dinu Maria<sup>3\*</sup>, Pintilie I.<sup>4</sup>

KEY WORDS: Raykat, foliar treatment, genotypes, ascorbic acid

#### ABSTRACT

The use of bio-stimulating substances in vegetable growth plays an important role in achieving certain bio-chemical processes that have a positive impact on the nutritional quality of vegetables.

The foliar treatment was applied to six genotypes of Capsicum annuum L. (Long of Isalnita, Bogdan, Siret, L54, LTP and Cosmin), cultivated in an area protected by a solar type polyethene foil. As a result of a treatment with Raykat bio-stimulator, there was an increase in the levels of ascorbic acid present in the fruits of Caspicum annuum, compared to the untreated samples.

#### INTRODUCTION

The human body benefits a great deal from nutrition with vegetable fruits, which beyond the 76-94% water contain a considerable amount of vitamins and minerals, organic substances and have an excellent energetic value (Dumitrescu, 1971; Chilom, 2002; Bita et al., 2009). The amount and nature of vitamins in vegetables are net superior many other products in our nutrition, particularly those of animal origin. They provide a large range of mineral salts, predominantly chemical compounds with base tendencies: Ca, K, Na, Mg, Fe etc, which by their alkaline character neutralise the acidity induced by a high protein nutrition (meats, bread, eggs, milk, etc.).

Products of animal origin, cereal, and sugary products lack Vitamin C; the only natural sources of Vitamin C are fresh fruit and vegetables. The amount of Vitamin C in vegetables and fruits depends on the plant, the particularities of the variety, the climate it grew in, soil conditions, the time elapsed from harvest to consuming it and the conditions the plant was stored in.

Having a highly nutrient soil is very important in the assimilation process of the plant and its growth. A balanced fertilization mandates creating and maintaining a good ratio between the nutritional elements. The highest standards are required in the period when the edible fruits are formed.

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Applying nutrients through foliar sprinkling is an unconventional and non polluting technological stimulation, applied in small doses throughout the growth period in a plants metabolic life (Bireescu et al., 2003; Dinu et al., 2009). Supplementing the nutrition of a plant via its folio contributes not only to make up for some deficiencies in this sense but also generates an uplift in production for crop plants; this occurs even without any additional soil fertilization (Boote et al., 1978; Neumann et al., 1981; Dinu et al., 2009).

#### MATERIAL AND METHODS

The experimental activity was acted out on 6 genotypes of long pepper (Lung de Isalnita, Bogdan, Siret, L.54, L.TP, Cosmin) grown in an area protected by solar polyethene foil, before the start of the maturing process of the fruits.

The foliar treatment with RAYKAT 0.5% was applied in the beginning of the growth process of the fruit (fine and even sprinkling), and every 10 days after that. Three treatments were applied. Outlining the role had by the Raykat treatment was done by determining the content of Vitamin C in the *Caspicum annuum*.

*The dosage of Vitamin C by means of iodine-metric methods* (Bita & Marinescu 2008, Ianculov et al. 2003)

#### Principles

Vitamin C (ascorbic acid) is extracted using HCl 2%, by adding to the vegetal matter quarts sand and HCl 2%, by means of titration using a potassium iodine solution N/250, in the presence of potassium iodide and starch. The resulting oxidising reaction, takes place as indicated below:

$KIO_3$ + 6 HCl +5 KI	$\rightarrow$ 6 KCl + 3 I <sub>2</sub> + 3H <sub>2</sub> O
$C_6H_8O_6 + I_2$ —	$\rightarrow$ C <sub>6</sub> H <sub>6</sub> O <sub>6</sub> + 2HI Acid
ascorbic	dehidroascorbic

# Reactives and solutions

Chlorhydric acid 2%, potassium iodine N/250, potassium iodide 1%, starch;

#### Method

A sample of 5-10g from the vegetal matter, previously having had added quartz sand, is passed through a 100ml measured glass balloon, via a solution of chlorhydric acid 2%; you shake the mixture. After sedimentation, the total mixture is filtered into a dry glass. From the sediments in the filter, a 10ml sample is taken and put in a Berzelius glass; distilled water is added and 5ml of potassium iodide 1% and 1ml of starch; it is then titrated with potassium iodine N/250, while gently shaking until the colour becomes blue.

Calculations Vitamina C mg %= 352. n.f / G where: n - ml used for titration; f - potassium iodine N/250 factor;

G – weight of the sample in grams;

#### **RESULTS AND DISCUSSION**

Applying the foliar treatment with Raykat bio-stimulator has resulted in a variation of the concentration of ascorbic acid of 6 and 9mg/100g fresh product, for the fallowing samples: Lung de Işalniţa, Bogdan, Siret şi Cosmin.

For the  $L_{.54}$  sample, the concentration of ascorbic acid doubles, while for L.TP the concentration decreases from 87.5 la 96 mg/100g fresh pepper, as noted in Table 1.

Table 1.

	Ascorbic acid, mg/100g fresh product					
Sample name	Untreated	Treated				
Lung de Işalniţa	156	162				
Bogdan	140	149				
Siret	104	110				
L.54	75	146				
L.TP	96	87,5				
Cosmin	169	175,5				

The concentration of ascorbic acid for pepper samples treated with Raykat

### CONCLUSIONS

By applying the foliar treatment to the six genotypes of pepper a difference to the untreated samples in the concentration of ascorbic acid occurs.

The small difference, noted for the Lung de Işalniţa, Bogdan, Siret, L.TP şi Cosmin genotypes, concludes that using Raykat bio-stimulator is not recommended for these varieties.

The doubling of the concentration of ascorbic acid for the L.54 genotype, implies a recommendation to succesfully utilize the fertilizer for incereasing the levels of Vitamin C.

Although for the Lung de Işalniţa, Bogdan, Siret, L.TP şi Cosmin samples, the tratment has not brought about a major difference in the levels of ascorbic acid. We therefore recommend further research on the influence of this prodct on other chemical compounds of the pepper.

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# ON ISCHYROPSALIS MANICATA L. KOCH, 1865 (ARACHNIDA, OPILIONES) FROM ROMANIA

Babalean Anda<sup>1\*</sup>, Bălescu Carmen

**KEY WORDS:** Ischyropsalis manicata, systematic position, taxonomic characters, geographical distribution

#### ABSTRACT

The paper presents some data concerning the species Ischyropsalis manicata L. Koch, 1865 in Romania: historic, the main synonymies, the most important taxonomic features and their variability, the range and the geographical distribution in Romania.

#### INTRODUCTION

Ischyropsalidae family is represented in Romania only by *Ischyropsalis manicata*. Some other Ischyropsalis species were also recorded: *Ischyropsalis helwigii* Panzer (Cardei, 1943, 1947, 1960); *Ischyropsalis taunica* Muller (Cardei, 1960); *Ischyropsalis carli* Lessert (Cardei and Bulimar, 1962). Avram (1964) mentioned on the romanian territory another 5 species: *Ischyropsalis helwigii* Panzer, *Ischyropsalis herbstii* C.L. Koch, *Ischyropsalis taunica* Muller, *Ischyropsalis carli* Lessert and *Ischyropsalis dacica* Roewer but their real existance in Romania is uncertain.

The large variability of the most important taxonomic features has had 2 consequences: erroneous identifications and descriptions of new species later synonymised. The highly intraspecific variability is well illustrated by the number of synonymies.

The morphological variability on *Ischyropsalis manicata* from romanian caves was studied by Avram who also brought valuable informations on the geographical distribution and ecology of this species.

#### MATERIAL AND METHODS

a) bibliographical data

b) 2 males, 2 females – Parang Mountains-Ranca- 17.08.1997- leg. det. Anda Babalean

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#### **RESULTS AND DISCUTIONS**

Taxonomic position

Ischyropsalis manicata L. Koch, 1865

Ord. Opiliones Subord. Palpatores Suprafam Ischyropsalidoidea Fam. Ischyropsalidae

First record in Romania

L. Koch, 1865- Siebenburgens= locus typicus (Martens, 1978)

#### Synonyms

Ischyropsalis dacica Roewer, 1916 Ischyropsalis helvetica milleri Kratochvil, 1934 Ischyropsalis kastneri Roewer, 1950 Ischyropsalis austriaca Roewer, 1950 Ischyropsalis balcanica Roewer, 1950

Taxonomic features and variability

The most important taxonomic features are:

- 1. the length of the body
- 2. the number of denticles on the toracal tergite I
- 3. the length of the first cheliceral article
- 4. the length of the first cheliceral article related to the body length
- 5. the number of spines on the first cheliceral article in dorsal position, ventralmedial position, ventral-lateral position
- 6. the morphology of the penis
- 7. the ovipositor and receptaculae seminis

All these characters are more or less variable. In table number 1 it is shown such a variability as results from some arachnological literature and from personal investigations-2 females (F1, F2) – Parang Mountains- Ranca. Some morphological characters are also figured: fig. 1a, b, c; fig. 2d, e, f.

# Geographical distribution

(Kolosvari, 1929; Kolosvary, 1943; Cardei, 1943; Cardei, 1960; Cardei and Bulimar, 1962; Kolosvary, 1963; Avram, 1964; Avram and Dumitrescu, 1969; Dumitrescu, 1972; Dumitrescu, 1973, 1976; Martens, 1978; Weiss, 1996; Babalean, 1997; Lengyel and Pall Gergely 2009, 2010).

In relation to zoogeographical distribution, *Ischyropsalis manicata* is a Carpathian endemite: Poland, Czech Republic, Slovak Republic, Romania, Ukraina.

Chronologically speaking, *Ischyropsalis manicata* is a relic of Tertiary Neogene period – a Pliocene age element. Is therefore a paleoendemite (Drugescu 1994).

Ischyropsalis manicata lives preponderant in caves (troglophyllous species- Avram & Dumitrescu 1969), but also in very humid endogean and epigean environement (hemiedaphic hygrophyllous species- Avram 1964).

Distribution of Ischyropsalis manicata in Romania is:

*Est Carpathians*: Rodnei Mt.; Gurghiu- Harghita Mt.; Persani Mt.; Postavaru Mt.; Obcina Mare Mt.; Rarau Mt.; Ceahlau Mt.; Garbova Mt.; Vrancei Mt.

South Carpathians: Bucegi Mt.; Parang Mt.; Ciucas Mt.; Fagaras Mt.

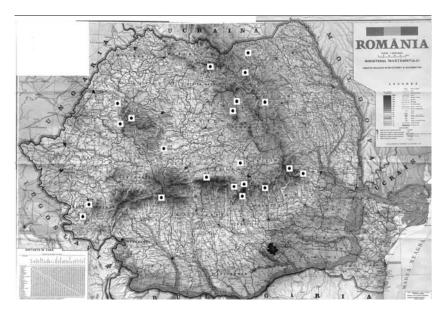
West Carpathians: Aninei Mt.; Bazinul Vaii Barzava, Bihorului Mt. (Sighistel Valley, Vladeasa Mt.); Bazinul Somesului Cald; Padurea Craiului Mt.

Transilvania: Brasov, Mures, Toplita regions.

Table 1

Features	Roewer	Šilhavý	Martens	Avram	F2	F1
a. body length (lg. mm)	8	6,5-8	6,3-7,7	4,6-7	7,7	7
b. lg. of the 1 <sup>st</sup> cheliceral	3,5	3-3,5	-	3,1	3	3,1
article (mm)						
c. $\frac{1}{2}$ body length (mm)	-	-	-	-	3,85	3,5
d. 2/3 body length (mm)	-	-	-	-	5,12	4,66
e. b-c	-	b<=c	-	b>c	b <c< td=""><td>b<c< td=""></c<></td></c<>	b <c< td=""></c<>
f. b-d	-	-	-	b <d< td=""><td>b<d< td=""><td>b&gt;d</td></d<></td></d<>	b <d< td=""><td>b&gt;d</td></d<>	b>d
g. number of spines on the	4	4-5	-	3-5	5	7
first cheliceral article -						
dorsal						
- ventral-medial	5-6	4-5	-	5-8	5	6
- ventral-lateral	3-4	excresc	-	3-6	6	5
		ences				

Ischyropsalis manicata - taxonomic features and variability



Map 1. The geographical distrbution of Ischyropsalis manicata in Romania

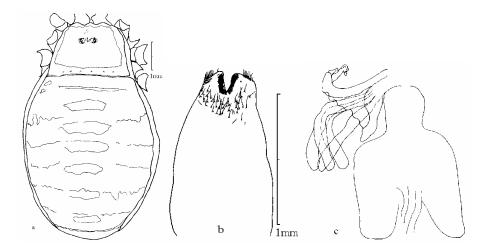


Figure 1. *Ischyropsalis manicata* (Parâng-Rânca, 17.08.1997) a-female – dorsal view, b-ovipositor, c-receptaculae seminis

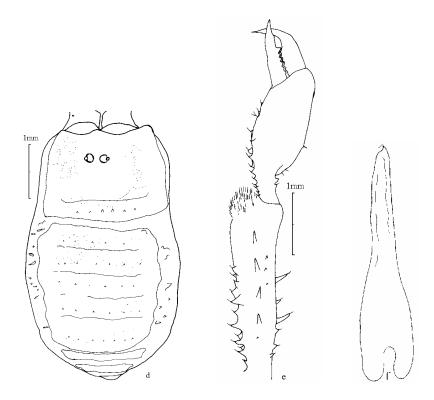


Figure 2. *Ischyropsalis manicata* (Parâng-Rânca, 17.08.1997) d-male – dorsal view, e-male chelicerae, f-penis

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

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### ASPECTS ON APPLYING BORON ORGANIC COMPOUNDS AS FERTILIZERS ON TOMATOES PLANTS

Badea Florentina<sup>1\*</sup>, Popa Daniela<sup>2</sup>, Mitrut Mihaela<sup>3</sup>

KEY WORDS: natural fertilizers, boron, tomatoes, quality, production

#### ABSTRACT

Boron is a micro-nutrient, essential for plant growth. After the experiments performed regarding the foliar and on soil application of fertilizers with boron organic compounds, it was observed that the average height of plants indicated a significant growth for the treated variants, the values being within 25-62.5%. Compared to the witness variant, the average number of plant inflorescences was larger for those variants where fertilizers were applied. The percentage of fruit connection on the plants indicated an increase by applying the mixed treatment (root + foliar spraying) compared to the variants treated only radicularly and compared to the witness, the values for those variants with mixed treatment being within 88.2 and 92.4. The production of tomatoes was directly influenced by mixed fertilization (root + foliar spraying). For the variants with mixed fertilization, productions were within 124.55 and 136.98 t/ha.

#### **INTRODUCTION**

The nutritive elements forming the nourishment factor influence a good development and growth of horticultural plants, by their presence in sufficient quantities and easily assimilable forms and under a practical aspect, they are materialised by:

- Quantitative and qualitative increase of the production;
- Shortening the vegetation period.

The role of the macro-elements: N, P, K, Ca, Mg, Al, etc. is more broadly shown, and that of the micro-elements: B, Cu, Mn, Mo, Zn, Fe, Al, etc., in a narrower way; the nutritive complex could not lack any of them, but they should not be in excess either, in both cases generating undesired changes (Ciofu et al., 2003).

Boron is recognised as an essential micro-nutrient for plants and animals, with a very important role in growth and development (Warington, 1923; Lewis, 1980 quoted by Scorei, 2006). It has a favorable effect onto flowering and fruiting of plants, as it stimulates the rapid germination of pollen (Davidescu, 1981; Neamțu, Câmpeanu and Socaciu, 1995, quoted by Scorei, 2006).

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Romania is one of the European countries with a low content of boron in the soil and phreatic waters, which implicitly determines a deficiency of Boron in plants and animals' and people's nourishment (Scorei, 2005).

A series of researches shows that boron is a bioactive elements for the human body (Nielsen, 2008). But the effect of boron onto the human body is not fully elucidated (Armstrong, 2001).

The issue of boron deficiency in the soil does not only limit to the effect onto the harvest quantity, but it also affects the quality of leaves, as well as fruit quality (Dong et al.; Zude et al. quoted by Scorei, 2006).

Applying the nutritive elements by means of foliar spraying represents an unconventional and non-pollutant stimulation technological element, with small doses along the vegetation of the plant metabolism (Bireescu et al., 2002).

Calcium fructoborate is a boron and fructose compound, a carbohydrate that appears naturally in many fruit and vegetables and is a stable compound (Scorei, 2007).

Fertilization with natural compounds of boron represents advantages connected to the ready accessibility at the level of plants and natural fertilizers may also be used unrestrictedly in order to obtain ecological products even in agriculturally disadvantaged areas or which have soils indicated nutrition deficiencies.

Tomatoes are included into the technological group of *solano-fruitful vegetables*. The plants in this group are part of the botanic family *Solanaceae*. Tomatoes are largely cultivated on great surfaces into the field and in protected areas. It is cultivated by means of seedling or sowing directly on the field and need special attention regarding the hydric and nutritional regime. Tomatoes have a high nutritive value, only thanks to the contents of fruit in vitamins, sugars, mineral substances, amino-acids and organic acids. (Singureanu, 2008).

#### MATERIALS AND METHODS

The experiments were performed at S.D. Banu Maracine, Dolj County, inside a solarium, in four repetitions, having the following experimental variants:

V1 = untreated witness;

V2 = radicular treatment with Calcium Fructoborate;

V3 = radicular treatment with Calcium Glucoborate;

V4 = radicular treatment with Calcium Fructoborate + foliar treatment with glucose Folibor;

V5 = radicular treatment with Calcium Fructoborate + foliar treatment with fructose Folibor;

V6 = radicular treatment with Calcium Glucoborate + foliar treatment with glucose Folibor;

V7 = radicular treatment with Calcium Glucoborate + foliar treatment with fructose Folibor;

The applied commercial fertilizers were produced by S.C. Natural Research S.R.L., calculated at the surface unit of 51/ha; the concentration of the solution for each foliar fertilizer was of 1%, and for the radicular fertilization, a solid product quantity of 200kg/ha was applied. Radicular fertilisation was done 10 days before planting the tomatoes.

A series of two foliar treatments each was applied for every individual variant, during the vegetation period:

- The first treatment when the tomato plants had 8-10leaves formed and normally developed;
- The second treatment in 15 days since the first application

Antalia tomato hybrid was used as biological material.

Within the experiment, determinations and biometric measurements were performed (average height of the plant, average number of inflorescences per plant) and production measures (average number of fruits per plant, production obtained at the surface unit).

The results presented in this paper represents partial results of an ample study regarding the effect of the products with boron organic compounds as fertilizers on tomatoes plants. The novelty of the study consists in testing, for the first time in Romania, the efficiency of applying the boron based organic compounds on the soil. It should be mentioned that the existing results deals only with their foliar application.

### **RESULTS AND DISCUSSIONS**

The growing and fruiting processes of tomato plants were influenced by the way of fertilization (Table 1). Thusly, it can be found out that the height of plants registered a slight increase in the case of mixed fertilization compared to the radicular one and even a greater difference compared to the witness.

The average height of plants indicated a significant increase, from 25.0% to 62.5% compared to the control, for all experimental variants. The largest difference was recorded at V5 (radicular treatment with Calcium Fructoborate + foliar treatment with fructose Folibor) and V6 (radicular treatment with Calcium Glucoborate + foliar treatment with glucose Folibor) (circa 62.5%) and smaller in V2 (radicular treatment with Calcium Fructoborate) and V<sub>3</sub> (radicular treatment with Calcium Glucoborate) (circa 25%).

The average number of inflorescences on the plant indicated an increase, by applying the fertilizers, compared to the witness variant, due to the better development of plants, the largest number of inflorescences being recorded for variant V5.

The percentage of fruit connection on the plants indicated an increase by applying the mixed treatment (*root* + *foliar spraying*) compared to the variants treated only radicularly and compared to the witness variant, the highest value was recorded for the variant V6 (for the 7 inflorescences).

The tomato production was directly influenced by the mixed fertilization (root + foliar spraying). Thusly, for those variants with mixed fertilization, productions were within 124.55 and 136.98t/ha.

From a statistical point of view, the production differences (Table 2) were in between insignificant (V3 - radicular treatment with Calcium Glucoborate), to significant (V2 - radicular treatment with Calcium Fructoborate, V4 = radicular treatment with Calcium Fructoborate + foliar treatment with glucose Fructoborate), distinctly significant (radicular treatment with Calcium Glucoborate + foliar treatment with fructose Folibor) and to very significant (V5 - radicular treatment with Calcium Fructoborate + foliar treatment with fructose Folibor and V6 - radicular treatment with Calcium Glucoborate + foliar treatment with reatment with glucose Folibor).

Table 1

Variant	t Average height of the plant		Average number of inflorescence			% binding
	cm	%	on plant	Flowers	Fruit	
V1 = untreated control	160	100.0	8	40.1	32.1	80.1
V2 = radicular treatment with Calcium fructoborate	200	125.0	9.1	46.4	40.4	87.1
V3 = radicular treatment with Calcium glucoborate	200	125.0	9	46.8	40.7	86.9
V4 = radicular treatment with Calcium fructoborate + foliar treatment with glucose Folibor	240	150.0	9.8	47.8	43.1	90.2
V5 = radicular treatment with Calcium Fructoborate + foliar treatment with fructose Folibor;	260	162.5	10.1	53.3	48.1	90.2
V6 = radicular treatment with Calcium Glucoborate + foliar treatment with glucose Folibor;		162.5	10.0	51.1	47.2	92.4
V7 = radicular treatment with Calcium Glucoborate + foliar treatment with fructose Folibor;		150.0	9.8	50.0	44.1	88.2

Growing and fruiting of tomato plants under the influence of the fertilization manner

# Tabelul 2

The influence of the fertilization manner onto the tomato production

V	Production obtained	Difference $\pm$ t/ha	Relative	Significance
	t/ha	from control	Production %	
V1	105,45	-	100,00	
V2	122,75	17,30	116,41	Х
V3	116,43	10,98	110,41	
V4	124,55	19,10	118,11	Х
V5	136,98	31,53	129,90	XXX
V6	134,20	28,75	127,26	XXX
V7	129,15	23,70	122,48	XX

DL 5% = 13,68 t/ha

DL 1% = 22,78 t/ha

DL 0,1% = 23,89 t/ha

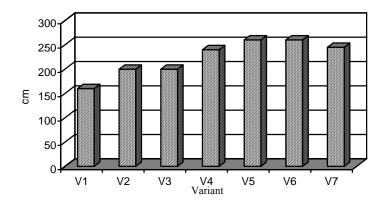


Figure 1: Average height of tomato plants

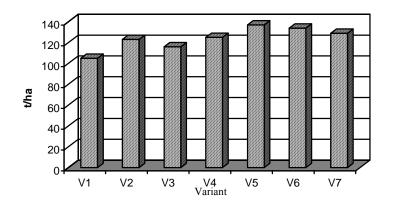


Figure 2: Total production of tomatoes

# CONCLUSIONS

The average height of the tomato plants registered a significant increase at the variants radicularly + foliarly treated with fertilizers based on boron organic compounds, compared to the control, the values being within 25-62.5%.

► Compared to the control variant, the average number of plant inflorescences was larger for those variants where fertilizers were applied, due to a better development of plants.

The percentage of fruit connection on the plants indicated an increase by applying the mixed treatment (radicular + foliar) compared to the variants treated only radicularly and compared to the witness variant, the values for the variant with mixed treatment being in between 88.2 and 92.4.

► The tomato production was directly influenced by the mixed fertilization (radicular + foliar). Thusly, for those variants with mixed fertilization, productions were within 124.55 and 136.98t/ha.

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Seria: 🖌 Biologie

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🖌 Tehnologia prelucrării

produselor agricole

🖌 Ingineria mediului

Vol. XV ( XLXI ) - 2010

#### CONTRIBUTIONS REGARDING THE MORPHOLOGY OF THE ENCEPHALON IN *CAPOETA TINCA* (HECKEL, 1843) AND *CIRRHINUS REBA* (HAMILTON, 1822) (PISCES, TELEOSTEI, CYPRINIDAE)

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KEY WORDS: morphology, encephalon, variability, lobes, tubercles

#### ABSTRACT

The present study comparatively presents the macroscopic characteristics of the encephalon in two species of fish belonging to the Cyprinidae family: Capoeta tinca and Cirrhinus reba. It was observed a variation both intraspecific and interspecific regarding the shape of the encephalon according to the body length and the way of life. In some Capoeta tinca individuals, it was observed an asymmetry of the cerebral hemispheres, which we consider to be of mechanic nature. In what concerns the development degree of the vagal lobes and of the facial lobe, the studied species fall into the first group, where Cirrhinus reba is placed, having the facial lobe and the vagal lobes little developed and the second group, to which Capoeta tinca belongs, having the facial lobe developed.

#### **INTRODUCTION**

At the present time, in the world, there are many papers that discuss the brain of the teleostei fish. Few authors have studied the encephalon in unitary groups of fish (Davis and Miller, 1957; Miller and Evans, 1965; Popescu-Marinescu, 1968; Kotrschal et al., 1988, 1991, 1992, 1998) etc.

In our country, the morphology of the encephalon in cyprinidae fish was studied by the following researchers: Bănărescu (1949), Necrasov et al. (1955, 1960, 1968 etc), Dornescu et Marcu (1973). Bălescu Carmen also made similar papers (2000-2005).

We have not found data in the specialty literature that may confirm the fact that the encephalon of *Capoeta tinca* and *Cirrhinus reba* has been studied. The cyprinidae have about 2070 species (Nelson, 1994), thus it is very difficult to analyze the structure of the brain in all fish. Therefore, the aim of this paper is the comparative analysis of the 5 vesicles of the encephalon, both between the 2 species and among the individuals of the same species in strong connection with the environment conditions specific to the biotope that they occupy. We pursue to find out which one of the divisions of the encephalon presents a greater variability of the brain.

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#### MATERIAL AND METHODS

The analyzed species, *Cirrhinus reba* and *Capoeta tinca*, belong to the Cyprininae subfamily. They are freshwater rivers species, originary from the Asia. *Cirrhinus reba* is found in India, Bangladesh, Nepal (Talwar and Jhingran, 1991). It is a reophile species and it swims in the water mass. It has an elongated body and laterally compressed; the inferior mouth with a pair of rostral barbels. It is omnivorous and bentophag. *Capoeta tinca* has a wide distribution in western of Asia (Anatolia). It prefers streams. It is a benthopelagic and omnivorous species. The mouth is equipped with two pairs of barbells (Turan et al., 2006).

The material for the processing, preserved in alcohol, was supplied from the collection of Acad. dr. doc. Petru Bănărescu. We worked on 5 samples belonging to the *Capoeta tinca* species, having body sizes between 6,15 cm and 4,72 cm and 2 samples belonging to the *Cirrhinus reba* species, whose length was of 10,3 cm and 9,9 cm. For each exemplary there were made measurements regarding the body length and the length of the encephalon and its vesicles. The fish were weighed with the analytical balance. The encephalon, taken out of the skull by classic methods, was placed in a crucible with water in order to avoid dehydration. It was drawn at camera clara. The details were stressed with the help of the hand held magnifying glass and of the binocular. The main vesicles and divisions of the encephalon (the telencephalon, the diencephalon, the mesencephalon, the myelencephalon) were comparatively analyzed.

#### **RESULTS AND DISCUSSIONS**

#### Telencephalon

It is developed in both species. In 2 exemplaries of *Capoeta tinca* we observed an asymmetry of the 2 telencephalic hemispheres – one of the hemispheres is bigger than the other (Fig. 1. a, b). We consider the cause to be of mechanic nature, being influenced by the skull. It is difficult to draw a conclusion regarding this asymmetry, because of the small number of exemplaries that we had at our disposal. The asymmetry can also be genetically explained or ascribed to the way of life and the environment. Sulci and tubercles, which vary in what concerns the aspect and the number, can be noticed on the dorsal surface of the hemispheres (Fig. 1.). In *Capoeta tinca*, the tubercles clearly stand out. Also, the olfactory tracts are very short, so that the olfactory bulbs come into contact with the rostral part of the hemispheres. In *Cirrhinus reba*, the olfactory tracts are long and the olfactory bulbs are oval.

#### Diencephalon

We analyzed the lobes of the hypothalamus, among the divisions of the encephalon. In both species, the lateral lobes of the hypothalamus are big, caudally close, and rostrally they include the median lobe between them. It has the rostral surface rounded in *Cirrhinus reba* and almost plane in the individuals belonging to the *Capoeta tinca* species. The longitudinal, median and mamilar sulci can be observed on the surface of the lateral lobes. The hypophyse above the median lobe can be distinguished in the individuals belonging to the *Capoeta tinca* species.

#### Mesencephalon

The optic lobes are big and they appear under different aspects both intraspecific and interspecific. In both *Cirrhinus reba* individuals, the optic lobes do not touch themselves on the median line. They are slightly spaced between themselves in the rostral

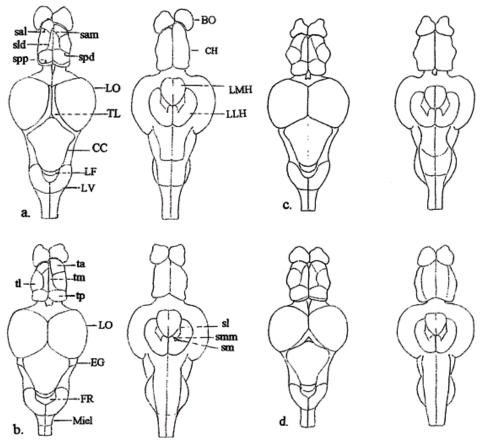
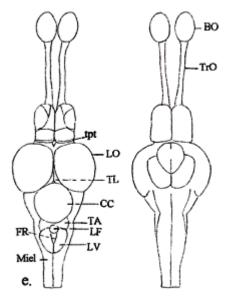


Fig.1. Dorsal view (left) and ventral view (right) of the encephalon in 2 species: Capoeta tinca - in



individuals having the body size different: a. L=6,15 cm; b. L=5,58; c. L=5,45 cm; d. L=5 cm and Cirrhinus reba: e. L=10,27 cm. BO = bulbus olfactorius, TrO = tractus olfactorius, CH = cerebral hemispheres, LO = lobus opticus, TL = torus longitudinalis, LMH = lobus medianus hipotalamic, LLH = lobus lateralis hipotalamic, CC = corpus cerebelli, EG = eminentia granularis, TA = tuberculum acusticum, Miel = mielencephalon, LF = lobus facialis, LV = lobus vagus, FR = fossa rhomboidea, ta = tuberculum (t.) anterius, tl = t.laterale tm = t. medianum t., tp = t. posterius, tpt = t. postremus, sal = sulcus (s.) anterolateralis, sam = s. anteromedianus, sld = s. laterodorsalis, spd = s. posterodorsalis, spp = s. posteropostremum, sl = s. longitudinalis, smm = s. mammillaris, sm = s. medianus Scale: 4,33:1 (a.), 4,27:1 (b., e.), 4,57:1 (c.), 4,45:1 (d.).

part, and a bit more spaced in the caudal part. The semicircular toruses can be noticed at this level (Fig. 1.e.). Three situations were observed in Capoeta tinca individuals:

- when the optic lobes are united on the entire median surface (Fig. 1 b., c.);
- when the optic lobes are rostrally united and caudally spaced. At this level there can be noticed the longitudinal toruses. (Fig. 1.d);
- when the optic lobes are much spaced on the entire median line, the longitudinal toruses\_clearly being distinguished on the whole median length (Fig. 1.a).
   Metencephalon

The body of the cerebellum presents interspecific variability. In *Cirhhinus reba*, it has spherical shape. In *Capoeta tinca* it is big, rounded pentagonal, elongated, with well-developed granular eminences. The valvule of the cerebellum has different shapes in what concerns the two species (Fig. 2). In *Cirrhinus reba*, the lateral portions of the valvule are equipped with a sulcus on their dorsal surface. They are close to one another on the anterior part, thus exceeding the median portion at this level. In *Capoeta tinca*, the lateral portions are little spaced between themselves at their rostral portion.

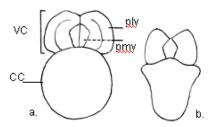


Fig. 2. Valvula cerebelli (VC) in *Cirrhinus reba* (a.) and *Capoeta tinca* (b.). CC –corpus cerebelli; plv –the lateral part of the valvula cerebelli; pmv

the median part of the valvula cerebelli. Scale: 6,25:1 (a.), 5,66:1 (b.)

#### Myelencephalon

The vagal lobes and the facial lobe associated with the gustatory sense are characteristic to the myelencephalon (Evans, 1931). The facial lobe associated with the external taste is small and rounded in Cirrhinus reba, and in Capoeta tinca is big, curved and spherical. A small portion from it can be distinguished without removing the body of the cerebellum. In Cirrhinus reba, the vagal lobes, associated with the internal taste, are slightly thrown into relief and a little rostrally spaced, so that the rhomboid fossa has the shape of a narrow triangle. In *Capoeta tinca*, the vagal lobes are much rostrally spaced, so that at this level the rhomboid fossa is large, having a semicircle aspect. The acoustic lobes are placed rostrally towards the vagal lobes. Its lateroposterior portions can be very well seen in *Cirrhinus reba*. The anteromedian portions of these lobes are wide and flat, because of the press exerted by the body of the cerebellum upon them. Due to the characteristics of the facial lobe and of the vagal lobes, this type of myelencephalon falls into 2 groups according to the classification made by Bănărescu (1949): the first group with reduced facial lobe and vagal lobes, where Cirrhinus reba is situated, and the third group with big, prominent facial lobe and moderately developed vagal lobes, where Capoeta tinca is situated

On the basis of the morphologic aspects correlated with the measurements of the divisions and subdivisions and also taking into account the specialty literature (Kotrschal et al. 1992, 1998), we found out that the studied species fall into 2 groups. The basic group of brains of the cyprinid type, which is characterized by the well-developed visual lobes, but with a moderate development of the gustatory and octavolateralis lobes in what concerns *Cirrhinus reba*. The group of the chemosensory brain characterized by large gustatory lobes and less developed visual and octovolateralis lobes in what concerns the *Capota tinca* species.

#### CONCLUSIONS

An intraspecific variability in what concerns the shape and the dimensions according to the size of the fish is observed in the *Capoeta tinca* and *Cirrhinus reba* species.

The dorsal surface of the telencephalon is furrowed by sulci which delimitate tubercles, more prominent in *Capoeta tinca*. The olfactory tracts are long in *Cirrhinus reba* and very short in *Capoeta tinca*, which makes the olfactory bulbs come into contact with the rostral part of the telencephalon. In some individuals of *Capoeta tinca*, we can associate the asymmetry of the two halves of the cerebral hemispheres with a mechanic cause, determined by the skull.

In *Capoeta tinca*, the hypothalamus is better developed, so that the hypophisis can be seen. The spacing of the optic lobes on the median line, with total or partial prominence of the longitudinal toruses is influenced by the development of the cerebellum valvule.

According to the development degree of the lobes at the level of the myelencephalon, the species fall into the first group: *Cirrhinus reba* – having the facial lobe and the vagal lobes moderately developed and into the third group: *Capoeta tinca* – having the facial lobe very well-developed and the vagal lobes moderately developed.

The shape variability of the different divisions and subdivisions of the encephalon is more accentuated among the individuals belonging to the *Capoeta tinca* species than between the individuals belonging to the *Cirrhinus reba*.

By the morphological criterion, the encephalon of the species *Cirrhinus reba* belongs to the group of primary brain of cyprinid type. The encephalon of the species *Capoeta tinca* belongs to the group of the chemosensory brain.

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### SETTING OUT METODOLOGY FOR A SEWAGE INFRASTRUCTURE SECTION OF SÂNTANA LOCALITY, ARAD COUNTY

Bârliba Livia Luminița<sup>1,\*</sup>, Bârliba C.<sup>1</sup>, Peptan Carmen<sup>1</sup>, Eleş G.<sup>2</sup>

**KEYWORDS:** setting out, total station, edilitar equipment, graphical measurements,

#### ABSTRACT

Whatever their purpose, constructions require topographical works for all stages of execution: the planning activity is made on digital plans having level contours obtained by topofotogrammetric works. The execution stage involve stetting out operation control and reception measurements, however the exploitation stage require the following behavior of constructions for a certain period of time. The overall objectives subject is engineering topography.

Setting out, as the main execution operation, concern the transposition in the field of the characteristic points of constructions, planimetric and altimetric according to the project. The operation require the draft plotting, computing the necessary elements and the drawing pieces necessary to set out the axes, contours and characteristic details of the project.

#### **INTRODUCTION**

An important role in achieving aspirations to achieve, Regional Development is considering a settlement that is part of the border areas and other localities are lower or higher, with different problems.

Development involves a coherent thought, forecasting, identification and responsible allocation of resources and collaboration (Badea, 2002).

In this way, to achieve these goals, the necessity of obtaining financial resources through European and government funding programs, cooperative programs and partnerships, and not at least the long term credit programs (Stan, 2006).

Development and improvement of rural and urban infrastructure includes construction and upgrading of roads and bridges, construction and modernization of water supply, construction of sewerage water through investments related to water treatment plants (David et al., 2005).

Setting out the constructions involves the field application of topographic factors (elements), as compared with a support network, using processes and tools appropriate to the situation on the ground and precision required (Eleş, 2010).

Topographical elements that apply in the plan and height are: Distance (inclined or horizontal), horizontal angles (directions), level differences and lines of certain slope. These are deducted in advance *by graphical* measurements on the situation plan, or *analytical* way, by calculation of known or deduced rectangular coordinates on the original

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plan. (Basil Savitsky et al., 2008).

The support network, essential for setting out and execution, consists of points of known coordinates marked on the ground and the plane. Basically, it is recommended to use a single network, for planimetric and altimetric setting out. (Belc, F., 2004) The latter goal, very important must be taking into account since the survey. In some cases rely on a special network for setting out or the possibly to use a different altimetric and planimetric network (Boş, 2007).

# MATERIAL AND METHOD

Working methods and procedures differ among themselves as to objective nature, field conditions, support network density, accuracy, efficiency, etc.

Area: Sântana built village, Str. Ciocârliei (figure 1).



Figure 1. Str.Ciocârliei Sântana

Subject of study: Setting out the municipal sewage works Str.Ciocârliei, Sântana built village.

Sântana is a city situated in the North-West area of Arad county, at a distance of 28 km. from Arad city and at 22 km. from Chişineu-Criş city. Throughout the city are taking on a very important element "Tisa Plain" where is located entirely. In terms of its extent, Santana occupies 10,714 hectares (figure 2).

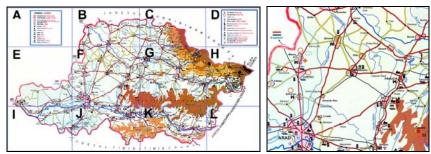


Figure 2. Framing zone of Sântana city

An important element is that the communication paths available that help to develop economic relations with the rest of the towns and surrounding areas. Thus railway Timişoara–Arad–Oradea passes through Sântana, split to Brad and Cermei, the road Arad – Oradea passes at 7 Km from the village by the county road Pâncota–Caporal Alexa–

Sântana-Curtici.

Sântana city has a clogged domestic waste channel located along the civic center to the existing Wastewater Treatment Plant site.

Wastewater treatment station is physically and morally not able to provide the water treatment requirements. Also it is located at a distance less than 300 m (distance required by law) to the inhabited area.

The technological process includes only a partial settling.

Waste water is draining into a ditch, envoy without continuous flow.

For this reason it required the creation of a centralized sewerage system and wastewater discharge, covering the entire area.

In order to achieve a centralized sewerage and sewage disposal, feasibility studies for investment "Sewage Infrastructure and wastewater treatment plants at Sântana, Arad county." were done.

The feasibility study was completed with the maximum daily flow of the wastewater discharged from 2000 cubic meters per day.

The proposed flow sheet consists of the following items:

- Main collectors system;

- Secondary collectors system;

- Vacuum and pumping stations;

- Discharge pipes to the treatment plant;

- The advanced sewage treatment plant for removing nitrogen and phosphorus

Equipping urban development and sewer infrastructure of the city wastewater treatment Sântana:

Sewage systems consist of main canals, secondary canals and discharge pipes.

Main collector networks will be made of PE 100-HD, SDR 17.

Secondary collector networks will be made of PVC-SDR 41 (SN 4), respective PE 100-SN4.

Connections to households and public buildings will be made of pipes PVC – SDR 41(SN 4) in total length of 54.128 ml, with D=160 mm.

Secondary sewers will be made of PVC pipe and will have D = 160mm

Total length of secondary collectors will be 21.382 ml from PVC SDR 41 (SN4) D = 160 mm and 5130 ml din PE 100 - HD, D = 90 mm SDR17

Diameters used to make major collectors are vacuum D 110 mm D 125 mm D 160 mm. length of the diameter will be:

PE 100 – HD Dn 110 SDR 17 – 38.600 ml'

PE 100 – HD Dn 125 SDR 17 – 12.270 ml,

PE 100 – HD Dn 160 SDR 17 – 7.700 ml,

PE 100 – HD Dn 110 SDR 17 – 12.270 ml.

Vacuum stations are underground tank construction type and building on top is a brick wall.

Construction goes on two levels, ground and underground tank

External size of the vacuum stations:

- L ext = 11,8 m B ext = 7,58 m H ext = 3,22 m Internal size of tank:

- L int = 10 m B int = 6,5 m H int = 3,36 m

The vacuum underground station size:

- L ext = 6,7 m B = 2,8 m (compare with NTN) H ext = 3,22 m.

Vacuum tank is cylindrical, horizontally mounted, stainless steel interior and exterior treated with epoxigudronice paints.

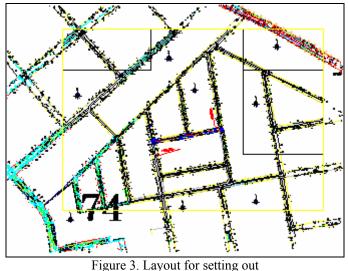
Vacuum homes are prefabricated concrete construction, cylindrical, assembled on site. The walls of houses are mounted in a prefabricated tube of a truncated cone with a diameter reduction from 0.8 m to 1m head of cast iron fireplace.

From each vacuum station a pipe to discharge wastewater towards the treatment plant was provided. The main pipeline between STV1 - STV2 intersection with the pipeline - the pipeline crossing the STV3 – Wastewater Treatment Plant. This pipeline will be the three sections mentioned diameters of 200 mm, 250 mm and 315 mm. From the STV1 to the intersection the diameter will be D 200 and the from the STV3 to the intersection the diameter will be D = 180 mm the pipes will be made from PE 100 - HD SDR 17

# **RESULTS AND DISCUSSIONS**

Using, integrated AutoCAD environment, the plan for setting out was drawn up (Figure 3). Location and position limits of the demarcating points were determined by using the following menus AutoCAD plotting the coordinates of points required (Table 1)

	COORDINATES TABLE (St	tereo-70) Table 1
Point number/description	X [m]	Y[m]
BIS.SANTANA	543936,428	230518,278
100	543759,037	231493,363



The setting out plan, will provide all data required for materialization points in field sight lengths, angles and points coordinates in the stereographic system 1970 (Coşarcă C., 2003). Having as support point of knowing coordinates in the stereographic system 1970, station point 100 and first bearing sight on the Sântana Church the necessary elements for setting out has been determined.

Based on points coordinates need to be materialized on field, using AutoCAD software we obtain the horizontal angles and horizontal distances from the point station to the setting out points. On field the instrument has been set up on the station point #100(figure 4).

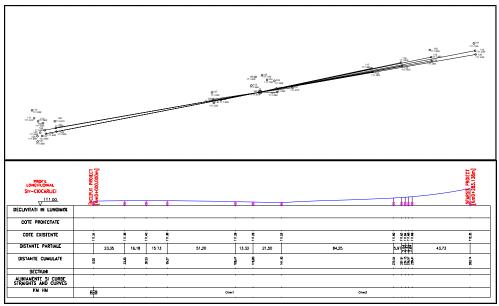


Figure 4. Longitudinal profile of the street and setting out sights

The total station has been bearing on the SÂNTANA Church  $(311^{\circ} 45^{\circ} 64^{\circ\circ})$  after that using the recording movement we set the sight at the appropriate setting out angles. From repeated measurements, the first point position has been determined. Using the same procedure all 37 points were set out.

After verify the set out the first remark was that the point position in the field has fulfilled the demands concerning the accuracy. The set out accuracy was superior resembling the imposed tolerances.

The setting out proposal of both alignment was chose in such manner that the limit position to fall into the provided area of the project and these alignments fall strictly so as not to overlap other elements related to underground infrastructure and the future path of the pipeline can remain in the archives of City Hall for future interventions or investments.

#### CONCLUSIONS

In conclusion, it was considered to comply with all wishes shaping imposed by the designer and owner and, therefore, endorsed the project proposal complies with plotting the Cadastre and Land Registration Office of Arad County

Given the issues presented in the paper, it appears some of the benefits of using modern equipment in this field to derive the following conclusions:

Knowledge and planning how to use the land can be achieved only on the basis of the general cadastre information system.

In the last decade human settlements, urban and rural areas have continuously upgraded through accessing European funds in various areas, especially infrastructure development.

Current urban development, offers specialist surveyor to the problems of increasingly complex.

Therefore assigned to future increasingly important, requiring a very good management and organization, and not least keeping pace with technological development

of increasingly complex.

Surveying and setting out topographic work runs through simplified techniques that do not require additional effort from operators and affected during the work is much reduced.

Modern geodetic methods have replaced virtually all conventional methods of lifting, as have the advantage that in addition to lifting quantitative planimetric mapping, high efficiency allows a computerized inventory of work done.

Future developments will be towards obtaining higher precision existing in a short time and at lower cost.

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### DRAWING UP A 3D PLAN NECESSARY TO ACHIEVE A DEVELOPMENT PROJECT OF QUARRIES IN THE AREA OF BATA, ARAD COUNTY

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KEY WORDS: topographic plan, total station, cadastral works, smart station, pantograph

#### ABSTRACT

The final phase of topographic surveys and fotogrammetric works is drawing up plans. Topographic surveys can be executed independently of geodetic network of the country or support network. In the first case, the preparation is done independently of the network plan map, and in the second case is made on the Stereo 1970 trapeze or on cadastral sections (in regions that have replaced local project works). Drawing up plans consists of the execution of successive operations related to each other, which generally are similar to the two categories of surveys.

Development work was based on results of a topographic surveys and altimetry a work area to carry out a land character in Bata village, Arad County.

The paper release was based on results of topographic surveys for an area in order to carry out a cadastral work in Bata village, Arad County.

#### **INTRODUCTION**

The last achievements, concerning the combination GPS & total station – Smart station, the 3D laser scanner, means new opportunities and hopes in the continuous evolution of modern techniques, having spectacular results, hard glimpsed.

The sequence of operations is as follows: preparation of drawing paper and determining the format; setting up the coordinates axes, points reporting, setting up the limits and the details (points connection); marking execution, landscape representation; staining (Niţu C., 1992).

Because of the advantages, the map is the traditional representation form and most common representation format for spatial data.

In its ,clasic" format the map is used both for spatial data storage and for submitting them to different users, but the mapping automation, storage and representation are two different concepts.

Data can be stored in a high degree of detail and then, depending on needs, "implemented" on paper at different scales and different degrees of detail.

In our country, round availability of such tools can now be considered satisfactory, the new equipment currently being used, the total expression level or in some cases, as an experiment.

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Topographic assistance during construction ensures contractor, and beneficiary of proper implementation, as project work. Topographic measurements, followed by modeling of the relief, help in assessing and planning work in mining, quarries and gravel pits (Dreuille, C., 1999).

#### MATERIAL AND METHOD

The paper purpose is to manage an area of public interest and economically for the area of Bata, regarding the setting of a stone quarrying, preparing a 3D plan necessary to achieve a development project.

1. This investment helps to develop and bring attention to this area and other potential investors. The maps and topographic plans (scale 1:50000,1:5000) helped for a better area identification, and based on the topographic survey the plans, profiles and the model has been achieved (Omura, 2006).

To implement a 3D plan is required precise topographic map, or documents showing the land altitudes as dense and detailed as possible, either by height points, or by sections of land, or more simply by contours. Then, using stereoscopic aerial photographs is often useful to represent areas with rugged terrain in mountain regions, for example, but for specific details and variations in slope or contour interval of the height points.

After determining the overheight coefficient or the descending overheight curve (figure 1), the map establishing is preceded by a training that consists of a strengthening of the main contours, in a draw where the successive contours to be distinguished through different colors in order to avoid any error identification an possibly in some complementary details; buckling curves in plane areas, ridges, rise and curve in massive rock (Bonn, 1996).

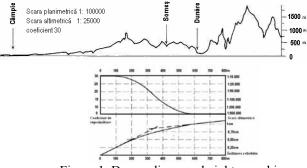


Figure 1. Descending overheights graphics

Simplified various manual procedures, can be take into consideration in order to build up a model in unique terrain models (figure 2).

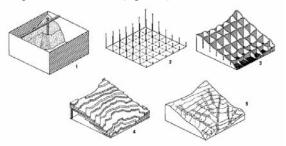


Figura 2. Methods of producing terrain models

Dipstick deep: referring to travel down a graduated dipstick moving in the horizontal plane formed by the edges of a sandbox proceed step by step through the fill and purge.

Steak is pushed into a thick plank nails or rods so that their height is equal to the altitude of the considered point or more precisely with the drum from the reference plan materialized by plank: then filling is done with sand, plaster or clay.

Scant profiles: is done by cutting out cardboard shapes perpendicular or wood; profiles are then broken and the filling is made with any material or strips of cloth bill (M.C. Girard et al., 1998).

Superimposed floors: after drawing contour on sheets of cardboard, plywood or plastic, of thickness of choosing equidistance and considering the overheight, successive floors are cut on the outline courses. It is possible to use only three areas, performing cutting on alternative crowns, on first surface after 1-4-7-10-etc. curves, on the second after 2-4-6-11-etc. curves, on the third after 3-6-9-12-etc. curves, obtain coverage of two intervals that is sufficient to carry overlapping floors, setting them with glue or nails and possibly inserting some support pillars under peaks. Contours, then filled with a material that is suitable for modeling (Bârliba, 2006).

Gradene cut (figure 3): we are obtaining an identical result cutting out the exterior shapes of the landforms needed to be preserved by successive slices, from a homogenous block of clay material, soft paste wax model or a cutting pantograph, from a block of plastic sheets overlaid with a cutting pantograph. There are a lot of pantographs: with overlapping, with lateral translation, with similarity; generally template curves are followed by a peak situated at an arm extremity that commands the cutter movement situated at another arm extremity, on other systems, the curve is followed by a sight mark, by moving the stereotyped from a cylinder, by translation and rotation, and the movement control movement gypsum, placed in a holder under a fixed head miller. Continuous modeling of slopes, is obtained either by filling, considering that the top of the gradens materializes the curves and adding material either by modeling considering the curve at the bottom of gradenas and removing material. This last method is more extensive, but allows precise and neat polish, that highlights the essential lines of the relief, ridge lines, change of slope, thalweg bottoms, while filling tends to calm sudden slope variations.

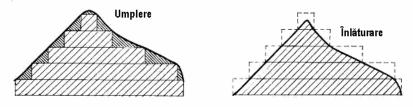


Figure 3. Cutting gradene

Pressing or stamping a sheet of plastic; this method is used especially when the terrain is not well known, especially when we have not any kind of structural elements, without ending altitudes, starting from a map by recognizing the land, or an aerial photo. It is, in cold forming plastic sheet on the back of main ridge lines, on main thalwegs, then on the reverse side ridges, and thus to minor elements. This process gives excellent results for obtained such relief models designated photographic obdurate in dune areas, when the plastic sheet is pressed on a natural granular, for instance a granular paper, grain produced on plastic allows to achieve charts of obscuration by straight photography without any grid

# (Moldoveanu C., 2005).

All the above processes require further reporting by planimetry approximation, doing like in case of dipstick method of depth, by sliding down a pole related to a horizontal rule, or more precisely through an optic system that project the planimetry image on the relief model.

### **REZULTS AND DISCUSSIONS**

The interest area for setting the stone quarries is situated in the Noth part of built village Bata, in the neighborhood of Mureş River. Considering altitude, this is varying between level contours of 145 and 240 meters (Black Sea reference system), which requires the necessity to execute a situation plan with level contours, but to make work properly asked to draw up a plan for surveying situation. The cadastral plan has been achieved at 1:1000 scale based on the field surveys, in the mean time a series of transversal profiles through quarries has been achieved to determine the excavated stone volume.

The field data gathering by total station Leica TC 407 were downloaded through specific software, Leica Geo Office into the computer and later on processed by using software as Notepad, Excel, Word, AutoCAD 2004.

By using all gathering field data the situation plan was achieved and all verifying procedures for this kind of works have been done.

To exemplify how the paper was making we broke the working project on the main stages and also we are exemplify the sequence of editing operations of project:

- use the existing plans for the area;



Figure 4. Framing plan at 1:25000 scale - processing the heights and drawing the heights contours to an equidistance of 1 m:

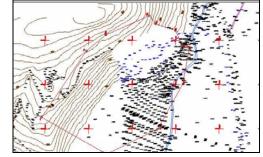


Figure 5. Situation plan containing heights contours at 1:1000 scale - setting the interest area on the situation plan containing heights contours:



Figure 6. Location and demarcation plan of the interest area With heights contours at 1:1000 scale

- the transversal and longitudinal profile execution:

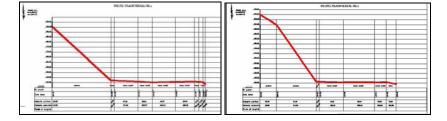


Figure 7. Transversal profiles at 1:500 scale

To achieve the model, it was necessary to dispose of preparing drawings in stages, each with a definite purpose in the final project. The planimetric scale chose depend by the demands that must be satisfied from the beneficiary requests, and the altimetry scale has been established related to the relief importance in realizing the future arrangements. Therefore it was necessary to exaggerate the altimetry scale at 1:20 ratio. So it was necessary to exaggerate heights, how is also made when drawing the terrain sections beginning from the large scale and this overheight grows inversely with the flattering from the reduction in ladder planimetry.

In order to achieve the project, common materials based on polystyrene, polyvinyl acetate, cardboard and plastic elements to render planimetry details identically represented to natural. Representation of relief was based on sections of plan with heights contour lines by cutting and pasting material overlapped from 10 to 10 cm. Other elements of planimetry, were made with colors depending on the destination and vegetation was volumetric represented.

#### CONCLUSIONS

If passed, the use of maps and plans was limited, particularly for geographical orientation and less on administrative or political needs, today's maps have invaded almost all technical fields but also social.

The evolution of thinking and technology development facilitated the transition from classical study of topographic maps in interpreting aerial photographs, satellite images, to create experimental models at different scales, from detailed knowledge of the elements of land, the establishment of mathematical relationships and computer programs concerns the genesis and evolution of the various components that help to achieve maps.

If two decades ago, almost all the maps were made on paper with pencil and toner, and drafting work required a considerable period of time, usage of PC computers and various computer programs significantly reduced the time required to achieve maps and has increased precision, quality and appearance representations maps and topographical plans.

For better handling and maintenance them, they started storing digital maps and plans and listing them with plotters (Bârliba L. et al., 2009).

Making plans and topographic maps, are carried out with programs such as AutoCAD, Autodesk Map, ArcGIS, Photo Shop and others, which give a very accurate representation of land space and other details of planimetry.

Unfortunately, currently there is no version of a 3D making, except the virtual presentation of the land as it may be true.

Carried out and the project layout, location area consisting of a representation of future stone quarries in the terrain according to the European standards draw attention of potential investors to the area.

Execution model with all details of land, it could achieve through proper implementation of the topographic survey and through compiling topographic plan based automated specialized programs that faithfully reproduce all details of planimetry and relief.

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### THE PRODUCTIOVE POTENTIAL MANIFESTATION OF SOME COURGETTE HYBRIDS UNDER THE IMPACT OF SOME CULTURE TECHNOLOGIES MODERNIZATION IN CONDITIONS OF COLD SOLARIUMS

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KEY WORDS: Technology, hybrids, solariums, fertilizers, vegetation

#### ABSTRACT

Courgette (zucchini) is a vegetable belonging to Cucurbitaceae Family to whom it hasn't been given too much attention if we consider the other species, such as cucumbers (long, semi long, cornichon type) or melons and watermelons, which had different modifications in their culture technologies in the past years, meaning their improvement, some of them being considered modern technologies compared to the classical ones.

The researches made had as a goal to show their impact upon the productive potential of two hybrids (Cavili F1 and Ambasador F1), after modernizing some culture methods in cold solariums, that is those referring to the culture systems and using in vegetation some foliar fertilizers specific for this botanical family.

#### **INTRODUCTION**

Courgette, though it is a species spread in our country, of which we consume the young fruits (in bloom squash) and in other areas even the more developed fruits while they are still fresh, didn't get a special attention as other species belonging to *Cucurbitaceae* Family. In this sense, we can example with cucumbers (long, semi long, cornichon type) and also with watermelons and melons which's culture technology in the past years had big transformation, in the sense of improving them, some of them being even modern technologies compared to the classical ones.

Obtaining early or very early productions as an effect of some culture protection systems by using shelters of plastics with new properties (Horgoş, 2003), obtaining quantitative productions close to the theoretical potential of the used cultivars and the organoleptic special features of the new varieties and hybrids concerning the taste, smell and core colour, lack of seeds (watermelons), using drip irrigation and fert-irrigation are all technological elements which reveal the modernism of this new technologies.

The alimentary values of courgette (we could say ignored until now), because of the high content of carbon hydrates, proteins, calcium salts, phosphorus and iron and also vitamins A and C (Pârvu, 2003), (with their extremely good role in human metabolism) will have the deserved place in human alimentation in the conditions where natural remedies and the concept of alternative alimentation have more and more adepts and the nutritionists give more and more attention to the researches made in this domain.

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#### MATERIAL AND METHODS

The study concerning modernizing some technological processes in courgette's culture in solariums was made in a family association from Arad County, specialized on early vegetable crops from *Cucurbitaceae* family (squash, semi long and cornichon cucumbers) and *Brasicaceae* family (cabbage, cauliflower, broccoli).

The experiment was done on two courgette hybrids (Cavili F1 and Ambasador F1, some of the most cultivated at this time) cultivated in cold solariums, in different culture and extra-root fertilization systems. The culture was settled in cycle I of production 2009, the experiment being a polyfactorial one (Săulescu and Săulescu, 1975).

FactorA – The hybrid: $a_1$  – Cavili  $F_1$ ;  $a_2$  – Ambasador  $F_1$ 

Factor B – The culture system:  $b_1$  – culture on shaped field on build up layers with the canopy height of 104 cm and mulching with PE sheet + drip irrigation;  $b_2$  – culture on plane fields, not shaped, in equidistant rows + drip irrigation

Factor C – Foliar fertilizers used for stimulating plants' growth and development: c<sub>1</sub> – Control variant, not foliar fertilized; c<sub>2</sub> – Foliar fertilization with Bionex; c<sub>3</sub> – Foliar fertilization with Bostim.

The applied culture technology distinguished by the fact that for assuring plants' humidity and their fertilization there was used the drip irrigation system, which helped to do the fertilization during the vegetation period (fert-irrigation) (Ciofu et al., 2003). There were applied chemical modern fertilizers like Kemira (Cropcare – complex fertilizers for the bases fertilization and Ferticare, starter and phasial with microelements for fer-irrigation and foliar fertilization). The researches made had as goal improving the culture technologies of this species with culture surfaces extending, by following aspects like: applying a new culture system (on shaped field and using PE sheet mulch, easy to apply); assuring an extra-root fertilization (foliar) as a completion to the root one, considering the culture in cold solariums (Bionex – foliar fertilizer with plant extracts and Bostim – biostimulent and bioregulator specific for cucurbits).

### **RESULTS AND DISCUSSIONS**

Out of table 1 and the correspondent figure it results that for factor A (the hybrid), the variation limits are very close of 23.3 t/ha  $(a_2) - 24.3$  t/ha  $(a_1)$ , a difference of +1 t/ha, which is +4.3% in favour of a<sub>1</sub>-Cavili F<sub>1</sub>. To the experimental average (Mx-a<sub>3</sub>) the differences are smaller of ±0.5 t/ha, respectively ±2.1% in per cents.

By analysing the influence of factor B (culture system) with the two graduations –  $b_1$  (shaped field + mulch) and  $b_2$  (plane field, not mulched) – we observe more accentuate variation limits of production than factor A (the hybrid) and less accentuated limits than factor C (foliar fertilizer). In concrete, these limits are between 20.2 t/ha ( $a_2b_2$ ) and 27.6 t/ha ( $a_1b_1$ ), a difference of +7.4 t/ha, which means +26.8%, the average being of 20.6 ( $b_2$ ) – 27.0 ( $b_1$ ) t/ha, that is 23.7%. The percentage differences in hybrids' case vary between 24.3% and 23.5%, which shows the significant impact of shaped field and its' mulching with PE sheet upon the obtained production. Factor C graduations (foliar fertilizers) show the most accentuated variation of courgette production. In percentage, the extreme limits are 118% ( $a_1b_2c_3$ ) and 128.6% ( $a_2b_1c_2$ ), than the control variant not foliar fertilized. On the experiment average (Mx –  $a_3$ ) the extreme limits are of 21.5 t/ha – 120.5% ( $a_3b_2c_3$ ) and 29.5 t/ha – 128.3% ( $a_3b_1c_2$ ). On the analyse made for each hybrid ( $a_1$  – Cavili F<sub>1</sub> and  $a_2$  – Ambasador F<sub>1</sub>) in correlation with the culture system ( $b_1$ -shaped field + mulch and  $b_2$  – plane field not mulched) and also on the analyse made for the experiment average (Mx –  $a_3$ ) different for  $b_1$  and  $b_2$ , we can see that the highest production than the control variant - not

fertilized was obtained where it was used Bionex (c<sub>2</sub>) foliar fertilizer which gave an increase of 5.7 t/ha – 127.0 % for Cavili F<sub>1</sub> (a<sub>1</sub>) and of 5.6 t/ha – 127.9% for Ambasador F<sub>1</sub> (a<sub>2</sub>). For the average value, there was observed an increase of 5.65 t/ha – 127.7%, being followed by Bostim (c<sub>3</sub>) foliar fertilizer with +4.6 t/ha – 122.5%. We conclude that Bionex (c<sub>2</sub>) and also Bostim (c<sub>3</sub>) give production increases by foliar application compared to the control variant, which was not fertilized. Factor B (culture system) by its' two graduations (b<sub>1</sub> – shaped field + mulch and b<sub>2</sub> – plane field, not mulched) shows an accentuated differentiation of courgette's production in favour of b<sub>1</sub> (shaped field + mulch) of 27.0 t/ha (100.0%) compared to b<sub>2</sub> (plane field, not mulched) with 20.6 t/ha (76.3%). The increase is of 6.4 t/ha, meaning +23.7%. The conclusion is of organising the field for courgette's culture shaped in build up rows with the canopy height of 104 cm and mulching with PE sheet. Factor A (the hybrids) with its two graduations (a<sub>1</sub> – Cavili F<sub>1</sub> and a<sub>2</sub> – Ambasador F<sub>1</sub>) in our experiment does not give significant increases of production, which means that the productive potential of the two hybrids is very close. The difference of 1 t/ha (4.1%) between Cavili F<sub>1</sub> (a<sub>1</sub>) and Ambasador F<sub>1</sub> (a<sub>2</sub>) is not significant.

Table 1

Synthesis of the production results of courgette hybrids cultivated in cold
solariums in cycle 1 2009, under the impact of the interactions between the experimental
factors

Ex	p.fa	act.	Av	erage	produc	tion/plant and l	nectare for:	Ave	erage productio (t/ha and %			e
						Factor C		Fa	actor B	F	Factor A	
А	В	С	Kg/pl.			t/ha (%) for		$a_{1-3}b_{1-2}$	$a_{1-2}b_1$ and	a <sub>1-3</sub>	% to	% to
			Kg/pi.	c <sub>1</sub>	-c <sub>3</sub>	b <sub>1-2</sub> c <sub>1-3</sub>	$a_{1-2} c_{1-3}$	u1-3 01-2	$a_{1-2}b_2$	u1-3	a <sub>1</sub>	Mx
		$c_1$	1,888	23,6	100,0	$b_{1-2}c_1$		<b>07</b> (				
	$b_1$	$c_2$	2,416	30,2	127,9	20,9 (100,0%)	$a_{1-2}c_1$ 20,4 (100,0%)	27,6 (100.0%)				
		$c_3$	2,328	29,1	123,0	$b_{1-2}c_2$			a <sub>1-2</sub> b <sub>1</sub>	24.2	100.0	102 1
$a_1$		$c_1$	1,464	18,3	100,0	26,6 (127,0%)			27,0 (100,0%)	24,3	100,0	102,1
	$b_2$	$c_2$	1,832	22,9	125,1	$b_{1-2}c_3$	$a_{1-2}c_2$	20,9 (75,7%)				
		$c_3$	1,728	21,6	118,0	25,4 (121,3%)	26,1 (127,7%)	(75,770)				
		$c_1$	1,792	22,4	100,0	b <sub>1-2</sub> c <sub>1</sub>						
	$b_1$	$c_2$	2,304	28,8	128,6	19,9 (100,0%)		26,4 (100,0%)				
		$c_3$	2,232	27,9	124,6		$a_{1-2}c_3$		$a_{1-2}b_2$	<b>2</b> 2.2	95,9	97,9
a <sub>2</sub>		$c_1$	1,384	17,3	100,0	25,5 (127,9%)	25,0 (122,5%)		20,6 (76,3%)	23,3	95,9	97,9
	$b_2$	$c_2$			127,7	$b_{1-2}c_1$		20,2 (76,5%)				
		$c_3$	1,704	21,3	123,1	24,6 (123,6%)		(70,570)				
		$c_1$	1,840	23,0	100,0	b <sub>1-2</sub> c <sub>1</sub>	$a_3c_1$		a <sub>3</sub> b <sub>1</sub>			
	$b_1$	$c_2$	2,360	29,5	128,3	20,4 (100,0%)	20,4 (100,0%)	27,0 (100,0%)	27,0 (100,0%)			
		$c_3$	2,280	28,5	123,9	$b_{1-2}c_2$	$a_3c_2$			23,8	07.0	100,0
a <sub>3</sub>		$c_1$	1,424	17,8	100,0	26,1 (127,7%)	26,1 (127,7%)		$a_3b_2$	23,8	<i>31,</i> 9	100,0
	$b_2$				126,4		$a_3c_3$	20,6 (76,3%)	20,6 (76,3%)			
		$c_3$	1,716	21,5	120,5	25,0 (122,5%)	25,0 (122,5%)	(,.,.,.)				



Photo 1. Courgette culture in cold solariums in the first phases of growth and development

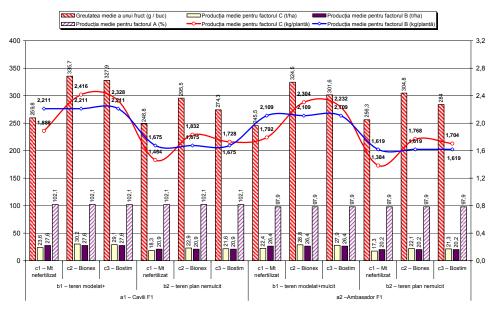


Fig.1. Production results of courgette hybrids cultivated in cold solariums in cycle 1 2009

In table 2 we present the significance of the production differences under the unilateral impact and interaction of experimental factors.

Out of table 2 we can conclude the following: concerning the impact of the hybrid upon the production there is no significance between the productions of Ambasador F<sub>1</sub> and Cavili F<sub>1</sub> and between the experimental average and each hybrid, which shows the close value considering the productive potential (point 1); the impact of culture system (b<sub>1</sub> – shaped field + mulch and b<sub>2</sub> – plane field, not mulched) upon the production is extremely good, compared to b<sub>1</sub> the significance being very significant negative, the obtained production being statistically assured (point 2); considering the unilateral impact upon production of the foliar fertilizers, the obtained productions are statistically assured, the differences being very significant positive; this shows the good effect of using foliar fertilizers upon the production compared to the control variant, not fertilized (point 3); the interactions impact from points 4-12 show the superiority of c<sub>2</sub> and c<sub>3</sub> association (products for foliar fertilization) with any culture system and any hybrid and also the inexistence of a good effect for any culture system and the control variant, not fertilized.

# Table 2

		consu	me maturity		
Variant	Average p		Relative	Difference	Significance
v ur fuitt	(kg/		production (%)	(± t/ha)	Significance
		<u>^</u>	the hybrid upon t		1
a2-a1	23,30	24,28	95,95	-0,98	-
a3-a1	23,82	24,28	98,08	-0,47	-
a3-a2	23,82	23,30	102,22	0,52	-
	%= 1,31		1%=1,98	DL 0,1%= 3	,18
		<u>^</u>		on the production	
b2-b1	20,59	27,01	76,25	-6,41	000
	%= 0,45		1%=0,62	DL 0,1%= 0	,85
		· ·	· · · · · ·	on the production	I
c2-c1	26,01	20,41	127,44	5,60	***
c3-c1	24,98	20,41	122,37	4,57	***
c3-c2	24,98	26,01	96,03	-1,03	0
	%= 0,94		1%=1,28	DL 0,1%=1	
	<u>^</u>	-		ne or different cultu	ire systems
a2b1-a1b1	26,37	27,63	95,42	-1,27	-
a2b2-a1b2	20,23	20,93	96,66	-0,70	-
a2b2-a1b1	20,23	27,63	73,22	-7,40	000
	‰=1,42		1%=2,11	DL 0,1%=3	/
				ifferent culture sys	tems
a1b2- a1b1	20,93	27,63	75,75	-6,70	000
a2b2- a2b1	20,23	26,37	76,74	-6,13	000
	%=0,78		1%=1,07	DL 0,1%=1	
6. The	interaction im	pact of the s	ame hybrid and d	ifferent foliar fertil	izers
alc2-alc1	26,55	20,95	126,73	5,60	***
alc3-alc1	25,35	20,95	121,00	4,40	***
a1c3- a1c2	25,35	26,55	95,48	-1,20	-
a2c2- a2c1	25,45	19,85	128,21	5,60	***
a2c3- a2c1	24,60	19,85	123,93	4,75	***
a2c3- a2c2	24,60	25,45	96,66	-0,85	-
	‰=1,63		1%=2,21	DL 0,1%=2	
7. The inte	raction impac	t of the same	culture system a	nd different foliar f	
b1c2-b1c1	29,51	23,01	128,25	6,50	***
b1c3-b1c1	28,50	23,01	123,85	5,49	***
b1c3-b1c2	28,50	29,51	96,57	-1,01	-
b2c2- b2c1	22,51	17,81	126,39	4,70	***
b2c3- b2c1	21,46	17,81	120,46	3,64	***
b2c3- b2c2	21,46	22,51	95,31	-1,06	-
DL 5	%=1,33	DL	1%=1,81	DL 0,1%=2	,41
8. The interaction	n impact of di	fferent cultur	re system and the	same or different f	oliar fertilizers
b2c1-b1c1	17,81	23,01	77,40	-5,20	000
b2c2-b1c2	22,51	29,51	76,28	-7,00	000
b2c3-b1c3	21,46	28,50	75,28	-7,04	000
b2c2-b1c1	22,51	23,01	97,83	-0,50	-
	%= 1,18		1%=1,60	DL 0,1% = 2	15

The unilateral impact and interaction of experimental factors upon courgette production at

9. The interac	ction impact o	f different hy	brids and the san	ne or different foliar	fertilizers
a2c1-a1c1	19,85	20,95	94,75	-1,10	-
a2c2- a1c2	25,45	26,55	95,86	-1,10	-
a2c3- a1c3	24,60	25,35	97,04	-0,75	-
a2c2- a1c1	25,45	20,95	121,48	4,50	***
DL 59	∕₀=1,86	DL	1%=2,65	DL 0,1%= 3	88
10. The interaction	on impact of t		rid and the same or tilizers	culture system and d	ifferent foliar
alb1c2-alb1c1	30,20	23,60	127,97	6,60	***
alb1c3-alb1c1	29,10	23,60	123,31	5,50	***
alb1c3-alb1c2	29,10	30,20	96,36	-1,10	-
a2b2c2- a2b2c1	22,10	17,30	127,75	4,80	***
a2b2c3- a2b2c1	21,30	17,30	123,12	4,00	***
a2b2c3- a2b2c2	21,30	22,10	96,38	-0,80	-
	%= 2,31		1%=3,13	DL 0,1%=4,1	
11. The interaction	on impact of t		rid and different of fertilizer	culture system and th	ne same foliar
alb2c1-alb1c1	18,30	23,60	77,54	-5,30	000
a2b2c2- a2b1c2	22,10	28,80	76,74	-6,70	000
a3b2c3- a3b1c3	21,47	28,50	75,32	-7,03	000
DL 59	%= 2,04	DL	1%=2,77	DL 0,1%= 3,	72
12. The interaction	on impact of d		ids and the same fertilizer	culture system and t	he same foliar
a2b1c1-a1b1c1	22,40	23,60	94,92	-1,20	-
a2b1c2- a1b1c2	28,80	30,20	95,36	-1,40	-
a2b1c3- a1b1c3	27,90	29,10	95,88	-1,20	-
a2b2c1- a1b2c1	17,30	18,30	94,54	-1,00	-
a2b2c2- a1b2c2	22,10	22,90	96,51	-0,80	-
a2b2c3- a1b2c3	21,30	21,60	98,61	-0,30	-
DL 5%= 2,35 DL 1%= 3,29 DL 0,1%= 4,67					

# CONCLUSIONS

1. The biological value of the two hybrids is very close, the production differences between them being insignificant.

2. The culture system on shaped field on build up layers and mulching with PE sheet gives production increases of 23.7%.

3. Bionex foliar fertilizer and Bostim bioregulator have a very good impact upon the production, giving increases of 27.7% and, respectively 22.5%.

4. We recommend the continuity of researches in order to know better the technological aspects studied in this current experiment.

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### STUDY UPON THE IMPACT OF COVERING LAYER'S COMPOSITION AND HEIGHT UPON THE PRODUCTIVE POTENTIAL OF SOME AGARICUS BISPORUS MUSHROOMS

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**KEY WORDS:** Mushroom culture space, mixture, layer, mycelium, efficiency

#### ABSTRACT

Introducing and settling the nutritive culture substrate (compost) in the production space was one of the technological processes that suffered adaptations, together with the operation of coverage meaning the composition and the quantity used, quantified in its' thickness above the nutritive culture substrate, localized as being the fructification space of the mycelium.

In this article we present the influence of the coverage mixture of different compositions and of its thickness after the coverage process upon the productive efficiency of two Agaricus bisporus strains (Sylvan A-15 and Lambert).

## INTRODUCTION

Producing mushrooms in our country after the 90's suffered important transformations because the production system in existent mushroom spaces in the organized economy of the state (places built especially in this purpose) stopped their activity because of economical reasons.

In the past 10 years *Agaricus bisporus* culture registered some decrease, the intern production of mushrooms coming from their culture in spaces which initially had other purposes, more frequently being those for poultry, stables or storerooms. Normally these transformations took place because of some investments made by those with financial capital. The culture technology, in parallel with this adaptation for the new using conditions of the existing spaces suited for mushroom culture, needed also some modifications or adaptations concerning some technological processes.

Introducing and placing the nutritive culture substrate (the compost) in the culture space was one of the technological processes that suffered adaptations together with the coverage process, meaning coverage layer's composition and the quantity used, quantified in its thickness above the nutritive culture substrate, localized as being the fructification space of the mycelium.

According to the system of culture used nowadays for mushroom culture, the intensive one, monozonal, bizonal and plurizonal, we can distinguish culture as plane layers and culture in polyethylene sacks or other kinds of recipients (Mateescu et al., 1997).

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Mushrooms' culture in polyethylene sacks has the following advantages: the nutritive substrate from sacks is protected by nematodes infestation compared to the one on the soil; the filling of sacks is done more easily than doing the billons; the culture space is hardly infested with specific diseases and pests even after doing some more culture cycles; introducing and evacuating the sacks in and out of the culture space are done better than in the case of billons and plane layers; the nutritive substrate can be transported to longer distances than the place where it was made the compost etc.

In the intensive culture system placing the nutritive substrate can be done by three methods: on shelves – for monozonal culture; in crates – for bizonal and plurizonal cultures; concentrated culture (now in experimentation) –  $1 \text{ m}^2$  can be equal with  $10 \text{ m}^2$  of culture from the industrial mushroom culture places (Horgos, 2002).

Nowadays, in the private mushroom culture places that activate in our country are used as recipients mainly the PE sacks and the parallelepiped recipients.

After placing the nutritive culture substrate in recipients, in PE sacks or parallelepiped recipients, there have to be assured optimum culture conditions in the culture place (temperature, relative humidity of air, ventilation etc.) for mycelium incubation. This is done only if mycelium's incubation hasn't been done by the producer. For the moment the compost necessary for mushroom culture in the private mushroom culture places from Romania is being bought from specialized Hungarian societies.

These societies can deliver the compost with the incubated or not incubated mycelium in it, as the buyer wants, and he will do the incubation in his culture space. Indispensable, even if mycelium's incubation in the nutritive culture substrate was done by the producer (nutritive culture substrate prepared in phase III) or by the buyer, after this technological process the nutritive culture substrate has to be covered with coverage mixture (Mateescu and Mitroi, 1969).

Preparing the coverage mixture is done by different prescriptions, and after disinfection there are used different methods of placing it over the nutritive culture substrate with the incubated mycelium, by respecting a certain thickness of it, because in it takes place mycelium fructification (Mateescu et al., 1972).

### MATERIAL AND METHOD

The researches upon the productive potential of some mushroom strains, which are intensively cultivated at this time, under the influence of two different prescriptions for the covering mixture for different thickness values, were done at S.C. Champignon S.R.L. Arad, a private society that settled the mushroom culture place in the halls of an old poultry farm (Avicola – Arad).

For the moment this society has all the attributes necessary for a modern mushroom culture space, having a heating and cooling installation (for winter and summer), the production cycle developing in a continuous flux, with mechanical ventilation installation and humidity assurance in the microclimate of the culture chambers, all of them being computerized in a programme established in relation with the culture technology.

In this sense it was initiated a trifactorial experiment where the experimental factors are: Factor A – Mushroom strain:  $a_1$  – Sylvan A-15;  $a_2$  – Lambert; Factor B – Coverage mixture prescription:  $b_1$  – prescription I – mixture made with additive peat;  $b_2$  – prescription II – mixture made of garden soil 50% + black peat 50%; Factor C – Thickness of the coverage mixture:  $c_1$  – thickness of the coverage mixture (G.s.a.)  $\geq 2.0 - < 2.5$  cm;  $c_2$  – thickness of the coverage mixture (G.s.a.)  $\geq 3.0 - < 3.5$  cm;  $c_3$  – thickness of the

coverage mixture (G.s.a.)  $\ge 4.0 - < 4.5$  cm; c<sub>4</sub> – thickness of the coverage mixture (G.s.a.)  $\ge 5.0 - < 5.5$  cm; For both prescriptions and different thickness of the coverage mixture there was used the nutritive culture substrate (compost) incubated in phase III, which allowed 8 culture cycles in one calendar year.

According to the phase of compost preparation, the period of one cycle can be of: 60 days when the compost is prepared in phase II, of which 54 incubation days until phase III and harvest and 6 days for evacuating the used nutritive substrate and for preparing a new production cycle; 365 days/year : 60 days/cycle = 6 cycles of production/year; 46 days when the compost is prepared in phase III, of which 40 days until harvesting and 6 days for preparing a new production cycle; 365 days/year : 46 days/cycle =  $7.9 \approx 8$  cycles of production/year.

# **RESULTS AND DISCUSSIONS**

In table 1 are presented the experimental results obtained after cultivating the two *Agaricus bisporus* strains, Sylvan A-15 ( $a_1$ ) and Lambert ( $a_2$ ) using for culture PE sacks, in conditions of applying two different coverage mixtures ( $b_1$  and  $b_2$ ) of four different thickness values ( $c_1$ - $c_4$ ).

Table 1

	Exp								Ave	rage pro	duction	for:				
			No.		Factor	C/cycl	e		Factor B/cycle			Factor A/cycle				
А	в	С	mushr. /recip. (piece)	Pro recip		kg/ 100	kg/m <sup>2</sup> surf.		od/ pient	kg/ 100 kg	kg/m <sup>2</sup> surf.	Pro recip		kg/ 100 kg	kg/m <sup>2</sup> surf.	<u> </u>
			(piece)	Kg	%	kg n.c.s. or %	culture chamb.	$I \alpha = 0/.$	n.c.s.		Kg	%	n.c.s. or %	culture chamber	%	
		$c_1$	121,5	3,365	100,0	17,7	16,81									
	b <sub>1</sub>	$c_2$	186,3	5,551	166,0	29,2	27,74	1 770	100,0	25,9	24.62					
	01	$c_3$	231,6	7,156	230,5	40,8	38,76	4,770	100,0	23,9	24,62					
		$c_4$	108,2	3,041	90,4	16,0	15,20					4,725	100,0	25,3	23,98	98,9
$a_1$		$c_1$	121,9	3,231	100,0	17,0	16,15					4,723	100,0	23,3	23,90	90,9
	$b_2$	$c_2$	194,3	5,304	164,1	27,9	26,50	4,672	07.8	97,8 24,6	24,6 23,34					
	02	$c_3$	237,0	7,205	222,9	37,9	36,00	4,072	97,0							
		$c_4$	105,6	2,946	91,1	15,5	14,72									
		$c_1$	116,2	3,346	100,0	17,6	16,72									
	b <sub>1</sub>	$\mathbf{c}_2$	192,0	5,684	169,9	29,9	28,40	5 014	100,0	26,4	25.08					
	01	$c_3$	254,9	7,851	234,6	41,3	39,23	5,014	100,0	20,4	25,00					
a 2		$c_4$		3,174		16,7	15,86					4,902	103,7	25,8	24,51	101,1
u 2		$c_1$	120,8	3,250	100,0	17,1	16,24					4,902	105,7	23,0	24,51	101,1
	$b_2$	$c_2$	194,9	5,361	164,9	28,2	26,79	4,790	95,5	25,2	23,94					
	02	$c_3$	250,7	7,547	232,1	39,7	37,71	1,770	,0,0	20,2	23,71					
		$c_4$	106,1	3,003	92,4	15,8	15,01									
a <sub>3</sub>		$c_1$	· · · ·	3,356	· · · ·	17,7	16,77					4,814	101,9	25,6	24,25	100,0
Mx	b <sub>1</sub>	$\mathbf{c}_2$	· · · ·	5,618		29,6	28,07	4 896	100,0	.0 26.2	24,85					
	01	$c_3$	242,8	7,504	232,2	41,1	38,99	.,070	100,0 26,2	20,2	21,00					
		$c_4$	109,4	3,108	92,7	16,4	15,53									

Experimental results concerning *Agaricus bisporus* production cultivated in polyethylene sacks with the nutritive culture substrate (n.c.s.) prepared in phase III (incubated)

	$c_1$	121,4	3,241	100,0	17,1	16,20									
b <sub>2</sub>	$\mathbf{c}_2$	194,8	5,333	164,3	28,1	26,65	4,731	96,6	24,9	23,64					
02	$c_3$	243,4	7,376	224,0	38,8	36,86	4,751	90,0	24,9	25,04					
	$c_4$	105,9	2,975	91,8	15,7	14,87									
(	21	120,0	3,299	100,0	17,4	16,49	*	*	*	*	*	*	*	*	
(	$c_2$	192,0	5,478	169,0	28,9	27,36	*	*	*	*	*	*	*	*	*
(	C <sub>3</sub>	243,1	7,440	229,3	39,9	37,93	*	*	*	*	*	*	*	*	
(	C <sub>4</sub>	107,7	3,042	92,5	16,1	15,20	*	*	*	*	*	*	*	*	

We can see the differentiation of production for each strain under the impact of the two coverage mixture prescriptions and thickness of coverage mixture, which are of 29.2-40.8 % in  $a_1b_1c_{2-3}$  and of 27.9-37.9% in  $a_1b_2c_{2-3}$  for  $a_1$ -Sylvan A-15 in comparison with  $a_2$ -Lambert, of 29.9-41.3% in  $a_2b_1c_{2-3}$ , respectively 28.2-39.7% in  $a_2b_2c_{2-3}$ .

Efficiency's differentiation for both strains is clear under the impact of prescription I (additive peat) and thickness graduations  $c_3 (\ge 4.0 - < 4.5 \text{ cm})$  and  $c_4 (\ge 5.0 - < 5.5 \text{ cm})$  compared with prescription II (garden soil 50% + black peat 50%).

The average production obtained under the impact of factor B is of 25.9 % (b<sub>1</sub>), respectively 24.6 % (b<sub>2</sub>) in  $a_1$  – strain Sylvan A-15 and of 26.4 % (b1) and 25.2 % (b<sub>2</sub>) in  $a_2$  – strain Lambert.

Of the same table we can see the total productions obtained under the impact of interaction between the three factors A (mushroom strain), B (prescription) and C (thickness of the covering mixture). The highest productions for both strains  $(a_1 - Sylvan A-15 and a_2 - Lambert)$  under the impact of  $b_1$  (prescription I – additive peat) were of 25.9 Kg/100 Kg n.c.s. in  $a_1$  and of 26.4 Kg/100 Kg n.c.s. in  $a_2$  in comparison with 24.6 Kg/100 Kg n.c.s. in  $a_1$  and of 25.2 Kg/100 Kg n.c.s. in  $a_2$  under the impact of  $b_2$  (prescription II – garden soil 50% + black peat 50%). In percentage, the productions obtained under the impact of  $b_2$  (prescription II) are with 2.2-4.5 % lower than those obtained under the impact of  $b_1$  (prescription I).

The experimental average for  $b_1$  (prescription I) is of 26.2 Kg/100 Kg n.c.s. than 24.9 Kg/100 Kg n.c.s. for  $b_2$  (prescription II), which is with 3,4 % higher.

The highest productions obtained under the impact of factor C (thickness of the coverage mixture) are obtained in graduations  $c_2 \ge 3.0 - < 3.5$  cm and  $c_3 \ge 4.0 - < 4.5$  cm varying between 27.9-41.3 Kg/100 Kg n.c.s., in percentage 164.1-232.1 % than  $c_1 \ge 2.0 - < 2.5$  cm (100 %).

The average production for  $c_2 \ge 3.0 - < 3.5$  cm is of 28.9 Kg/100 Kg n.c.s. – 169.0 % and for  $c_3 \ge 4.0 - < 4.5$  cm of 39.9 Kg/100 Kg n.c.s. – 229.3 % in comparison with  $c_1 \ge 2.0 - < 2.5$  cm, while for  $c_4 \ge 5.0 - < 5.5$  cm of 17.4 Kg/100 Kg n.c.s. respectively 16.1 Kg/100 Kg n.c.s., which is 100.0 % respectively 92.5 %.

Table 2

The synthesis of the annual experimental results concerning *Agaricus bisporus* production under the impact of coverage mixture prescription and thickness

Ext	), fa	ctors		Average production /calendar year:									
				Factor C		Factor B				Factor A			
			kg/	kg/m <sup>2</sup>	t/culture	kg/	t/culture	kg/m <sup>2</sup>		kg/ 100	t/culture	kg/m <sup>2</sup>	
Α	В	С	100 kg	surf.	chamber	100 kg	chamber	surf.	%	. 0	chamber	surf.	%
			n.c.s.	culture	of 240	n.c.s.	of 240	culture	70	kg n.c.s. or %	of 240	culture	70
			or %	chamber	m <sup>2</sup>	or %	m <sup>2</sup>	chamber		01 70	m <sup>2</sup>	chamber	
$a_1$	$b_1$	<b>c</b> <sub>1</sub>	17,7	134,5	32,3	25,9	47,3	197,0	100,0	25,3	46,1	191,9	100,0
		$c_2$	29,2	221,9	53,3								
		<b>c</b> <sub>3</sub>	40,8	310,1	74,4								

		$c_4$	160,0	121,6	29,2								
		c <sub>1</sub>	17,0	129,2	31,0								
	$b_2$	$c_2$	27,9	212,0	50,9	24,6	44,8	186,8	94,8				
	02	C3	37,9	288,0	69,1	21,0	11,0	100,0	71,0				
		$c_4$	15,5	117,8	28,3								
		<b>c</b> <sub>1</sub>	17,6	133,8	32,1								
	$b_1$	$c_2$	29,9	227,2	54,5	26,4 48,1 20	200,4	100,0					
	01	$c_3$	41,3	313,8	75,3	20,4	40,1	200,4	100,0				
2		$c_4$	16,7	126,9	30,5					25,8	47,0	195,9	101,9
a <sub>2</sub>		<b>c</b> <sub>1</sub>	17,1	129,9	31,2					23,8	47,0	195,9	101,9
	h	$c_2$	28,2	214,3	51,4	25,2	45,9	191,5	95,6				
	<b>b</b> <sub>2</sub>	<b>c</b> <sub>3</sub>	39,7	301,7	72,4	25,2	43,9	191,3	95,0				
		$c_4$	15,8	120,1	28,8								

In table 2 there are presented the obtained productions in one calendar year for  $1 m^2$  surface from the culture chamber and for the whole chamber under the impact of the three experimental factors.

Under the impact of factor C (thickness of the coverage mixture) the highest productions were obtained in the three-factor combinations  $a_2b_1c_{2.3}$  of 74.4 t/chamber of 240 m<sup>2</sup>, respectively 75.3 t/chamber, and showing up strain  $a_2$  – Lambert and  $b_1$  – prescription I for preparing the coverage mixture (additive peat).

The average production/culture chamber under the impact of factor B (the prescription for preparing the coverage mixture) is of 47.7 t/chamber – 100 % (b<sub>1</sub>) and 45.4 t/chamber meaning 95.2 % (b<sub>2</sub>). Under the impact of factor A (mushroom strain) the difference of production is not significant, of 1.1 t/chamber (46.1 t/chamber in  $a_1$  and 47.0 t/chamber in  $a_2$ ), in percentage this is 101.9 % than  $a_1 - 46.1$  t/chamber – 100.0 %.

In table 3 according to the statistical calculations, specific to the variance analyses method, the significance of the production differences, made in comparisons as an effect of interdependence of the experimental factors, are being concretized.

Table 3

The unilateral impact and the interaction between the experimental factors impact upon *Agaricus bisporus* production, the strains Sylvan A-15 and Lambert

Variant	Average pro (Kg/100 Kg		Relative production (%)	Difference (± Kg/100 Kg n.c.s.)	Significance			
	1. The unilateral impact of strains upon the production							
a2-a1	25,79	25,25	102,13	0,54	**			
			DL 1% = 0,48					
2. The unilate	eral impact of pro	escription	for preparing the	coverage mixture upon the	e production			
b2-b1	24,89	26,16	95,17	-1,26	**			
	DL 5% = 0	,74	DL 1% = 1,01	DL 0,1% = 1,40				
3. The u	nilateral impact	of the thic	kness of the cover	age mixture upon the proc	luction			
c2-c1	28,81	17,36	165,97	11,45	***			
c3-c1	39,94	17,36	230,12	22,58	***			
c4-c1	16,01	17,36	92,22	-1,35	00			
c3-c2	39,94	28,81	138,65	11,13	***			
c4-c2	16,01	28,81	55,56	-12,80	***			
	DL 5% = (	),84	DL 1% = 1,13	DL 0,1% = 1,52				

4. The interaction	n impact betwee	n the same	strain and differe mixture	ent prescriptions for prepa	rig the coverage
a1b2- a1b1	24,58	25,93	94,79	-1,35	0
a2b2- a2b1	25,20	26,38	95,55	-1,18	-
	DL 5% = 1,2		DL 1% = 1,76	DL 0,1% = 2,42	•
5. The interac	tion impact betw	veen the sa	me strain and diff	ferent thickness values of	the coverage
	_		mixture		
alc2-alc1	28,55	17,35	164,55	11,20	***
alc3-alc1	39,35	17,35	226,80	22,00	***
alc4-alc1	15,75	17,35	90,78	-1,60	0
a1c3-a1c2	39,35	28,55	137,83	10,80	***
alc4-alc2	15,75	28,55	55,17	-12,80	000
alc4-alc3	15,75	39,35	40,03	-23,60	000
a2c2- a2c1	29,05	17,35	167,44	11,70	***
a2c3- a2c1	40,50	17,35	233,43	23,15	***
a2c4- a2c1	16,25	17,35	93,66	-1,10	-
a2c3- a2c2	40,50	29,05	139,41	11,45	***
a2c4- a2c2	16,25	29,05	55,94	-12,80	000
a2c4- a2c3	16,25	40,50	40,12	-24,25	000
	DL 5% = 1,4	45	DL 1% = 1,96	DL 0,1% = 2,62	

Out of the unilateral analyse of the experimental factors from points 1, 2, and 3 (table 3) it results: the productions obtained under the impact of factor A (mushroom strain) have statistically assured, the difference of production being distinct significant positive showing a kind of superiority of Lambert strain than Sylvan A-15 strain; the productions obtained under the impact of factor B (the prescription for preparing the nutritive mixture) are statistically assured, the difference of production being distinct significant negative, showing up the great impact of prescription I (additive peat) upon the production; the productions obtained under the impact of factor C (thickness of the coverage mixture) are statistically assured, the difference of production being very significant positive in four cases and significant negative in only one case.

By analysing the impact of the interaction between the experimental factors from points 4-5 we can see that in most of the cases the obtained productions are statistically assured, having significant, distinct or very significant positive or negative differences. There also is a great number of situations where the productions were not statistically assured so there were no significances between the differences of production.

### CONCLUSIONS

1. The average production obtained under the impact of factor A (mushroom strain) is of 25.3% in  $a_1$  – Sylvan A-15 and 25.8% in  $a_2$  – Lambert, the productions being statistically assured. The efficiency is higher in  $a_2$  (Lambert) than in  $a_1$  (Sylvan A-15) with 0.5%, fact that does not exclude the cultivation of Sylvan A-15 strain.

2. The average production obtained under the impact of factor B ( $b_1$  – prescription I: mixture made of additive peat and  $b_2$  – prescription II: garden soil 50% + black peat 50%) are statistically assured, showing that the additive peat gives higher productions and is also easily to manipulate, excluding the effort made for preparing the coverage mixture.

3. We strongly recommend to use the additive peat for covering the nutritive substrate, but we do not exclude the use of the mixture made of garden soil 50% + black peat 50% (according to prescription II).

4. The thickness of the coverage mixture is determined according to the obtained production, so that the one used in  $c_3$ :  $\geq 4.0 - < 4.5$  cm gave the highest production of 39.9 Kg/100 Kg n.c.s., which is 229.3 % than the control variant – 100.0% ( $c_1$ :  $\geq 2.0 - < 2.5$ ).

5. We recommend the continuity and thoroughgoing study concerning the optimum prescription for preparing the coverage mixture and the optimum thickness value for the coverage layer.

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### UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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#### THE ANALYSIS OF SOME *BOMBINA VARIEGATA* (AMPHIBIA) POPULATIONS FOUND IN WESTERN POIANA RUSCĂ MOUNTAINS, ROMANIA

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KEY WORDS: Bombina variegata, Poiana Ruscă Mountains, features, western Romania

#### ABSTRACT

The 8 studied populations of Bombina variegata, from western Poiana Ruscă Mountains, as well as other populations of this species, found in western Romania, show some characteristics of the Bombina bombina species. The percentage of Bombina variegata characters varies in these populations, with values between 71,31 % and 83,67 %, which are higher than the ones in other populations from other mountains, from western Romania. These results confirm the existence of some differences between the populations of Bombina variegata from different mountains in Romania; these differences are determined by their different history. The dissimilarities between the 8 studied populations are not determined by the altitude at which they are found, but by the morphology of their habitats.

### **INTRODUCTION**

According to literature data, the populations of Bombina variegata found in Romania are not pure, they also present, in a certain amounts, characteristics of the congenial species, Bombina bombina (Covaciu-Marcov et al., 2003, 2007, 2009a, 2010a, Dimancea & Covaciu-Marcov 2009, Kovacs & Covaciu-Marcov 2009). This situation seems to be also applicable to the overall areal of the species, and, also for the congenial species (Stugren 1980, Covaciu-Marcov et al., 2002, Groza et al., 2007). This situation arises due to the fact that these 2 species of the Bombina genus, that are present in Europe, are not reproductively isolated, hybridizing in different areas in which their areal are in contact (Szymura, 1993). The 2 species occupy different areal, Bombina bomina is present in plain areas and *Bombina variegata* in higher grounds (Fuhn, 1960). The contact of the 2 species is not just a present phenomenon, it has been occurring several times in successive interglacial periods, so that the two species have been exchanging genes constantly (Maxon & Szymura, 1979). This has not allowed the establishment of reproductive isolation, probably contributing to the presence in each species' genotype of different alleles, expressed according to the conditions (Stugren, 1980). Although, until now, the studies referring to the percentage of Bombina bombina characteristics in populations of Bombina variegata were made only in certain areas of Romania (Covaciu-Marcov et al., 2003, 2007, 2009a, 2010, Kovacs & Covaciu-Marcov. 2009), the differences were discernible in

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populations of this species from the Eastern and Western Carpathian Mountains (Covaciu-Marcov et al., 2009a), and further more in other areas, like the Danube Gorge (Covaciu-Marcov et al., 2007). In context, our studies aim was the analysis of some populations of *Bombina variegata* from an area in Romania where this kind of studies weren't done before, namely the Poiana Ruscă Mountains.

# MATERIALS AND METHODS

Field researches were done in the spring of 2010. We have analyzed 224 specimens of *Bombina variegata* colected from 8 localities situated in the western area of the Poiana Ruscă Mountains, in Timiș and Caraș-Severin Counties.

Poiana Ruscă Mountains are located in western Romania, they are small mountains, belonging to the Western Carpathian Mountains (Posea & Badea, 1984). The maximum altitude of the mountain is smaller than the other units of the Western Carpathian Mountains, reaching only 1374 m in Padeşu peak (Tufescu, 1986). Towards the west, the mountain is bordered by the Western Plains. All the investigated habitats, with the exception of the one from Nădrag, are represented by temporary pools and open areas, formed by small flow streams. In Nădrag the yellow-bellied toads were populating a large pool area with vegetation.

The studied specimens were captured by hand or with the help of a net with a metallic handle. After the study was realized, all the captured specimens were set free into their original habitats. The establishment of each population's affiliations, from the western part of the Poiana Ruscă Mountains has implied the usage of a method that needs an analysis of the main morphological and chromatic characteristics of the 2 species, grouped in 2 grills. The characteristic used are the most important diagnose characteristic of the two species and several authors used them (Stugren, 1980, Ghira & Mara, 2000, Ghira et al., 2003, Gollmann et al., 1993, Szymura & Barton, 1991).

We analyzed 20 features, using two grids, each grouping 10 characteristic. The first grid analyzed the morphology, the dimension and the ratios of light ventral spots, colored in red at *Bombina bombina* and in yellow at *Bombina variegata*. The degree of confluence or separation of different ventral spots is scored for 10 chromatic groups (table 1). If the light spots are separated among them by black pigment the character belongs to the *Bombina bombina*. When the spots are united among them and the light pigment on the body appears uniform the character belongs to *Bombina variegata*. The second grid analysis 10 features as well and was used by Stugren (1980) and modified by Ghira & Mara (2000) (Table no. 1).

Both of the grids use a binary system (0, 1). For both grids each feature receives a mark: 1 if it is expressed like *Bombina variegata* and 0 if it is expressed like *Bombina bombina*. Summing the marks obtained for each characteristic, a certain individuals can receive on each grid a score ranging from o to 10; the score equal to 0 means a pure *Bombina bombina*, the score equal to 10 means a pure *Bombina variegata*. After assessing the score for each individual, we calculated the average score of all individuals of each population, for each grind and then the average of the two grind. This method allows the transfer of the feature into percentages and their statistical interpretation. The final mark indicates the amount of *Bombina variegata* species features.

These grills were recently used in the studies of several populations from Romania (eg: Covaciu-Marcov et al., 2002, 2003, 2007, 2009 a,b, Groza et al., 2007, Ferenți et al., 2008, Vesea et al., 2004, Dimancea & Covaciu-Marcov, 2009, Kovacs & Covaciu-Marcov, 2009, Sas et al., 2009). The use of this unique, standardized method, has allowed the gradual

emphasizing of the differences between the populations of *Bombina variegata* from different areas of Romania, and the formulations of some zoo-geographical speculations (Covaciu-Marcov et al., 2009a).

Table no. 1.

Cha	racter	Bombina bombina	Bombina variegata
	Grid 1 (the characteri	stic of the ventral patte	ern)
1	Chin – chin	Separated	United
2	Chin – chest	Separated	United
3	Chest – chest	Separated	United
4	Chest – shoulder	Separated	United
5	Shoulder – arm	Separated	United
6	Chest – abdomen	Separated	United
7	Abdomen – abdomen	Separated	United
8	Abdomen – basin	Separated	United
9	Basin – basin	Separated	United
10	Basin – thigh	Separated	United
	Grid 2 (after Stugren,	Ghira & Mara, modifi	ied)
1	Color of light ventral spots	Red, orange,	Yellow
		yellowish	
2	Color of the top of fingers	Black	Yellow
3	Dorsal color	Black	Pale gray
4	Relation between tarsal and plantar light spots	Separated	United
5	Ventral color	Orange spots on black background	Black spots on yellow background
6	Relation between the head length and width	Length > width	Length < width
7	The drawing of the lateral and ventral	White spots around	Without white spots
	parts	the verrucae	around the verrucae
8	Aspect of the dorsal black tubercles	Regulated	Scattered or absent
9	The aspect of the dorsal verrucae	Lens-shaped, squatted	Sharp, rough
10	Ratio of tibia-tarsian joints when the legs segments are parallel	Not touching	Touching

The two Grids of differentiation of the European species of Bombina

## **RESULTS AND DISCUSSIONS**

All of the 8 studied populations belong to the *Bombina variegata* species. This fact was predictable, the populations were found in a mountain area, at altitudes higher than 200 m, all characteristic to the species (Cogălniceanu et al., 2000). Yet, none of the studied populations were pure *Bombina variegata*, the congenial characters of *Bombina bombina* were present in all cases. The amount of *Bombina variegata* characteristics in the studied populations varied between 71,31 % and 83,67 % (Table no. 2).

Although the studied populations also presented characters of *Bombina bombina*, their ratio seems to be smaller than the one recorded in other populations of western Romania, found at similar altitudes, or even higher (Vesea et al., 2004, Covaciu-Marcov et al., 2003, 2009 a, b, Kovacs & Covaciu-Marcov, 2009). Therefore, the populations found at higher altitudes than 500 m in Salaj County, do not reach beyond 80% of the ratio of the

species characteristics. This situation is applicable to the populations found in the Apuseni Mountains (Vesea et al., 2004, Covaciu-Marcov et al., 2009 a), or in the Jiu Gorge (Covaciu-Marcov et al., 2010a). The only populations from western Romania that come close to 80 % and to the ones found in Poiana Ruscă, are the populations found in the Danube Gorge (Covaciu-Marcov et al., 2007). Although the populations found at Portile de Fier are not that pure as the ones in Poiana Ruscă Mountains, they are located at just 100 m altitude, obviously under the inferior altitude limit of the species found in Romania. Therefore the high share of *Bombina variegata* characteristics found in these is even more remarkable as they descend to the lowest altitudes in Romania (Covaciu-Marcov et al., 2009 c).

Table no. 2.

Locality	Rușchița	Oțelu Roșu	Maciov a	Nădrag	Drăgsi- nești	Drinova	Luncani de Jos	Fărășeș ti
No. of studied toads	30	24	20	34	30	28	34	34
Altitude (m.)	497	325	238	333	249	230	338	267
Average grid 1	73,26	68,54	73,07	69,11	78	80	78,52	80,58
Average grid 2	77,43	74,08	76,07	74,11	83,66	82,14	85,29	86,76
Average of grids	75,34	71,31	74,57	71,61	80,66	81,07	81,9	83,67

The affiliation of the studied populations

The raised ratio of Bombina variegata characteristics found in the populations of western Poiana Ruscă Mountains seems to support the idea of existing differences between the populations of this species, from different mountain areas of Romania (Covaciu-Marcov et al., 2009a). Also, our results support the speculation that these differences are caused, to a great extent, by the different history of the 2 species on today's western Romanian territory (Covaciu-Marcov et al., 2009a). This way the higher ratio of Bombina variegata characteristics are demonstrated in the populations of this species from the south-western mountains of Romania. This is consistent with the assumption of the existence of a glacier refuge of this species in this region of Romania (Hofman et al., 2007). Therefore, the populations from the Poiana Ruscă Mountains are in the vicinity of the populations from the refuge, at only 100-150 km away. Also, there is a direct connection between the 2 areas through some mountain peaks, which facilitated the rapid and direct colonization of the Poiana Ruscă Mountains, from the refuge in the Danube Gorge. This fact explains the high ratio of Bombina variegata characteristics found in the populations from the 2 areas in comparison with more northern areas, where the migration of the species took more time and the contact with the congenial species was easier (Covaciu-Marcov et al., 2009a). The rapid colonization of the southern mountain is sustained by the hypothesis of the existence of a secondary glacier refuge for some species, related to the colder climate, like the alpine newt (Covaciu-Marcov et al., 2010b).

In the case of the studied populations there are no connections between the characteristic's ratio and their altitude, although, in general, the increasing altitude triggers the rise of the *Bombina variegata* characteristic's ratio (Vesea et al., 2004, Covaciu-Marcov

et al., 2009b). Therefore, the purest population, from Fărăşeşti, is found at only 267 m altitude, whereas the population from Otelu Rosu, found at 325 m, show 12.31 % more *Bombina bombina* characteristics. In this case the differences can be explained only by the different morphology of their habitats. Similar situations were reported in other limited and leveled regions, geographically and geomorphologically, as well (Kovacs & Covaciu-Marcov, 2009a).

The absence of the differences determined by the altitude, or in any case, the absence in the studied populations, show, once again, a quick colonization, from a single direction, made in time, between different areas of the mountain, according to the habitats. For example, the higher ratio of *Bombina bombina* characteristics in the case of the populations found in Nădrag is explained through the characteristics of their habitat. Even if the habitat is found in higher grounds, in the middle of the mountain, it is more similar to the demands of the *Bombina bombina* species, being represented by a wide pool area, with rich vegetation and mud. The population from Nădrag is the only one in which an individual presents the characteristics of the 2 species in equal proportions. The characteristics of the habitats affect the aspect of the hybrid populations between *Bombina bombina and Bombina variegata* (MacCallum et al., 1998, Covaciu-Marcov et al., 2003).

Regarding the ratio of expression in each of the 20 studied characteristics, they are generally similar, as in the case of other populations from the western area of Romania (Covaciu-Marcov et al., 2009a,b, Kovacs & Covaciu-Marcov., 2009). There are, in both grilles, characters that all 8 studied populations express majorly as in the *Bombina bombina* species (table 3).

Table no. 3

Locality	Rușchița	Oțelu Roșu	Maciova	Nădrag	Drăgsi- nești	Drinova	Luncanii de Jos	Fărășești
Feature								
1	100	100	100	100	100	100	100	100
2	13.33	8.33	7.69	11.76	13.33	32.14	20.58	11.76
3	23.33	12.5	23.07	11.76	33.33	42.85	35.29	35.29
4	86.60	81.25	98.07	76.47	93.33	96.42	88.23	94.11
5	100	100	100	100	100	100	100	100
6	38.33	20.83	30.76	17.64	53.33	42.85	47.05	55.88
7	100	95.83	100	94.11	100	100	100	100
8	96.66	78.26	75	79.41	86.66	85.71	94.11	100
9	100	100	96.15	100	100	100	100	100
10	98.33	100	100	100	100	100	100	100
Feature			•					
1	100	100	100	100	100	100	100	100
2	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100
4	75	66.66	57.69	47.05	90	92.85	64.70	91.17
5	100	100	100	94.11	100	100	100	100
6	36.66	4.16	19.23	23.52	33.33	28.57	52.94	58.92
7	0	0	0	0	13.33	0	35.29	17.64
8	93.33	100	88.46	76.47	100	100	100	100
9	100	100	100	100	100	100	100	100
10	96.66	100	96.15	100	100	100	100	100

The features' ratio in two grids of the studied populations

The ratio of *Bombina bombina* species is generally lower that in the case of other populations of *Bombina variegata* from western Romania. The fact that the same characters are responsible for the presence of *Bombina bombina* characters in the *Bombina variegata* populations from western Romania, show their overall uniformity. Therefore, these populations probably come from the same refuge and the differences between them are indeed the result of the different history following the last ice age (Covaciu-Marcov et al., 2009a).

### CONCLUSIONS

All 8 *Bombina variegata* populations from western Poiana Ruscă Mountains also show characters from *Bombina bombina*. However, their ratio is lower than in other populations from western Romania. Some of the studied populations are purer then the conspecific populations found at higher altitudes in other Romanian Mountains. The higher *Bombina variegata* characteristic's ratio is probably a result of the species history from the post-glacial period, the populations found in Poiana Ruscă Mountains are located in the vicinity of the level of those found in the Danube Gorge, the region being colonized by the species very rapidly and easily, due to the warming-up of the climate. The differences in the morphology of their habitats. In spite of the high ratio of *Bombina variegata* characteristics, there are some characteristics which in the quotient of most individual in all populations express themselves as in the *Bombina bombina*. These are the same characteristics found in other western Romanian populations.

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# THE STEM ANATOMY OF ALCHEMILLA CONNIVENS AND ALCHEMILLA CRINITA SPECIES

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KEY WORDS: Alchemilla, rhizome, flowering stem, anatomy, Romania

#### ABSTRACT

The paper presents the results of histo-anatomical investigations on underground stem and flowering stem from two species of the Alchemilla genus (A. connivens Buser and A. crinita Buser). Histo-anatomical descriptions are accompanied by original photographs and micrometer measurements.

# **INTRODUCTION**

The anatomy of vegetative organs to *Alchemilla* is less approached in the consulted literature. Early histo-anatomical investigations on a species of *Alchemilla* were made in Romania by Toma and Rugină, 1998 and refers to species *Alchemilla vulgaris* L. and the petiol structure is missing its description. *A. vulgaris* is presented in the summary paper "Anatomy of the Dicotyledons" (Metcalfe and Chalk, 1950), but here we find only some references on the structure of this species.

In this context, the paper presents the stem anatomy (the underground stem and flowering stem) of *Alchemilla connivens* and *A. crinita*, aiming at identifying new diagnems and completing the descriptions of studied species. Histo-anatomical researches revealed some structural differences between the taxa analyzed.

#### MATERIAL AND METHODS

The material investigated in histo-anatomical point of view, represented by rhizomes and flowering stems, comes from the Parâng Mountains. It has been preserved in grain alcohol 70% to the full flowering of the plants.

For species identification *Alchemilla connivens* and *A. crinita* we used the specialty literature (Assenov, 1973; Buia, 1956; Ciocârlan, 2009; Walters and Pawłowski, 1968) and the genuine material from collections for a comparison.

For making of sections and microscopic preparations was taken into account the methodology of Tarnavschi et al., 1974.

Making of microscopic preparations was made by cutting the hand microtome. There were realized transversal sections through the median zone of rhizome and flowering stem. Clarification and staining sections were made according to the methodology of Andrei and Paraschivoiu, 2003.

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The preparations have been analyzed with a Nikon microscope and photography with a Nikon camera. Measurements were made on MC-3 microscope. There were 10 measurements each (the wood vessel diameter, the liber vessel diameter). Have been considered the highest individual value (V<sub>M</sub>), the minimum individual value (Vm) and average ( $\frac{\gamma}{\gamma}$ ) which is the arithmetic average of the 10 individual values.

# RESULTS AND DISCUSSIONS

# ALCHEMILLA CONNIVENS BUSER

Rhizome (Figure 1)

It is an underground stem with secondary structure, which strongly emphasizes, in particular, the central cylinder.

From outside to inside to the following areas:

The unilayer epidermis, interrupted here and there, without cuticle, thickness of  $31.5 \,\mu$ m.

*The crust itself* is pluri-layered, consisting of 13 to 14 (-15) batches of spheroidal or ovoid cells, with irregular intercellular spaces. Thickness about 405  $\mu$ m.

*The endodermis* (the last layer of bark) and the pericycle (first layer of the central cylinder) no longer stands out clearly as the pericycle was formed the felogen which gave rise outwards of suber, and inwards in several layers of cells that are the feloderm.

*The suber* is easily recognized by the 8-10 radial lines of tabulation cells walls thicken. The suber thickness of 135  $\mu$ m.

The secondary conducting tissues: by activity the cambium formed the secondary phloem (liber) outwards, which is presented only as soft liber, with thin walls. It is therefore very difficult to distinguish the secondary phloem of the primary. The phloem thickness of 112,5  $\mu$ m. The secondary xylem (wood) formed inwards side.

In the secondary xylem visible and much thicker than the secondary phloem, the wood vessels are arranged in orderly radial rows, with the lumen becoming narrower toward medullary parenchyma. Diameter the wood vessels:  $V_M = 40.5 \ \mu\text{m}$ ;  $V_m = 13.5 \ \mu\text{m}$ ;  $\overline{\chi} = 23,85 \ \mu\text{m}$ . The secondary xylem in its entirety, thickness of 360  $\mu\text{m}$ .

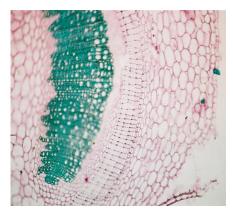


Figure 1. *Alchemilla connivens* – rhizome in transversal section: the epidermis, the crust itself, the endodermis, the suber, the secondary phloem, the secondary xylem, the medullary parenchyma (Oc. 10x, Ob. 10, Original)

*The medullary parenchyma* occupies all central rhizome. It is composed of ovoid or spheroidal cells, with intercellular spaces. Some of them contain simple crystals of calcium oxalate.

# Flowering stem (Figure 2).

Presents a secondary structure evident especially in the central cylinder.

The epidermis is unilayer, with thickness of 13,5  $\mu$ m, covered with a cuticle of 0,9  $\mu$ m thickness.

The crust itself is differentiated into an angular colenchim composed of 2-3 layers of cells, situated beneath the epidermis. The parenchymatous bark follows a much more developed, consisting of spheroidal and ovoid cells, with intercellular spaces. The crust thickness is  $360 \mu m$ .

The internal layer, *the endodermis*, is composed of cells containing the statolitic starch and therefore is called amilipherous sheath. This is thickness of 11,25  $\mu$ m.

*The central cylinder* begins with a row of cells making up the pericycle, in some places more clearly revealed.

The activity cambium formed secondary phloem elements and xylem. The secondary phloem (liber) is composed of vessels with thin cell walls (the liber soft) and has a thickness of 45  $\mu$ m. The secondary xylem is much thicker (101,25  $\mu$ m) and is composed the xylem vessels with large lumen, arranged in radial rows, accompanied by xylematic parenchyma with slightly thickened walls and wood fibres (the libriform), more bold, with lumen very small.

Inboard side of the secondary xylem there are still remnants of the available the primary xylem unordered.

*The medullary parenchyma* consists of large, ovoid or spheroidal cells, with thin walls, and large intercellular spaces. In some cases disappears the medullary parenchyma and formed a relatively large aeriferous cavity.

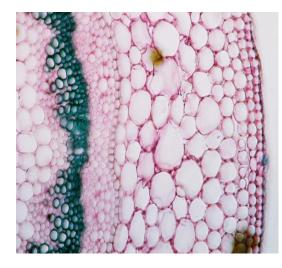


Figure 2. *Alchemilla connivens* – Flowering stem in transversal section: the epidermis, the crust itself, the endodermis, the pericycle, the secondary phloem, the secondary xylem, the medullary parenchyma (Oc. 10x, Ob. 20, Original)

#### ALCHEMILLA CRINITA BUSER

**Rhizome** (Figure 3)

Secondary structure present in the central cylinder.

The epidermis is unilayer, composed of large cells, elongated in the tangential sense, thickness of 13,5  $\mu$ m. The external walls of the cells are easily thickened and covered with a cuticle of 1,35  $\mu$ m.

The crust is pluri-layered, consisting of 10 to 11 layers of spheroidal or ovoid cells with intercellular spaces. Thickness of 150,75  $\mu$ m. The endodermis is poorly demarcated, interrupted.

The suber have different thickness on the circumference, is composed of 7-8 layers of tabular cells with available radial, closely attached. The suber average thickness is 36  $\mu$ m.

The conducting tissues generated of the cambium: the secondary phloem outwards, with thickness of 67,5  $\mu$ m, interrupted here and there. The riddled tubes and cells annexes are narrower than phloematic parenchyma cells. The secondary xylem (the secondary wood) has greater thickness and is composed of vessels, parenchyma and

wooden fibres (the libriform). Diameter wood vessels:  $V_M = 22,5 \ \mu\text{m}$ ;  $V_m = 11,25 \ \mu\text{m}$ ;  $\overline{X} = 15,3 \ \mu\text{m}$ .

*The medullary parenchyma* is composed of parenchymal cells with slightly thickened walls.

# Flowering stem

In a transversal section (Figure 4) highlighted the epidermis, bark with the endodermis, the secondary conducting tissues and the medullary parenchyma.

*The epidermis* is unilayer, uninterrupted, composed of isodiametric cells, with thickened internal and external tangential walls. Average thickness of 15,75  $\mu$ m and is covered with a cuticle of 1,57  $\mu$ m.

Here and there we can notice tector hairs that thick wall, and lumen narrow. Brushes are surrounded by the epidermal cells, above its level and that form a bulb-like formation.

*The crust itself* is pluri-layered. The first layer below the epidermis is an angular colenchim. Other layers of cells are parenchymatous, cell diameter increases towards the central zone of crust and again falls to the endodermis. Thick crust itself is on average of 216 µm.

*The endodermis* (the amilipherous sheath) is thickness of 6,75  $\mu$ m. The cells are orderly arranged around the central cylinder and are elongated in the tangential sense.

The conducting tissues. The secondary phloem (the secondary liber) has the form of a continuous ring with a thickness of  $45 \,\mu\text{m}$ .

Diameter liberian vessel:  $V_M = 9 \ \mu m$ ;  $V_m = 4.5 \ \mu m$ ;  $\overline{X} = 6.3 \ \mu m$ .

The parenchymal cells have smaller diameters. The secondary xylem (the secondary wood) is prepared under the liber with continuous ring-shaped, with larger vessels with lumen to the medullary zone, and to liber lower cells walls thickened (wooden fibres). The wood vessels are radially arranged and accompanied by wood parenchyma.

Diameter the wood vessels:  $V_M = 13,5 \ \mu\text{m}$ ;  $V_m = 9 \ \mu\text{m}$ ;  $\overline{\mathcal{X}} = 11,7 \ \mu\text{m}$ .

*The medullary parenchyma* is composed of large cells more or less isodiametric, with large intercellular spaces.

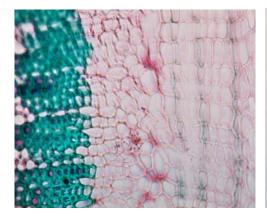




Figure 3. *Alchemilla crinita* – rhizome in transversal section: the endodermis, the suber, the secondary phloem, the secondary xylem (Oc. 10x, Ob. 40, Original)

Figure 4. *Alchemilla crinita* – Flowering stem in transversal section: the epidermis, the crust, the endodermis (Oc. 10x, Ob. 40, Original)

#### CONCLUSIONS

Between the two species examined are some small differences in structure:

The rhizome in *A. connivens* and *A. crinita* have the unilayer epidermis. It is thicker and without cuticle in *A. connivens*. In *A crinita* the epidermis is covered by a cuticle.

In *A. connivens* the crust is composed of several layers of cells compared with *A. crinita*.

The suber greater thickness in *A. connivens*. The phloem thickness greater in *A. connivens*.

Diameter wood vessels is higher in A. connivens.

In *A. connivens* some medullary parenchyma cells contain calcium oxalate crystals.

If the flowering stem of the two species analyzed is noted that:

- The epidermis is thicker in A. crinita;

- Thickness of the crust is higher in A. connivens;

- The endodermis is higher thickness in A. connivens;

- The secondary phloem is the same thickness in both species.

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# THE STEM HISTO-ANATOMY OF THE ALCHEMILLA GLAUCESCENS SPECIES

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KEY WORDS: anatomy, Alchemilla glaucescens, rhizome, flowering stem, Romania

#### ABSTRACT

The paper presents histo-anatomical characteristics of the underground stem (rhizome) and flowering stem from the Alchemilla glaucescens Wallr. species. There were realized transversal sections through the rhizome and flowering stem, histo-anatomical descriptions are accompanied by micrometer measurements and original photographs.

#### **INTRODUCTION**

In Romania, the species of *Alchemilla* can be found almost exclusively in the mountainous regions and the alpine ones.

The microspecies of *Alchemilla* are morphologically variable, and change is continuous, many of them having a separate status in the wild. Therefore, the morphological characters of vegetative and reproductive organs has sought to include histo-anatomical descriptions of them.

The anatomy of vegetative organs to *Alchemilla* is less approached in the consulted literature. Early histo-anatomical investigations on a species of *Alchemilla* were made in Romania by Toma and Rugină, 1998 and refers to species *Alchemilla vulgaris* L. and the petiol structure is missing its description. *A. vulgaris* is presented in the summary paper "Anatomy of the Dicotyledons" (Metcalfe and Chalk, 1950), but here we find only some references on the structure of this species.

This paper analyzed the histo-anatomical aspects of the stem from the *Alchemilla* glaucescens species, collected in Parâng Mountains, Romania.

*Alchemilla glaucescens* is a perennial, short-sized to medium plant, 5-20 (47) cm, glaucous, densely hairy, often sericeous. Erect stem, oblique-ascendant, procumbent, of 0,7-2 mm thick, often 2-5 times longer than the petiole basal leaves, with patent or erecto-patent hairs throughout.

For identification of the *Alchemilla glaucescens* species we used the specialty literature (Assenov, 1973; Buia, 1956; Ciocârlan, 2009; Walters and Pawłowski, 1968) and the genuine material from collections for a comparison.

Knowledge morpho-anatomical peculiarities of *Alchemilla* species is a necessary objective dictated that they have a difficult taxonomy.

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#### MATERIAL AND METHODS

The material investigated in histo-anatomical point of view, represented by rhizome and flowering stem on *Alchemilla glaucescens* species, comes from the Parâng Mountains. It has been preserved in grain alcohol 70% to the full flowering of the plants.

For making of sections and microscopic preparations was taken into account the methodology of Tarnavschi et al., 1974.

Making of microscopic preparations was made by cutting the hand microtome. There were realized transversal sections through the median zone of rhizome and flowering stem. Clarification and staining sections were made according to the methodology of Andrei and Paraschivoiu, 2003.

The preparations have been analyzed with a Nikon microscope and photography with a Nikon camera. Measurements were made on MC-3 microscope. There were 10 measurements each (the wood vessel diameter, the liber vessel diameter). Have been considered the highest individual value (V<sub>M</sub>), the minimum individual value (Vm) and average ( $\frac{\gamma}{\gamma}$ ) which is the arithmetic average of the 10 individual values.

# **RESULTS AND DISCUSSIONS** *ALCHEMILLA GLAUCESCENS* Wallr.

**Rhizome** (Figure 1)

In a transversal section presents a secondary structure evident especially in the central cylinder.

*The epidermis* is unilayer, composed of cells elongated in the tangential sense, with thickened internal and external walls. Average thickness of  $22.5 \mu m$ .

*The crust* is pluri-layered, consisting the felogen that differentiated in pericycle and generate 5-6 (-7) batches of flattened cells, with thickness of  $67,5 \text{ }_{\mu}\text{m}$ .

The secondary conducting tissues: clearly revealed in section, generated from the activity cambium. The secondary phloem is arranged as a continuous ring and consists of tubes riddled, cells annexes and more phloematic parenchyma cells. It have thickness of 112,5  $\mu$ m. The secondary xylem (the secondary wood) is clearly differentiated and it have thickness 585  $\mu$ m. Consists of radial rows of wooden vessels with narrow lumen, accompanied by abundant parenchymal cells and wooden fibres. Under the secondary wood one can notice here and there fascicles of the primary wood (6-7) in a fundamental parenchyma.

*The medullary parenchyma* occupies all the central part of rhizome and is composed of polygonal cells with slightly thickened walls and large intercellular spaces.

# **Flowering stem** (Figure 2)

In a transversal section presents a circular shape. The secondary structure is highlighted only in the central cylinder.

The epidermis is unilayer, with average thickness of 18  $\mu$ m and covered with a cuticle of 1,35  $\mu$ m.

*The crust* is composed of 5-7 (-9) batches of polygonal-rounded cells, which increases towards the center of the lumen. First cells layer of bark, prepared under the epidermis, is composed of small cells, arranged in neat, slightly elongated tangentially and colenchymatic walls. Remaining cells of bark are ovoid or spheroidal, untrimmed disposed and with intercellular spaces. Their lumen increases towards the center of crust and then begins to decrease to amilipherous sheath (the endodermis). *The endodermis* (Figure 3)

consists of small cells, slightly tangentially elongated, with slight thickening of the radial walls. This is thickness of  $11,25 \,\mu$ m.

The central cylinder has average thickness of 810  $\mu$ m. On the outside is the unilayer pericycle, whose cells alternate with the endodermis cells.

*The conducting tissue* consists of 10 to 11 fascicles of secondary and primary tissue, separated by medullary rays.

The primary and secondary liber (Figure 4) are arranged in packets separated by medullary rays two and three series of cells. Diameter the liberian vessels:  $V_M = 11,25 \mu m$ ;

$$V_{\rm m} = 6,75 \ \mu {\rm m}; \ \mathcal{X} = 8,325 \ \mu {\rm m}.$$



Figure 1. Transversal section through the rhizome: the epidermis by exfoliating; the crust itself; the endodermis; the suber; the secondary phloem; the secondry xylem; the medullary parenchyma; adventitious root zone (Oc. 10x, Ob. 10, Original)



Figure 2. Transversal section through the flowering stem: the epidermis; the angular collenchyma; the crust itself; the

endodermis; the secondary phloem fascicles; the secondary xylem fascicles; the medullary parenchyma (Oc. 10x, Ob.

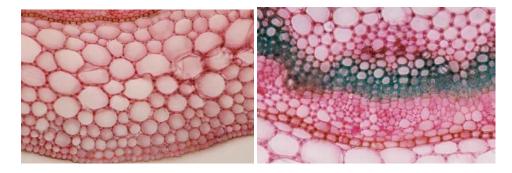


Figure 3. Detail the structure of the flowering stem at *Alchemilla glaucescens*: the epidermis; the angular collenchyma; the crust itself; the endodermis; the pericycle (Oc. 10x, Ob. 20, Original)

Figure 4. Detail the structure of the flowering stem at *Alchemilla glaucescens*: the endodermis; the pericycle; the secondary phloem; the secondary xylem; medullary rays; the medullary parenchyma (Oc. 10x, Ob. 40, Original) The secondary wood is composed of relatively narrow vessels with a circular shape, arranged in radial rows, each with medullary rays. Between vessels one can notice wood fibres. Diameter the wood vessels decrease in centrifugal sense:  $V_M = 15,75 \ \mu\text{m}$ ;  $V_m = 5,75 \ \mu\text{m}$ ;  $V_m = 12,275 \ \mu\text{m}$ ;  $V_m = 12,275$ 

= 5,75  $\mu$ m;  $\chi$  = 12,375  $\mu$ m.

Under the secondary wood is fundamental parenchyma, in which are arranged, from place to place the vessels which have belonged of primary wood.

*The medullary parenchyma* is a fundamental parenchyma, composed of polygonal cells.

#### CONCLUSIONS

The underground stem presents a secondary structure evident in the central cylinder.

The flowering stem in a transversal section presents a circular shape. The secondary structure is highlighted only in the central cylinder.

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# EVALUATION OF CULTIVARS AND PLANT SELECTIONS WITH BIOENERGETIC POTENTIAL FOR THE PROMOTION OF SHORT ROTATION COPPICE (SRC) TYPE CULTIVATION

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**KEY WORDS**: willow, poplar, energetic crops, short rotation coppice

#### ABSTRACT

Energetic crops with high yields of biomass grown as Short Rotation Coppice (SRC) are extending more and more around the world due to their role in the production of clean energy. University of Craiova through SCDP Valcea and in collaboration with CET Govora has tried for SRC cultivation a number of 19 selected genotypes from 5 genera of woody plants originating from different countries (Sweden, Italy and Romania). Out of the 19 genotypes 12 selections were identified in Romania (8 of willow, 2 of Myrobalan, one of common walnut and one of 'Paulownia 1'). At a density of 16,200 plants per ha in the second year from planting the highest annual growth, number of shoots per plant and highest wood volumes were recorded in the case of: 'Paulownia 1', 'Sacrau', 'Sven', 'Torra' and 'Inger'. Out of the Romanian selections, high growths were recorded for 'Cozia casanta' and 'Robesti 1', but these had lower production potential then the Swedish willow cultivars. The Romanian selections of Salix were identified in the local populations of natural hybrids, which have different phenotypes and have importance for their resistance to diseases and high degree of adaptability to the ecological conditions of the area and will be used for improvement programs. Due to these facts the selections can be used in the breeding programs. The selection 'Paulownia 1' has had the highest growth and volume of wood but the calculations were made with a density of 16,200 plants per ha, too large for this kind of plant with leafs of over 25 cm in diameter. The study will continue in the following years to determine the biomass production capacity for each genotype in full production.

#### INTRODUCTION

Short Rotation Coppice (SRC) is a coppice built up by different species grown as energy crops with duration of 20-30 years and which have as objective the production of large quantities of biomass per surface unit, which is used for the production of clean energy (Anonymous, 2004; Aylot et al., 2008).

These types of cultivations (SRC) require specific technologies and are not classified as either forest-related or agriculture-related cultivations (Danalatos, 2008; Lindegaard et al., 1996, 2001).

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This cultivation is employed in a number of countries (Sweden, Poland, England, Denmark, Italy, USA etc.) with the purpose of reducing carbon emissions by burning biomass in the place of fossil fuels (Tabbush and Parfitt, 1999).

The Kyoto Protocol mentions the reduction of gas emissions by 12.5% by 2010 and the transformation into electric energy of up to 10% of the total consumption.

The first research programs regarding selection and breeding of the most valuable plants for biomass production were launched in 1987 in Sweden, followed by England in 1991 (Cardias Williams and Thomas, 2006). Afterwards, many more countries have launched similar programs (Heinsoo, 2002).

At SCDP Valcea and CET Govora in 2009 a research program has been launched in order to evaluate foreign cultivars from different species and to select and/or bred new genotypes for the production of clean bioenergy using the resulting biomass (35-70 tons per ha per year).

#### MATERIALS AND METHODS

A trial has been set up in 2009 in the North of Oltenia region, at SCDP Valcea, which belongs to University of Craiova.

The biologic material used consists in different genera, species and clonal selections from Romania and from abroad.

The trial is composed of a number of 19 genotypes (Table 1); each genotype is represented by 50-100 plants. Planting distances are 1.5 m by 0.7 m or 0.6 m, the resulting density being approximately 16,000 plants per ha. It was established through planting of 20 cm long hardwood cuttings. After the first year the plants have been cut back at 10cm from ground level.

The soil was maintained clean of weeds by using herbicides and by mechanical tillage between the rows.

Fertilization has been done with 80 kg/ha nitrogen. The control of diseases and pests has been done through 5 phytosanitary sprayings.

# **RESULTS AND DISCUSSIONS**

The study has been carried out on a biologic material with various genetic and geographical origins.

The genetic origin is diverse and therefore 13 genotypes belong to the *Salix* genus, two genotypes to *Populus*, two belong to *Prunus*, one to *'Paulownia 1'* and one to *Juglans* genus. Nine of the genotypes used are the result of controlled crosses; the ten remaining ones were selected from natural hybrid populations. By geographical origin, a number of 5 genotypes come from Sweden, 2 from Italy and 12 from Romania (Table 1).

A number of 8 genotypes are named, registered or patented as cultivars; the remaining 11 ones are clonal selections.

The genotypes propagated through hardwood cuttings behave different in the next season after planting  $(2^{nd} \text{ leaf})$ , with the exception of 'J. regia 1' which propagated through seeds (Table 2). The average length of the annual growth has oscillated between 1.40 m ('Ladesti 1') and 3.74 m ('Paulownia 1'). The longest annual growths have been recorded for 'Paulownia 1' (3.74 m), 'Sacrau' (3.25 m), 'Sven' (2.95 m), 'Tordis' (2.94 m), 'Jorr' (2.87 m), 'Torra' (2.70 m), 'Inger' (2.60 m). The foreign cultivars of *Salix* and *Populus* surpass the average growths of all the Romanian selections. Out of the Romanian selections the most notable in growth length were: 'Cozia casanta' (2.0 m), 'Robesti 1' (1.97 m), 'Cozia 2' (1.90 m) and 'Cozia 1' (1.75 m).

The coefficients of variation for the average growth in length have values from 2.7% ('Sven') to 28.2% (H 17-18-91 Mir.). With the exception of H17-18-91 Mir. genotype and 'Torra', which have high variability coefficients, 9 genotypes have medium coefficients of variation and 8 ones low coefficients of variation; this shows uniformity of annual growth length of the plants.

The average number of annual growths on the plant is very different from one genotype to another and it decisively influences the volume, quantity and the quality of the biomass. The number of annual growths oscillates between 2.8 shoots per plant ('J. regia 1') and 22.5 shoots per plant ('Cozia 3'). In general, the number of annual growths is in a negative relationship with the length and width of annual growth. The lowest number of annual growths per plant was recorded for 'J. regia 1' (2.8 pcs.), 'Paulownia 1' (3.2 pcs.) and I 214 (3.8 pcs.).

The largest number of annual growths was observed for 'Cozia 3' (22.5 pcs.) and 'Cozia 2' (14.5 pcs.). The majority of the genotypes have 6.3 to 9.0 shoots per plant.

The average length of the annual growths and their number give the average sum of the annual growths per plant. This is between 4.3 m ('J. regia 1') and 37.1 m ('Cozia 3'). The average sum of the annual growths is high for 'Cozia 3' (37.1 m), 'Cozia 2' (27.6 m), 'Sven' (23.0 m), 'Inger' (22.1 m), 'Torra' (20.5 m) and 'Tordis' (18.8 m).

The studied genotypes present different daytime growth speed or vegetation period of the shoots. The daytime growth speed is between 1.02 cm/24h ('Ladesti 1') and 2.73 cm/24h ('Paulownia 1'). High growth speeds of over 2cm/day have been recorded for 'Paulownia 1', 'Sacrau', 'Tordis', 'Sven' and 'Jorr' (Table 3). Out of the Romanian genotypes the most significant from this point of vue were: 'Cozia casanta' (1.46 cm/day) and 'Robesti 1' (1.44 cm/day). The growth speed of the shoots on the plant is between 3.1 cm/24h ('J. regia 1') and 27.0 cm/24h ('Cozia 3'). The sum of the annual growths in 24 hours per ha is between 0.51 thousand m/24h/ha ('J. regia 1') and 4.38 thousand m/24h/ha, ('Cozia 3'). High growth speeds are also recorded for 'Cozia 2' (3.26 thousand m/24h/ha), 'Sven' (2.72 thousand m/24h/ha), 'Torra' (2.42 thousand m/24h/ha) etc.

The proportion between the daytime growth speed of the shoots and the growth speed for the whole plant depends on the length and the number of shoots per plant and is between 0.04 ('Cozia 3') and 0.36 ('J. regia 1').

Establishing the volume of wood produced by the plants in the second year is also dependent on the cross sectional area of annual growth (Table 4).

The trunk cross sectional area (TCSA) for the studied genotypes for the annual growths is between 0.42 cm<sup>2</sup> ('Cozia 3') and 3.40 cm<sup>2</sup> ('Sacrau'). The genotype 'Paulownia 1' presents average TCSA of 20.58 cm<sup>2</sup>, much larger than the other studied genotypes and this makes it an exception.

According to the TCSA and the average length of the annual growths it was determined the volume of wood per plant and the volume of wood per ha. This has oscillated between  $2.54 \text{ m}^3$ /ha ('Ladesti 1') and  $133 \text{ m}^3$ /ha ('Paulownia 1').

With the exception of genotype 'Paulownia 1' (very high growth) the other genotypes with high wood volume were: 'Sacrau' (53.70 m<sup>3</sup>/ha), 'Sven' (32.09 m<sup>3</sup>/ha), 'Torra' and 'Inger' (25.92 m<sup>3</sup>/ha), 'Cozia casanta' (16.70 m<sup>3</sup>/ha) and 'Tordis' (16.2 m<sup>3</sup>/ha).

Compared with control ('Sven'), only 'Paulownia 1' (414.4%) and 'Sacrau' (167%) managed to surpass it in wood biomass and 'Torra' and 'Inger' came close (80.8%).

The very large volume of biomass for 'Paulownia 1' brings a series of question marks for the future because this volume was determined for a density of 16,200 plants per ha and this density is too big considering the development of the foliage of the plant.

High growth of the plants in the  $2^{nd}$  leaf after planting in the context of 750 mm of annual rainfall, determines a high volume of biomass for the genotypes: 'Paulownia 1', 'Sacrau', 'Sven', 'Torra' and 'Inger'. The Romanian selections show a much lower growth and volume, with the exception of 'Cozia casanta' (16.7 m<sup>3</sup>/ha) and 'Robesti 1' (13.1 m<sup>3</sup>/ha).

The differences between the Swedish willow cultivars and the Romanian ones are not only in terms of volume of biomass but also in terms of growth type. The cultivars from *Salix* genus present vertical annual growths with very few anticipated shoots. The Romanian selections have a wide range of shoots, some with oblique position with a tendency of going vertical ('Cozia casanta', 'Robesti 1', 'Gibesti 1'), others with close to horizontal position ('Cozia 3') with a high number of anticipated bines etc.

The importance of the studied Romanian selection and of others which will be introduced in contest-ready comparative cultivations lies mainly in their use in improvement programs and for some even in their use in biomass cultivations. Their value is the high resistance to rust (*Melampsora* ssp.) and the high degree of adaptability to the ecological conditions from Romania.

# CONCLUSIONS

- 1. The cultivation of energetic crops of Short Rotation Coppice type in Romania offers economic and ecological perspectives regarding the reduction of carbon emissions.
- 2. Nineteen genotypes of various origins were planted and studied in a trial for biomass al SCDP Valcea.
- 3. The annual growths in the 2<sup>nd</sup> year after planting form the biomass yield. The longest annual growths have been recorded for 'Paulownia 1' (3.74 m), 'Sacrau' (3.25m), 'Sven' (2.95m), 'Tordis' (2.94 m), 'Jorr' (2.87m), 'Torra' (2.70m) and 'Inger' (2.60 m). The Romanian selections present lower growths but the best out of these are: 'Cozia casanta' (2.0 m), 'Robesti 1' (1.97 m), 'Cozia 2' (1.90 m) etc.
- 4. The number of annual growths per plant oscillates between 2.8 pcs. ('J. regia 1') and 22.5 pcs. per plant ('Cozia 3') and the majority of the genotypes have between 6.3 and 9 pcs. per plant.
- 5. The daytime growth speed of the shoots varied from 1.02 cm/24h ('Ladesti 1') to 2.73 cm/24h ('Paulownia 1') and the daily growth speed per ha is between 0.51 thousand m/24h/ha ('J. regia 1') and 4.38 thousand m/24h/ha ('Cozia 3').
- 6. The most important indicator along with the mass of wood is the volume achieved by each genotype. The genotype with the largest wood volume is 'Paulownia 1' (133m<sup>3</sup>/ha). Other notable volumes are: 'Sacrau' (53.70 m<sup>3</sup>/ha), 'Sven' (32.09 m<sup>3</sup>/ha), 'Torra' and 'Inger' (25.92m<sup>3</sup>/ha).
- 7. The Romanian genotypes differ from the foreign ones both in terms of growth type and in terms of lower wood volume ('Cozia casanta' with 16.73m<sup>3</sup>/ha and 'Robesti 1' with 13.1m<sup>3</sup>/ha). However, these genotypes are important for their resistance to diseases and for their high adaptability to the environmental conditions and can be used in the future breeding programs as genitors for resistance/tolerance and adaptability.

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Table 1

No.	Cultivar or selection	Genetic origin	Geographical origin	Type of plant
1	'Tordis'	(S. schwerinus x S. viminalis) x S. viminalis	Sweden	cultivar
2	'Torra'	S. schwerinus x S. viminalis	Sweden	cultivar
3	'Inger'	S. triandra x S. viminalis	Sweden	cultivar
4	'Jorr'	S. viminalis	Sweden	cultivar
5	'Ladesti 1'	Salix spp. (natural hybrid)	Romania	clonal selection
6	'Ladesti 2'	Salix spp. (natural hybrid)	Romania	clonal selection
7	'Cozia 1'	Salix spp. (natural hybrid)	Romania	clonal selection
8	'Cozia 3'	Salix spp. (natural hybrid)	Romania	clonal selection
9	'Cozia 2'	Salix spp. (natural hybrid)	Romania	clonal selection
10	'Cozia casanta'	Salix spp. (natural hybrid)	Romania	clonal selection
11	'Sven'	S. viminalis x (S. viminalis x S. schwerinus)	Sweden	cultivar
12	'Robesti 1'	Salix spp. (natural hybrid)	Romania	clonal selection
13	'Gibesti 1'	Salix spp. (natural hybrid)	Romania	clonal selection
14	H 17-18-91 Mir.	P. cerasifera	Romania	clonal selection
15	'Miroval'	P. cerasifera	Romania	cultivar
16	ʻI 214'	Populus deltoids x P. nigra	Italy	cultivar
17	'Sacrau'	Populus deltoids x P. nigra	Italy	cultivar
18	'Paulownia 1'	Paulownia tomentosa	· · · · · · · · · · · · · · · · · · ·	
19	'J. regia 1'	Juglans regia	Romania	clonal selection

# Genetic and geographical origin of the biologic material

# Table 2

Annual growth capacity for the plants designed for producing biomass in the 2<sup>nd</sup> leaf after planting

No.	Cultivar or	Average	Standard	Coefficient	Average	Average sum
	selection	length of	deviation	of	number of	of annual
		annual	(s)	variation	annual	growths per
		growths		(s %)	growths per	plant
		(m)			plant	(m)
1	'Tordis'	2.94	0.45	13.6	6.4	18.8
2	'Torra'	2.70	0.64	23.7	7.6	20.5
3	'Inger'	2.60	0.18	6.9	8.5	22.1
4	'Jorr'	2.87	0.46	16.0	5.8	16.6
5	'Ladesti 1'	1.40	0.12	8.6	6.3	8.8

6	'Ladesti 2'	1.65	0.10	6.1	7.7	12.7
7	'Cozia 1'	1.75	0.10	5.7	8.8	15.4
8	'Cozia 3'	1.65	-	-	22.5	37.1
9	'Cozia 2'	1.90	-	-	14.5	27.6
10	'Cozia casanta'	2.00	0.37	18.5	7.0	14.0
11	'Sven'	2.95	0.08	2.7	7.8	23.0
12	'Robesti 1'	1.97	0.10	5.1	8.5	16.7
13	'Gibesti 1'	1.90	0.19	10.0	9.0	17.1
14	H 17-18-91	1.52	0.43	28.2	9.0	13.7
	Mir.					
15	'Miroval'	1.70	0.12	7.0	6.4	10.9
16	ʻI 214'	2.18	0.39	17.9	3.8	8.3
17	'Sacrau'	3.25	0.26	8.0	9.0	10.4
18	'Paulownia 1'	3.74	0.60	16.0	3.2	12.0
19	'J. regia 1'	1.54	0.19	12.3	2.8	4.3

Table 3 Daytime growth speed of the plants and total shoots production per ha in the 2<sup>nd</sup> leaf after planting

No.	Cultivar or selection	Sum of annual growths (thousand m/ha)	Daytime growth speed of the shoots (cm/24 h)	Daytime growth speed per plant (cm/24 h)	Daytime growth speed per year/ha (thousand m/ha)	Proportion of daytime growth speed of the bines and daytime growth speed of the plant
1	'Tordis'	304	2.14	13.7	2.22	0.15
2	'Torra'	332	1.97	14.9	2.42	0.13
3	'Inger'	358	1.90	16.1	2.61	0.12
4	'Jorr'	269	2.09	12.1	1.96	0.17
5	'Ladesti 1'	142	1.02	6.4	1.04	0.16
6	'Ladesti 2'	206	1.20	9.2	1.50	0.13
7	'Cozia 1'	249	1.28	11.2	1.82	0.11
8	'Cozia 3'	601	1.20	27.0	4.38	0.04
9	'Cozia 2'	447	1.38	20.0	3.26	0.07
10	'Cozia casanta'	227	1.46	10.2	1.65	0.14
11	'Sven'	372	2.15	16.8	2.72	0.13
12	'Robesti 1'	270	1.44	12.2	1.97	0.12
13	'Gibesti 1'	277	1.38	12.4	2.02	0.11
14	H 17-18-91 Mir.	222	1.11	10.0	1.62	0.11
15	'Miroval'	176	1.24	7.9	1.29	0.15
16	ʻI 214'	134	1.59	6.0	0.98	0.26
17	'Sacrau'	168	2.37	21.3	1.23	0.11
18	'Paulownia 1'	194	2.73	8.7	1.42	0.31
19	'J. regia 1'	70	1.12	3.1	0.51	0.36

Table 4	4
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No.	Cultivar or	Surface of	Volume	Volume	Proportional	Classification
	selection	the section	of wood	of wood	percentage	by volume
		of bines at	per plant	per ha	compared	
		the base	(cm <sup>3</sup> /pl)	(m <sup>3</sup> /ha)	with control	
		$(cm^2)$			(%)	
1	'Tordis'	1.60	1003	16.20	50.5	7
2	'Torra'	2.37	1600	25.92	80.8	4
3	'Inger'	2.11	1600	25.92	80.8	5
4	'Jorr'	1.58	869	14.07	43.8	8
5	'Ladesti 1'	0.54	157	2.54	7.91	19
6	'Ladesti 2'	0.85	362	5.86	18.26	16
7	'Cozia 1'	0.59	301	4.87	15.2	17
8	'Cozia 3'	0.42	519	8.41	26.2	12
9	'Cozia 2'	0.63	580	9.40	29.3	11
10	'Cozia casanta'	2.21	1031	16.70	52.0	6
11	'Sven'	2.60	1981	32.09	100.0	3
12	'Robesti 1'	1.45	809	13.10	40.8	9
13	'Gibesti 1'	0.72	410	6.64	20.7	14
14	H 17-18-91 Mir.	0.59	269	4.36	13.6	18
15	'Miroval'	1.11	402	6.51	20.3	15
16	ʻI 214'	2.40	663	10.74	33.5	10
17	'Sacrau'	3.40	3315	53.70	167.3	2
18	'Paulownia 1'	20.58	8210	133.00	414.4	1
19	'J. regia 1'	3.23	464	7.52	23.4	13

Volume of wood per ha in year II from plantation for the energetic plants

#### UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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# EVALUATION OF WALNUT CULTIVARS IN THE CONDITIONS OF THE OLTENIA'S HILL AREA REGARDING FUNCTIONING POTENTIAL

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KEY WORDS: walnut, bearing, yield, cultivars, fruit trees

#### ABSTRACT

Modern growing of walnut in Romania is both a requirement and an objective necessity due to the high value of the fruits, the possibilities for commercialization and the historic tradition of cultivation. The main issue in modernizing walnut cultivation is providing a valuable assortment of cultivars, highly competitive on the international markets. In the sub-Carpathian area of Oltenia, which is one of the most favorable for walnut cultivation, the vielding potential was evaluated for 29 cultivars. The geographic origin of the cultivars is diverse (12 Romanian cultivars, 10 from the USA, 5 from France, one from Germany and one from Bulgaria). There are differences between cultivars regarding the fructification type (19 are terminal bearers and 10 are with lateral bearing) and the flowering dichogamy type (10 are protogynous and 19 are protandrous). The average fruit yield for all the cultivars during a period of 11-15 years from planting was 1.66 tons per hectare. The most productive cultivars were: Ferjean (2.4 t/ha), Vina (2.26 t/ha), Hartley (2.24 t/ha), Fernor (1.94 t/ha) and Lara (1.88 t/ha), all belonging to the lateral bearing cultivars group. Out of the cultivars with terminal bearing, the most productive were Valcor (1.86 t/ha), Jupanesti (1.82 t/ha), Velnita (1.74 t/ha) and Franquette (1.70 t/ha). Higher fruit yields for the cultivars with lateral bearing are due both to the genotypes and the favorable climatic conditions in the 2005-2009 period (minimum temperatures up to -20°C and no frosts at flowering time). In the conditions of the sub-Carpathian area of Oltenia walnut production is determined by the genotype (54.1%), the environment (8.9%), the interaction GxE (23.2%) and unknown factors (13.8%).

#### **INTRODUCTION**

The walnut has been cultivated in Romania since long time. The inhabitants of Dacia (nowadays Romania) knew about this crop and used the fruits of the walnut as a source of food.

The Latin poet Ovidius, exiled 200 years ago in Tomis (actual Constanta), wrote about the walnut that "it is not pretentious, it grows on the side of the road and does not fear nor the wind, nor the rain, nor the heat or the cold" (Bordeaianu, 1963 cited by Cociu et al., 2008).

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Starting with the XIVth century, the walnut appears in numerous historical documents but also in the names of several settlements (Nucet, Nucul, Valea Nucarilor, etc.) (Cociu et al, 2008).

Walnut cultivation has become during time very important in Romania for the fruit-growing area because of the multiple advantages that walnut products offer (fruits, wood, leafs etc.). With its 2,000 hectares of walnut orchards and over 5 million walnut trees on own roots, Romania has achieved in the last decade a production of 32 to 34 thousand tons of walnuts. This places it amongst the first 7 countries in the world regarding walnut production.

The favorable ecological conditions for walnut cultivation, especially in the Oltenia region, the historic tradition and the nutritional and economic interests create new perspectives for walnut cultivation in the following years (Botu et al., 2010).

The general tendency is to grow the surfaces cultivated with walnut and to modernize the growing techniques in order to get higher yields, better quality and competitivity in the worldwide trade. At present the world production of walnuts of 1,724,172 tons in 2008 (FAO Stat Database, 2010) is not sufficient to cover the demand.

For our country the main issue in the modernization of walnut growing is still the availability of a valuable assortment of cultivars. To this end, the aim is to evaluate in this paper a wide variety of walnut cultivars, with different geographic origin, regarding their yielding potential.

#### MATERIALS AND METHODS

The research has been conducted between 2004 and 2009 in a trial located at SCDP Valcea which belongs to the University of Craiova. The walnut trees in the trial have 11 and 15 years-old.

The biologic material is composed of 29 walnut cultivars (12 from Romania, 10 from the USA, 5 from France and 1 each from Germany and Bulgaria).

The cultivars are grafted on *Juglans regia* seedlings. Each cultivar is represented by 5-10 trees. Planting distance is  $9.0 \times 8.0 \text{ m}$  (139 trees per hectare).

Trees were trained as mixed pyramid. The soil between trees was sowed with grass. Fertilization has been done annually with NPK (100 kg of N, 20 kg of P and 20 kg of K per hectare). 6 to 7 phytosanitary treatments have been applied per year.

The research methods have been in relationship with the purpose of the work and included complex observations and determinations regarding trees' habit, growth and fruit-yielding phases etc.

Special interest was allocated for:

- establishing fruit yield of the cultivars;
- determining the type of fruit ripening;
- determining the influence of the genotype, environment and the interaction between genotype x environment on the fruit yield (using Two Way ANOVA with repetitions, according to Sohal and Rohlf, 1995).

The data collected has been analyzed biostatistically using:

- mean;
- standard deviation;
- amplitude and the variation coefficients;
- analysis of variance.

During the study period (2005-2009) the average annual temperature was  $11.3^{\circ}$ C (1.1 °C more than the multi-annual average) and the absolute minimum temperature was -

22.7 °C (2006). The absolute minimum temperatures and the lack of spring frosts have not caused damages to the catkins and female walnut flowers. The annual average of rainfall was 771.5 mm (59.5 mm more than the multi-annual average).

#### RESULTS

The walnut cultivars of different genetic and geographic origin studied in the North of Oltenia emphasized different behavior regarding growth and fruit ripening (Table 1).

In the 15<sup>th</sup> leaf a number of 25 cultivars showed high growth vigor, 2 cultivars emphasized very high vigor ('Argesan' and 'Geoagiu 65') and 2 cultivars show medium growth vigor ('Germisara' and 'Orastie').

The dichogamy of the Romanian walnut cultivars is of protogynous type (for 9 cultivars) and protandrous type (3 cultivars).

The foreign cultivars are mostly protandrous-type with the exception of 'Idaho' cultivar which is protogynous.

Flowering of the walnut cultivars takes place between 4<sup>th</sup> of April and 21<sup>st</sup> of May in the 5 year study period. During this period no late frosts were recorded. Between the lateral bearing cultivars and the terminal bearing ones there are no more than 4 to 10 days of flowering timing differences.

The walnut cultivars analyzed have two types of fruit bearing:

- terminal (19 cultivars out of which 12 are Romanian). The Romanian cultivar 'Germisara' has intermediary fructification but mostly of terminal type;
- lateral (10 cultivars 6 are from U.S.A. and 4 are French cultivars).

'Hartley' is considered to have intermediary bearing but most of its yield comes from lateral fructification buds.

Fruit yields of the walnut cultivars in the 11<sup>th</sup> to the 15<sup>th</sup> leaf is different (Table 2). At this age, the average yield for all the cultivars is 1.66 tons per hectare. From the 11<sup>th</sup> leaf till the 15<sup>th</sup> one the walnut yields is increasing for all the

From the  $11^{\text{th}}$  leaf till the  $15^{\text{th}}$  one the walnut yields is increasing for all the cultivars and is correlated with the growth of the trees. At this age the walnuts are covering the orchard area from 30.6% to 58.1% (in the case of 'Muscelean') of the total area. The yield increases as well when the foliage grows.

The highest yields have been recorded for the following cultivars: 'Ferjean' (2.4 tons per hectare), 'Vina' (2.26 t/ha), 'Hartley' (2.24 t/ha), 'Fernor' (1.94 t/ha), 'Lara' (1.88 t/ha) and 'Valcor' (1.86 t/ha), significantly comparable with control cultivar which was 'Franquette' (1.7 t/ha). Statistically distinctly significant yield was recorded in the case of 'Fernette' (1.84 t/ha) and significant for: 'Jupanesti', 'Payne' and 'Pedro' (1.82 t/ha).

Significantly negative yields have been recorded for: 'Uzlop 10' (1.18 t/ha), 'Howe' (1.06 t/ha), 'Adams 10' (1.24 t/ha), 'Muscelean'(1.48 t/ha), etc.

The cultivars with lateral bearing have had higher yields than the cultivar used as control ('Franquette').

Out of the cultivars with terminal bearing high yield potential has been recorded for 'Valcor', 'Jupanesti' and 'Velnita'. In the year 2009 it has been noticed that the cultivars with lateral bearing habits have much higher yields than in previous years (2.73 t/ha in 2009; 2.32 t/ha in 2008 and 1.87 t/ha in 2007). The same effect was noticed for the cultivars with terminal bearing but the differences are much lower (1.98 t/ha in 2009; 1.61 t/ha in 2008 and 1.22 t/ha in 2007).

The cultivars with lateral bearing were exposed during the 5 years of fructification to minimum temperatures of up to -22.7 °C and during flowering time they have not been affected by temperatures lower than  $-1^{\circ}$ C.

These cultivars are extremely sensitive to bacterial blight (*Xanthomonas campestris* pv. *juglandis*) and to low temperatures during flowering time. These are the reasons for which they should be grown in the future in the Oltenia region with extreme care.

It was observed also that for the walnut cultivars the variability of the yield is specific (Table 3).

For several cultivars the confidence interval (CI) and the coefficient of variation (CV) are high showing high variability ('Valcor', 'Muscelean', 'Geisenheim 139', 'Howe', 'Ferjean', 'Hartley', etc.). This phenomenon is mostly due to the fact that the cultivars have higher yield each year. Few cultivars have medium variability ('Sarmis', 'Valrex', 'Jupanesti', 'Orastie' and 'Franquette').

The walnut yields in the region of Oltenia hills depends on multiple factors (Table 4). Calculating the estimated variance  $(s^2)$  this shows the influence of the genotype (cultivar), environment (years) and the interaction GxE and the error. The F test indicates estimated value of the variance of these factors which are greatly higher than the variance of the error.

For determining the expected variance  $\sigma^2$  we have used the formulas adopted by Sokal and Rohlf (1995) as follows:

$$\sigma^{2} \operatorname{error} = s^{2} \operatorname{error} = 0.25$$
  

$$\sigma^{2}_{GxE} = n \ s^{2} \ G \ x \ E + \sigma^{2} \ C = \underbrace{S_{GxE}^{2} - \rho^{2}}_{n} = 0.42$$
  

$$\sigma^{2} E \ (\text{years}) = gs^{2} E + \sigma^{2} \ C + \sigma^{2}_{GxE} = \underbrace{S_{E}^{2} - \rho^{2} - \rho_{GxE}^{2}}_{g} = 0.16$$
  

$$\sigma^{2} G \ (\text{cultivar}) = Es^{2} + \sigma^{2} \ C + \sigma^{2} \ E = \underbrace{S_{G}^{2} - \rho^{2} - \rho_{E}^{2}}_{m} = 0.98$$

Where:

- n = pairs of years (2)

g = genotype or cultivars (29)

- m = years or environment (5)

From summing up the expected variances  $[\sigma^2 T = 1.81 \text{ and } \sigma^2 T = V_T (\text{phenotype})]$  $VT = VG + VE + V_{GxE} + V_{\varepsilon} = \sigma^2 G + \sigma^2 E + \sigma^2_{GxE} + \sigma^2_{\varepsilon}.$ 

In table 2 we calculated the quantitative and percentage yield at the level of the average yield for all the 29 cultivars in the 5 years of the study.

From these determination results the fact that the genotype of the walnut cultivated in Northern Oltenia is responsible for 54.1% of the total fruit yield while the environment influences by 8.9%, the interaction G x E by 23.2% and the rest of 13.8% is due to other factors or unknown errors.

By generalizing, each cultivar of walnut shows a production which is determined by the genotype, the environment and the interaction GxE.

The quantitative value of the yield determined by the genotype varies between 0.573 tons per hectare ('Howe') and 1.298 t/ha ('Ferjean') and the one determined by the environment varies between 0.094 t/ha ('Howe') and 0.214 t/ha ('Ferjean').

The interaction GxE with a value of 23.2% determines a quantitative value of 0.246 to 0.577 tons per hectare and represents the measure to which a cultivar responds positively to the ecological conditions of an area. In this case however, the high year-to-year yield also plays a role.

In the case of the analysis of these factors other influences were also recorded. These are known as errors and in this case they vary between 0.163 t/ha ('Geisenheim 139' and 'Uzlop 10') and 0.331 t/ha ('Ferjean'). The genotype is the main factor, followed by GxE.

The fruit yield for walnuts is dependent on quality as the cultivars are affected by pests and diseases.

# CONCLUSIONS

- Walnut cultivars with different genetic and geographic origin show in the sub-Carpathian area of Oltenia a differentiated behavior in the bearing process.
- The studied cultivars were grouped as follows:
  - a) by origin: 12 are Romanian cultivars and 17 are foreign cultivars;
  - b) by type of fructification: 19 are terminal bearers and 10 have lateral bearing;
  - c) by type of dichogamy: 10 are of protogynous type and 19 are protandrous.
- The average walnut yield for the 29 cultivars at ages between 11 and 15 years after planting was 1.66 tons per hectare.
- The most productive cultivars were: 'Ferjean' (2.4 tons per hectare), 'Vina' (2.26 t/ha), 'Hartley' (2.24 t/ha), 'Fernor' (1.94 t/ha), 'Lara' (1.88 t/ha) and 'Valcor' (1.86 t/ha).
- Fruit yields of cultivars with lateral bearing in climatic conditions lacking lo temperatures which could affect the trees has been superior to the average yield of terminal bearing cultivars (average of 1.97 t/ha compared with 1.49 t/ha).
- The yield has shown high or very high variance (s%= 20-43.4%) for the majority of the cultivars in the 5 years of the study; this was influenced both by the cultivar and also by the increasing yields each year.
- In the conditions of Northern Oltenia the walnut yield is determined by the genotype (54.1%), environment (8.9%), interaction Genotype x Environment (23.2%) and unknown factors (13.8%).

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No.	Cultivar	Origin	Plant vigor	Type of dichogamy	Type of bearing
1	'Sarmis'	Romania	high	protogynous	terminal
2	'Sibişel 44'	Romania	high	protogynous	terminal
3	'Valcor'	Romania	high	protogynous	terminal
4	'Valmit'	Romania	high	protogynous	terminal
5	'Valrex'	Romania	high	protogynous	terminal
6	'Jupânești'	Romania	high	protogynous	terminal
7	'Argeşean'	Romania	very high	protogynous	terminal
8	'Geoagiu 65'	Romania	very high	protogynous	terminal
9	'Germisara'	Romania	medium	protogynous	intermediate
10	'Muscelean'	Romania	high	protandrous	terminal
11	'Orăștie'	Romania	medium	protogynous	terminal
12	'Velnița'	Romania	medium	protogynous	terminal
13	'Adams 10'	USA	high	protandrous	terminal
14	'Chase D9'	USA	high	protandrous	terminal
15	'Geisenheim 139'	Germany	high	protandrous	terminal
16	'Franquette' (control)	France	high	protandrous	terminal
17	'Howe'	USA	high	protandrous	terminal
18	'Idaho'	USA	high	protogynous	terminal
19	'Uzlop 10'	Bulgaria	high	protandrous	terminal
20	'Ferjean'	France	high	protandrous	lateral
21	'Fernette'	France	high	protandrous	lateral
22	'Fernor'	France	high	protandrous	lateral
23	'Hartley'	USA	high	protandrous	intermediate
24	'Lara'	France	high	protandrous	lateral
25	'Payne'	USA	high	protandrous	lateral
26	'Serr'	USA	high	protandrous	lateral
27	'Tehama'	USA	high	protandrous	lateral
28	'Pedro'	USA	high	protandrous	lateral
29	'Vina'	USA	high	protandrous	lateral

Vigor, type of flowering and bearing of several walnut cultivars in Northern Oltenia

Table 2

			Fruit	t yield (	(t/ha)		Average	Diff.	
No.	Cultivar	2005	2006	2007	2008	2009	yield	±	Significance
							(t/ha)		
1	'Sarmis'	1.4	1.6	1.6	1.6	2.0	1.64	-0.060	ns
2	'Sibişel 44'	1.2	1.3	1.5	1.5	2.0	1.50	-0.200	000
3	'Valcor'	1.4	1.8	2.1	1.7	2.3	1.86	+0.160	***
4	'Valmit'	1.2	1.3	1.7	1.7	2.2	1.62	-0.080	ns
5	'Valrex'	1.3	1.3	1.5	1.6	2.1	1.56	-0.140	00
6	'Jupânești'	1.4	1.6	1.8	2.1	2.2	1.82	+0.120	*
7	'Argeşean'	1.2	1.3	1.5	1.6	2.0	1.52	-0.180	000
8	'Geoagiu 65'	0.8	1.2	1.4	1.4	1.9	1.34	-0.360	000
9	'Germisara'	1.0	1.4	1.8	1.7	2.1	1.60	-0.100	0
10	'Muscelean'	1.0	1.3	1.4	1.5	2.2	1.48	-0.220	000
11	'Orăștie'	1.2	1.6	1.7	2.1	1.7	1.66	-0.040	ns
12	'Velnița'	1.4	1.6	1.9	1.7	2.1	1.74	+0.040	ns
13	'Adams 10'	0.7	1.0	1.3	1.5	1.7	1.24	-0.460	000
14	'Chase D9'	1.0	1.4	1.5	1.6	1.9	1.48	-0.220	000
15	'Geisenheim 139'	0.5	1.2	1.0	1.3	1.9	1.18	-0.520	000
16	'Franquette' (control)	1.2	1.6	1.8	1.9	2.0	1.70	-	-
17	'Howe'	0.5	0.8	1.0	1.3	1.7	1.06	-0.640	000
18	'Idaho'	0.8	1.1	1.3	1.5	1.9	1.32	-0.380	000
19	'Uzlop 10'	0.6	0.9	1.2	1.4	1.8	1.18	-0.520	000
20	'Ferjean'	1.4	1.8	2.3	3.4	3.1	2.40	+0.700	***
21	'Fernette'	1.2	1.7	1.8	2.0	2.5	1.84	+0.140	**
22	'Fernor'	1.3	1.6	1.8	2.3	2.7	1.94	+0.240	***
23	'Hartley'	1.4	1.7	2.1	2.7	3.3	2.24	+0.540	***
24	'Lara'	1.3	1.7	2.0	2.8	1.6	1.88	+0.180	***
25	'Payne'	1.2	1.5	1.7	2.0	2.7	1.82	+0.120	*
26	'Serr'	1.3	1.5	1.7	1.7	2.6	1.76	+0.060	ns
27	'Tehama'	1.2	1.4	1.6	1.8	2.7	1.74	+0.040	ns
28	'Pedro'	1.2	1.8	1.7	1.8	2.6	1.82	+0.120	*
29	'Vina'	1.4	1.7	2.0	2.7	3.5	2.26	+0.560	***
	Mean	1.13	1.44	1.64	1.86	2.24	1.66	-	_

Walnut yields of cultivars aged between 11 and 15 years-old

LSD 5.0% = 0.098 LSD 1.0 % = 0.132 LSD 0.1 % = 0.155

Table 3

No.	Cultivar	Average yield (2005-2009) (t/ha)	Standard deviation (s)	Confidence interval (CI) (t/ha)	Coefficient of variation (CV) (s%)
1	'Sarmis'	1.64	0.22	1.42 - 1.86	13.4
2	'Sibişel 44'	1.50	0.30	1.20 - 1.80	20.0
3	'Valcor'	1.86	0.70	1.16 - 2.56	37.6
4	'Valmit'	1.62	0.40	1.22 - 2.02	24.7
5	'Valrex'	1.56	0.32	1.24 - 1.88	20.5
6	'Jupânești'	1.82	0.33	1.49 - 2.15	18.1
7	'Argeşean'	1.52	0.31	1.21 - 1.83	20.4
8	'Geoagiu 65'	1.34	0.39	0.95 - 1.73	29.1
9	'Germisara'	1.60	0.41	1.19 - 2.01	25.6
10	'Muscelean'	1.48	0.52	0.96 - 2.00	35.1
11	'Orăștie'	1.66	0.32	1.34 - 1.98	19.3
12	'Velnița'	1.74	0.27	1.47 - 2.01	15.5
13	'Adams 10'	1.24	0.39	0.85 - 1.63	31.4
14	'Chase D9'	1.48	0.33	1.15 - 1.81	22.3
15	'Geisenheim 139'	1.18	0.51	0.67 – 1.69	42.9
16	'Franquette' (control)	1.70	0.32	1.38 - 2.02	18.2
17	'Howe'	1.06	0.46	0.60 - 1.52	43.4
18	'Idaho'	1.32	0.41	0.91 - 1.73	31.1
19	'Uzlop 10'	1.18	0.46	0.72 - 1.64	39.0
20	'Ferjean'	2.40	0.84	1.56 - 3.24	35.0
21	'Fernette'	1.84	0.47	1.37 - 2.31	25.5
22	'Fernor'	1.94	0.56	1.38 - 2.50	28.8
23	'Hartley'	2.24	0.83	1.41 - 3.07	37.0
24	'Lara'	1.88	0.57	1.31 - 2.45	30.3
25	'Payne'	1.82	0.57	1.25 - 2.39	31.3
26	'Serr'	1.76	0.49	1.27 - 2.25	27.8
27	'Tehama'	1.74	0.58	1.16 - 2.32	33.3
28	'Pedro'	1.82	0.50	1.32 - 2.32	27.5
29	'Vina'	2.26	0.84	1.42 - 3.10	37.2
	Mean	1.66	0.42	1.24 - 2.08	25.3

Variance of walnut yield for some cultivars aged 11 to 15 years-old

Source of variation	Degree of SS freedom (Df)			Variance	Calculated average annual yield according to expected variance $(\sigma^2)$		
		(DI)	Estimated	F Test	Expected	Yield	Yield
			$s^2$		$\sigma^2$	(t/ha)	(%)
Genotype (cultivar)	177.42	28	6.33	25.32***	0.98	0.898	54.10
Environment (years)	21.44	4	5.36	21.44***	0.16	0.148	8.9
Genotype x Environment	170.59	112	1.52	6.08***	0.42	0.385	23.2
Error	72.01	144	0.25	-	0.25	0.229	13.8
TOTAL	441.46	288	-	-	1.81	1.660	100

Determination of the influence of the genotype and the environment on average yield with the help of variance analysis for the walnut cultivars

 $F_{G}\% (28/144) = 1.83$   $F_{E} 1\% (4/144) = 3.44$  $F_{GxE} 14\% (112/144) = 1.51$ 

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

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# THE EVALUATION OF BIOABSORPTION AND TRANSLOCATION FACTOR FOR P AND K AT SOME *LACTARIUS* SPECIES

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KEY WORDS: Bioabsorption, translocation, phosphorous, potassium, Lactarius

#### ABSTRACT

It were determinate the concentrations of chemical elements in some Lactarius species harvested from Dambovita forestry ecosystems. This paper is about Lactarius piperatus, Lactarius vellereus and Lactarius volemus, very common species in the forestry ecosystems from south of Romania. Biological samples were harvested from two forestry ecosystems of Dambovita county. Were determinate their content in biominerals as potassium and phosphorus by EDXRF method with ELVA-X spectrometer with fluorescence. The bioconversion and translocation factor was calculated for each case by mathematics equation. Also it was determinate the pH of macromycetes substrates being between 6,4 and 6,8 (soil). These researches put in evidence the possibility of using one or other species of Lactarius genus analyzed, as natural source for obtaining organic biominerals necessary for pharmaceutical or food industry biotechnologies.

#### **INTRODUCTION**

It is well known that there are very few vegetal sources for potassium especially so necessary to those who are health problems with heart and kidney diseases. On the other hand the organic form of biominerals is easier absorbed by organism and has no secondary effects that's why does it exist some studies concerning the chemical content of *Lactarius* genus species (Barros et al., 2007, 2008; Demirba, 2001). For example, Sesli et Tuzen (2006) find 22270-51455mg/kg potassium in fruiting body of some edible mushroom species as *Lactarius piperatus* and *Lactarius volemus* harvested from Artvin Province of Turkey. Demirba (2001) studied 18 mushrooms and among them *Lactarius piperatus* and *Lactarius sp.* species and find in their fruiting body 51 000mg/kg potassium. Another important biomineral for human nutrition is phosphorus. But the authors of this study cannot find references about phosphorus content of *Lactarius* genus. That's why it is important to propose new naturals sources rich in biominerals. Also in the literature studied cannot find information about bioabsorption and translocation factor for biominerals which could be important for biotechnologies area.

This paper is about some macromycetes species very common in forestry ecosystems of Dambovita County belonging to *Lactarius* genus. Generally they are edible in spite of their taste not to agreeable. So this paper goes to purpose as potassium and

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phosphorus natural source for pharmaceutical and food biotechnologies three *Lactarius* species, very common in Romania's forest.

#### MATERIAL AND METHODS

Biological samples consisted in three species of *Lactarius* genus, *L. piperatus*, *L. volemus* and *L. vellereus* harvested from two forests Mogoi and Bolboca area in Dambovita county. Were prelevated soil samples under the stipe of each exemplar. Mushrooms species where identifying using some guides very known in Europe, two of them published after 2000 (Bielli 2009; Jordan, 2004). Was determinate the pH of each soil samples.

Biological and soil samples were weighted and then dried at 60°C. After drying operation the samples were weighted again and grinded till become a very fine dusty. The elemental content of biological and environmental samples was determined using Elva-X spectrometer having a X-ray tube with Rh anode. The samples were excited for 300s and the characteristic X-rays were detected by a multichannel spectrometer based on a solid state Si-pin diode X-ray detector with a 140 mm Be window and a energy resolution of 200eV at 5.9 KeV 91,20. Elva-X software was used to interpret the EDXRF spectra (Arai, 2004; ASTM Standard D6247, 1998; Winerfordner, 1999). The accuracy and precision of results were evaluated by measuring a certified reference sample (NIST SRM 1571-Orchard biological samples) (EURACHEM/CITAC Guide, 2003). The results obtained by EDXRF method were exprimated in ppm. Every result represents the average of at least 2 and maximum 5 determinations. Bioabsorption factor for biominerals and their translocation factor find in cap and stipe of mushrooms was calculated after the following mathematics relations:

$$Bf\% = \frac{C_m}{C_s} \times 100$$

*were: Bf* % = bioabsorption factor;

Cm = metal concentration in mushroom;

Cs = metal content in substrate.

$$Tf = \frac{C_c}{C_s}$$

**were**: *Tf* = translocation factor;

 $C_c$  = metal concentration in cap;

 $C_s$  = metal concentration in stipe/soil.

Every result represents the average of at least 3 and maximum 6 determinations.

# **RESULTS AND DISCUSSIONS**

On can see in figure 1 that the concentrations of phosphorus were sensible higher in cap of mushrooms then the content of soil in all cases. Maximum was registered in *Lactarius vellereus* (1718,744 ppm) and minimum in *Lactarius piperatus*(558,753 ppm) harvested from Bolboca area. In case of stipe content of phosphorus the situation was different.

First the content of stipe was visible lower than the cap accumulation of phosphorus. Maximum content was in stipe of *Lactarius vellereus* (578,064 ppm) and

minimum was determinate in stipe of *Lactarius piperatus* (46,348 ppm) find in Bolboca area. *Lactarius piperatus* had very closed concentrations of phosphorus as well those in cap as those in stipe. The content of phosphorus in cap of *Lactarius volemus* was as value between that in cap of *Lactarius piperatus* and that in cap of *Lactarius vellereus*. Soil content of phosphorus was in trace.

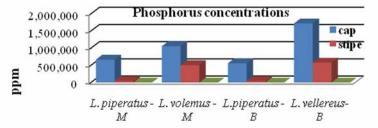


Figure 1. The content of phosphorous in Lactarius species

The best bioabsorption factor was calculated for cap in all cases with maximum value in cap of *Lactarius vellereus* (1719%) follow by *Lactarius volemus* cap (1057%) (Figure 2). The lowest bioabsorption factor had in all cases the stipe of *Lactarius piperatus* no matter the area of harvesting (46-47%).

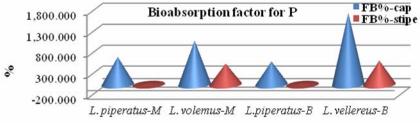


Figure 2. Bioabsorption factor for phosphorus in cap and stipe

The values of translocation rating of phosphorus from soil to stipe were very highest comparatively to that from stipe to cap in all samples, with some differences from case to case (Figure 3).

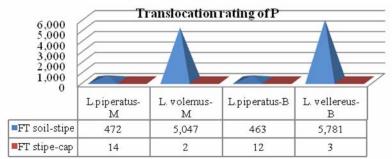


Figure 3. Translocation factor for phosphorus

But on can see a higher translocation factor between 12 and 14 at *Lactarius piperatus* from stipe to cap no matter of harvesting zone, comparatively to the others analyzed species of genus, which had higher content of phosphorous in cap.

In figure 4 on are representing the concentrations of potassium in the same biological and soil samples. Also the maximum content of potassium was registered in cap of *Lactarius vellereus* (34899,585 ppm) and minimum in cap of (22162,77 ppm) prelevated from Mogoi forest. But, in all caps of mushrooms analyzed the concentrations of potassium were higher, between 22162,77ppm and 23483,528ppm in case of *Lactarius* species harvested from Mogoi area and between 30,139,997ppm and 34899,585ppm in case of species prelevated from Bolboca forest.

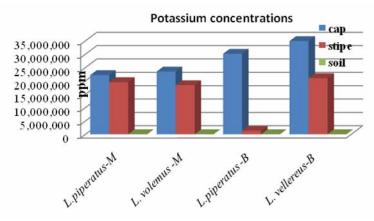


Figure 4. The content of potassium in Lactarius species

Bioabsorption factor for potassium in cap was very highest of ten thousand and million even in all cases calculated (Figure 5). Maximum which reached over one million had *Lactarius piperatus* from Bolboca area followed by *Lactarius volemus*. The bioabsorption factor of stipes inspite of great differences comparatively to those of caps was enough high in all cases but under one hundred percent.

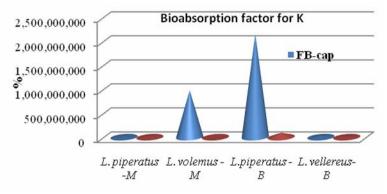


Figure 5. Bioabsorption factor for potassium in cap and stipe

The situation of translocation factor for potassium from soil to stipe is similar with that of phosphorous, much higher than that of translocation from stipe into cap (Figure 6).

On can relieve that the translocation factor for potassium from stipe to cap is double in case of *Lactarius* species harvested from Bolboca zone.

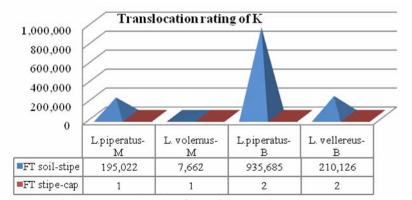


Figure 6. Translocation factor for potassium

Comparatively to some abroad studies (Demirba, 2001; Sesli and Tuzen, 2006) concerning the content of potassium this study come to prove once again that the species belonging to *Lactarius* genus are hyper accumulator for this bioelement so important for human organism.

#### CONCLUSIONS

• All species of *Lactarius* analysed had a great content of phosphorus in cap.

• The bioabsorption factor for phosphorous was highest as value in cap of *Lactarius vellereus*.

• The translocation factor for phosphorus was highest from stipe into cap of *Lactarius piperatus* no matter of growing zone.

All species had a very important content of potassium, impressive as value in caps.

• The best bioabsorption factor had the caps of *Lactarius piparatus* preleated from Bolboca forest.

• The highest value of translocation factor from soil into stipe had Lactarius piperatus grown in Bolboca area.

• The translocation factor from stipe into cap had the species *Lactarius piperatus* and *Lactarius vellereus* harvested from Bolboca.

• On can say that all three species studied are hyper accumulator for phosphorus and potassium, so they can be try to become a organic resource for food and pills.

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#### THE EFFECT OF ROOTING SUBSTATES ON THE DEVELOPMENT OF LEAF CUTTINGS OF SAINTPAULIA

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**KEY WORDS**: African violet, varieties, rooting substrates, multiplication methods

#### ABSTRACT

African violet is one of the most popular houseplants in the world and it is belonging to the genus Saintpaulia, Gesneriaceae family. The aims of these researches were to improve the assortment of indoor plants with new varieties and cultivars of Saintpaulia jonantha J. C. Wendl. The experiences regarding the effect of rooting substrates on the development of leaf cutting of Saintpaulia jonantha J. C. Wendl. were placed in the greenhouse of Floriculture Department, from University of Agricultural Sciences and Veterinary Medicine - Cluj Napoca. From the results, it appears that 'Arctic Frost' variety rooted the first (in 23 days), in the substrate composed by perlite. Varieties that were rooted at latest were 'Aloha Orchid' and 'Cinnamon Candy' in substrates composed by peat and garden soil in proportion of 1:1 (in 31 days). Root delay was observed at all studied species, in the substrate consisting by garden soil.

#### **INTRODUCTION**

Due to the high consumption of indoor plants in our country, determined us to investigate the possibilities of propagation technology of this species. Through studies and researches in this work was to fallowing the influence of rooting substrate at six varieties of African violets.

*Saintpaulia ionantha* J.C.Wendl. is a popular plant for trade, because is not a food plant, it is very difficult to collect comprehensive statistical data on production and its value. It is always located in the top 15 indoor plants, both in Europe and the USA (Holdgate, 1977) and world production is estimated at 30 million dollars per year. In the UK the production of *Saintpaulia* in 1986 was estimated at 4 million plants.

In the Sainpaulia genus are three different groups of species, categorized by plant shape. Some of them, as Saintpaulia grotei and Saintpaulia pendula are pendent, as others, like Saintpaulia pusilla and Saintpaulia strumensis are miniature shaped. The most common shape is rosette type; from this group are derived Saintpaulia ionantha and Saintpaulia grandifolia (<u>http://www.gesneriads.ca/gensaint.htm</u>). The range of varieties is extremely rich and includes three categories of plants in relation to their size: standard, medium and mini (Darbyshire, 2006).

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Violets excel trough a wide range of colors (white, red, pink, purple, blue) and the most requested color is blue in 60%, followed by pink in 20% (Şelaru, 2004). The aim of the researches was to improve the assortment of indoor plants with new varieties and cultivars of *Saintpaulia jonantha* J.C.Wendl.

High consumption of indoor plants in our country, determine us to investigate the possibilities of culture and propagation technology of *Saintpaulia jonantha* J.C.Wendl. It was followed the influence of the rooting substrate at 6 varieties of African violets.

Objectives that aimed to achieve its purpose were as follows: to study the rooting of cuttings belonging to six varieties of African violets, the influence of substrate on rooting varieties of African violets, the combined influence of experimental factors on *Saintpaulia jonantha* J.C.Wendl. varieties.

#### MATERIALS AND METHODS

The researches concerning the effect rooting substrates on the development of leaf cutting of *Saintpaulia jonantha* J. C. Wendl was placed in the greenhouse belonging to the Floriculture Department of University of Agricultural Sciences and Veterinary Medicine - Cluj-Napoca. The greenhouse is old, flat glass, with semi-automatic equipment. Rooting was carried out on tables in substrate and growing trays.

The biological material used in experiments with *Saintpaulia jonantha* J.C.Wendl was consisted by six African violets varieties, as following: "Hot Pink Bell" (pink, simple flower), "California Beauty" (pink, double flower), "Arctic Frost" (white, ruffled petals), "Aloha Orchid" (mauve, simple flower), "Astral" (blue, simple flower), "Cinnamon Candy" (purple-red, simple flower).

The studied varieties are derived by Standard groups.

The control of the experience was the variety "Hot Pink Bell".

The experiment was carried out during 2008-2009, was organized an experience with two factors and the analyzed factors were: varieties of African violets and rooting substrate (Figure 1):

Factor A: *variety*: a<sub>1</sub>, "Hot Pink Bell"

a<sub>2</sub> "California Beauty" a<sub>3</sub> "Arctic Frost" a<sub>4</sub> "Aloha Orchid" a<sub>5</sub> "Astral" a<sub>6</sub> "Cinnamon Candy"

Factor B: rooting substrates: b<sub>1</sub> garden soil

b<sub>2</sub> perlite

b<sub>3</sub> peat+perlite (1:1)

 $b_4$  peat+sand (1:1)

Through the combination of those two factors were obtained 24 experimental variants, which were placed in randomized blocks, in three repetitions.

For the propagation of African violets, on 30.01.2009 were collected 10 cuttings for each variant from the healthy mother plants, vigorous and free from diseases and pests. Cuttings were made from the middle-aged healthy leaves, the leafstalks was shortened to 2 cm. The prepared cuttings were treated with Radistim 1 for a better rooting.

Biological material prepared before had been planted in four rooting substrates, as follows: garden soil, perlite, peat + perlite (ratio 1:1) and peat + sand (ratio 1:1), the stalks being introduced in the substrate in an oblique position. Thickness of substrates for rooting was 8 cm.



Figure 1. Saintpaulia leaves in different rooting substrate

For a good rooting it was provided the following conditions: a temperature of 22 ° C, a relative humidity of 80% (Cantor, 2008). After the apparition of 2-3 leaves on each cutting was moved in the data of 27.03.2009, into a new substrate (Figure 2): 30% fibrous peat, 30% leaf compost, 20% fern compost, 10% perlite, 10% sand (Şelaru, 2006).



# Figure 2. Rooted cuttings transferred into plastic pots RESULTS AND DISCUSSIONS

Regarding the development of rooting system we can conclude that this process devolved different, depending to the variety (Table 1).

Table 1

Centralized table concerning the situation of rooting of Saintpaulia cuttings

Experimental variants	Data cuttings preparation	Data of cuttings rooting	No. of days of rooting
Hot Pink Bell+garden soil (Control)	30.01.2009	26.02.2009	27
Hot Pink Bell+ perlite	30.01.2009	25.02.2009	26
Hot Pink Bell+peat with perlite	30.01.2009	23.02.2009	24
Hot Pink Bell+peat with sand	30.01.2009	24.02.2009	25
California Beauty+ garden soil (Control)	30.01.2009	27.02.2009	28
California Beauty+perlite	30.01.2009	25.02.2009	26
California Beauty+ peat with perlite	30.01.2009	25.02.2009	26
California Beauty+ peat with sand	30.01.2009	26.02.2009	27
Arctic Frost+ garden soil (Control)	30.01.2009	25.02.2009	26
Arctic Frost+perlite	30.01.2009	22.02.2009	23
Arctic Frost+ peat with perlite	30.01.2009	23.02.2009	24
Arctic Frost+ peat with sand	30.01.2009	23.02.2009	24
Aloha Orchid+ garden soil (Control)	30.01.2009	02.03.2009	31
Aloha Orchid+perlite	30.01.2009	28.02.2009	29
Aloha Orchid+ peat with perlite	30.01.2009	27.02.2009	28
Aloha Orchid+ peat with sand	30.01.2009	25.02.2009	26
Astral+ garden soil (Control)	30.01.2009	01.03.2009	30
Astral+perlite	30.01.2009	27.02.2009	28
Astral+turbă cu perlit	30.01.2009	27.02.2009	28
Astral+ peat with sand	30.01.2009	28.02.2009	29
Cinnamon Candy+ garden soil (Control)	30.01.2009	02.03.2009	31
Cinnamon Candy+perlite	30.01.2009	27.02.2009	28
Cinnamon Candy+ peat with perlite	30.01.2009	28.02.2009	29
Cinnamon Candy+ peat with sand	30.01.2009	30.02.2009	31

The data from the upper table show that ,Arctic Frost' variety rooted at the earliest (23 days), in the perlite substrate. The substrates consist in peat + perlite and peat+ sand, the same variety rooted in 24 days. "Arctic Frost" was followed by "Hot Pink Bell" variety, which rooted in 24 days in the mixture consist of peat + perlite.

The varieties that have rooted at the latest were "Aloha Orchid" and "Cinnamon Candy", in the garden soil substrates and the mix of sand and peat (31 days). Root delay was observed at all studied varieties, in the garden soil.

In the second table are presents data concerning the total length of the analyzed cuttings. It appears that the most vigorous cuttings, of 11.57 cm were recorded at "Aloha Orchid,, which determine a difference of 2.93 cm, very significant compared to the control. "Arctic Frost" variety determines a distinct significant difference comparing with the control.

Table 2

Variety	The total length of cutting		±D (cm)	Signification	
variety	Absolute (cm)	Relative (%)	$\pm D$ (cm)	Signification	
Hot Pink Bell (C)	8.64	100.0	0.00	-	
California Beauty	8.77	101.4	0.12	-	
Arctic Frost	9.41	108.8	0.76	**	
Aloha Orchid	11.57	133.9	2.93	***	
Astral	10.11	117.0	1.47	***	
Cinnamon Candy	9.48	109.7	0.84	***	
DL (p 5%)			0.30		
DL (p 1%)			0.48		
DL (p 0.1%)			0.81		

Statistical dates concerning the total length of *Saintpaulia* cuttings

The best results concerning the rooting substrate were obtained in substrate consisting by peat+perlite and peat + sand. Both substrates have generated differences in length of 0.48 cm and respectively 0.71 cm. being very significant positive compared to the control, while the rooting in perlite was insignificant (Table 3).

Table 3

The unilateral influence of substrate on rotting of Saintpaulia cutting

Rooting substrates	The total lengt	$\frac{\text{gth of cutting}}{\text{blue}}$ ±D (cm) Signi		Signification
Rooting substrates	Absolute (cm)	Relative (%)	±D (cm)	Signification
Garden soil (Control)	9.34	100.0	0.00	-
Perlite	9.44	101.0	0.10	-
Peat+perlite	9.82	105.1	0.48	***
Peat+sand	10.05	107.6	0.71	***
DL (p 5%)			0.14	
DL (p 1%)			0.19	
DL (p 0.1%)			0.26	

The combined influence of experimental factors (rooting substrate x variety), results that "Bell Hot Pink" variety rooted best in the substrate composed by peat + sand and favorable in the substrate composed of peat and perlite. This variety recorded distinct differences positive respectively very significant positive compared to the control.

Remarks with very good results "California Beauty" variety which recorded significant respectively very significant differences, determining total length which exceeded the experience control with 2.5 to 7.5%.

"Arctic Frost" variety rooted favorable in the substrate composed of peat + sand recorded a difference of 0.49 cm. compared with controls.

"Aloha Orchid" was positively influenced by all rooting substrates. He has recorded significant and very significant differences compared with controls, recording total lengths between 11.45 to 11.94 cm. The substrate composed by peat + sand determines a difference of 0.45 cm at "Astral" variety significantly positive compared with the control.

Variety "Cinnamon Candy" in substrates composed of peat+perlite and peat + sand recorded positive difference but uninsured statistically (Table 4).

Table 4

Experimental variants	The total leng	th of cutting	±D (cm)	Signification
Experimental variants	Absolute (cm)	Relative (%)	±D (cm)	Signification
Garden soil x Hot Pink Bell (Control)	8.26	100.0	0.00	-
Perlite x Hot Pink Bell	8.46	102.4	0.20	-
Peat+perlite x Hot Pink Bell	8.86	107.3	0.60	**
Peat+sand x Hot Pink Bell	9.01	109.1	0.76	***
Garden soil x California Beauty (Control)	8.05	100.0	0.00	-
Perlite x California Beauty	8.50	105.6	0.45	*
Peat+perlite x California Beauty	9.06	112.5	1.01	***
Peat+sand x California Beauty	9.46	117.5	1.41	***
Garden soil x Arctic Frost (Control)	9.20	100.0	0.00	-
Perlite x Arctic Frost	2.94	100.4	0.04	-
Peat+perlite x Arctic Frost	9.50	103.3	0.30	-
Peat+sand x Arctic Frost	9.70	105.4	0.49	**
Garden soil x Aloha Orchid (Control)	11.05	100.0	0.00	-
Perlite x Aloha Orchid	11.45	103.6	0.40	*
Peat+perlite x Aloha Orchid	11.85	107.2	0.80	***
Peat+sand x Aloha Orchid	11.94	108.0	0.88	***
Garden soil x Astral (Control)	10.0	100.0	0.00	-
Perlite x Astral	9.90	99.0	-0.10	-
Peat+perlite x Astral	10.10	101.0	0.10	-
Peat+sand x Astral	10.45	104.5	0.45	*
Garden soil x Cinnamon Candy (Control)	9.50	100.0	0.00	-
Perlite x Cinnamon Candy	9.10	95.8	-0.40	0
Peat+perlite x Cinnamon Candy	9.55	100.5	0.05	-
Peat+sand x Cinnamon Candy	9.77	102.8	0.27	-
DL (p 5%)			0.35	
DL (p 1%)			0.47	
DL (p 0.1%)			0.65	

# The combined influence between substrate and variety on rooting of Saintpaulia cutting

As the length of the leaf cuttings studied at the six varieties of *Saintpaulia* it is remarks very significant lengths at "Aloha Orchid", "Astral" and "Cinnamon Candy which presents differences between 1.05 to 1.95 cm (Table 5).

Table 5

Length of leaves cuttings at Saintpaulia jonantha varieties

Variety	Leaves length		±D (cm)	Signification
v affety	Absolute (cm)	Relative (%)	$\pm D$ (cm)	Signification
Hot Pink Bell (Control)	4.07	100.0	0.00	-
California Beauty	4.13	101.5	0.06	-
Arctic Frost	4.15	101.9	0.08	-
Aloha Orchid	6.02	147.8	1.95	***
Astral	5.55	136.3	1.48	***
Cinnamon Candy	5.12	125.7	1.05	***
DL (p 5%)			0.17	
DL (p 1%)			0.26	

DL (p 0.1%)

Statistical data show that in the substrate composed of peat + perlite leaf length is larger, accounting differences of 0.20 cm very significant compared with the control (garden soil). It was registered positive and significant differences in substrates formed by perlite and peat + sand (Table 6).

Table 6

The length of leaves cuttings. under the influence of rooting substrate. a	t
Saintpaulia jonantha	

Rooting substrates	Leaves length		±D (cm)	Signification
Rooting substrates	Absolute (cm)	Relative (%)	$\pm D$ (cm)	Signification
Garden soil (Control)	4.73	100.0	0.00	-
Perlite	4.84	102.3	0.11	*
Peat+perlite	4.93	104.2	0.20	***
Peat+sand	4.85	102.4	0.11	*
DL (p 5%)			0.09	
DL (p 1%)			0.12	
DL (p 0.1%)			0.17	

Concerning the length of the root system at the six varieties of *Saintpaulia jonantha* reveals that all varieties records distinct respectively very significant differences. The variety "Arctic Frost" overtakes the control with 1.80 cm in length and "Aloha Orchid" with only 0.15 cm. Differences at all varieties are statistically assured (Table 7).

Table 7

The length of root system at the Saintpaulia jonantha varieties

Variety	Leaves	Leaves length		Signification
variety	Absolute (cm)	Relative (%)	±D (cm)	Signification
Hot Pink Bell (Mt)	1.60	100.0	0.00	-
California Beauty	2.23	139.1	0.62	***
Arctic Frost	3.40	212.5	1.80	***
Aloha Orchid	1.75	109.4	0.15	**
Astral	1.89	118.0	0.29	***
Cinnamon Candy	2.21	138.3	0.61	***
DL (p 5%)			0.09	
DL (p 1%)			0.14	
DL (p 0.1%)			0.24	

Table 8

The length of root system under the influence of rooting substrate at six variety of Sainpaulia *jonantha* 

Rooting substrates	Leaves	Leaves length		Signification	
Kooting substrates	Absolute (cm)	Absolute (cm) Relative (%)		Signification	
Garden soil (Control)	1.93	100.0	0.00	-	
Perlite	2.02	104.8	0.09	-	
Peat+perlite	2.31	119.9	0.38	***	
Peat+sand	2.47	128.1	0.54	***	
DL (p 5%)			0.15		
DL (p 1%)			0.21		

# DL (p 0.1%)

The studied rooting substrates that exceeded the control (garden soil) were mixtures consisting of peat+perlite and peat + sand. The difference of length in the first substrate was 0.38 cm (19.9%) and in case of peat+sand was 0.54 cm (28.1%) very significant compared to the control. Perlite recorded positive values (0.09 cm) but statistically uninsured (Table 8).

Analyzing the combined influence of two experimental factors we can conclude that the most prevalent significant differences were recorded by "Cinnamon Candy" variety in the substrate composed of peat + sand. The difference of 1.20 cm exceeded the control with 70.6%.

Distinct significant differences were recorded in the following combinations: "California Beauty,, in the substrate composed by peat + perlite; "Astral" in peat + perlite and peat + sand "Cinnamon Candy" in peat + perlite.

In case of "Aloha Orchid" and "Astral" varieties the recorded differences were significant. The substrate composed of perlite recorded positive difference but statistically uninsured (Table 9).

Table 9

The combined influence of experimental factors (rooting substrates x varieties) on length of
root system at the varieties of Saintpaulia jonantha

Experimentally variants	Leaves	length	±D (cm)	Signification
Experimentary variants	Absolute (cm)	Relative (%)	$\pm D$ (cm)	Signification
Garden soil x Hot Pink Bell (Control)	1.65	100.0	0.00	-
Perlite x Hot Pink Bell	1.65	100.0	0.00	-
Peat+perlite x Hot Pink Bell	1.15	69.7	-0.50	0
Peat+sand x Hot Pink Bell	1.95	118.2	0.30	-
Garden soil x California Beauty (Control)	2.05	100.0	0.00	-
Perlite x California Beauty	2.00	97.6	-0.05	-
Peat+perlite x California Beauty	2.65	129.3	0.60	**
Peat+sand x California Beauty	2.20	107.3	0.15	-
Garden soil x Arctic Frost (Control)	3.15	100.0	0.00	
Perlite x Arctic Frost	3.25	103.2	0.10	-
Peat+perlite x Arctic Frost	3.60	114.3	0.45	*
Peat+sand x Arctic Frost	3.60	114.3	0.45	*
Garden soil x Aloha Orchid (Control)	1.45	100.0	0.00	-
Perlite x Aloha Orchid	1.75	120.7	0.30	-
Peat+perlite x Aloha Orchid	1.85	127.6	0.40	*
Peat+sand x Aloha Orchid	1.95	134.5	0.50	*
Garden soil x Astral (Control)	1.55	100.0	0.00	-
Perlite x Astral	1.60	103.2	0.05	-
Peat+perlite x Astral	2.20	141.9	0.65	**
Peat+sand x Astral	2.20	141.9	0.65	**
Garden soil x Cinnamon Candy (Control)	1.70	100.0	0.00	-
Perlite x Cinnamon Candy	1.85	108.8	0.15	-
Peat+perlite x Cinnamon Candy	2.40	141.2	0.70	**
Peat+sand x Cinnamon Candy	2.90	170.6	1.20	***
DL (p 5%)			0.38	
DL (p 1%)			0.52	

117

DL (p 0.1%)

0.71

0.29

#### CONCLUSIONS AND RECOMMANDATION

Analyzing the obtained results from researches concerning the effect of rooting substrates on the development of leaf cutting of *Saintpaulia jonantha* J. C. Wendl using six different varieties and four rooting substrates results the following conclusions:

1. Concerning the development of rotting system we can conclude that this process devolved different depending to the variety. ,Arctic Frost' variety rooted at the earliest (23 days) in the perlite substrate. The substrates consist in peat + perlite and peat+ sand the same variety rooted in 24 days. "Arctic Frost" was followed by "Hot Pink Bell" variety which rooted in 24 days in the mixture consist of peat + perlite.

The varieties that have rooted at the latest were "Aloha Orchid" and "Cinnamon Candy" in the garden soil substrates and the mix of sand and peat (31 days). Root delay was observed at all studied varieties in the garden soil.

Root delay was observed at all studied varieties in the garden soil.

**2**. Analyzing the total length of cutting we can observe that the most robust variety with a length of 11.07 cm was "Aloha Orchid" which recorded a difference of 2.33 cm. being very significant compared with the control.

**3**. The very best results regarding the influence of rooting substrate about the total length of cuttings it was obtained in the substrates composed by peat+perlite and peat+sand. The both substrate produced length differences of 0.48 cm. respectively 0.71 cm. being very significant compared with the control.

**4**. It was studied the length of leaves at the six *Saintpaulia* varieties. Vary significant differences ware recorded at "Aloha Orchid". "Astral" and "Cinnamon Candy" which presents positive differences between 1.05-1.95 cm.

**5**. Under aspect of rooting substrate it was found out that the length of leaves in the substrate compound by peat+perlite is higher then controls (garden soil). It was recorded significant differences in the perlite and peat+sand too.

**6**. Regarding the length of root system at six *Saintpaulia jonantha* varieties we can show that all varieties record distinct and very significant differences. "Arctic Frost" exceeds the control with 1.80 cm. and "Aloha Orchid" with only 0.15 cm.

7. In the case of root system length the rooting substrates which exceed the control (garden soil) were peat+perlite and peat+sand. Length difference in the case of mix substrate (peat+perlite) was 0.38 cm (19.9 %) while at peat+sand was 0.54 cm (28.1 %) very significant compared with control. Perlite record positive values (0.09 cm), but statistically uninsured.

Based on these results obtained in the researches and the conclusions we can make the following **recommendations**:

1. Cultivation of studied varieties further because they have good resistance to pests and diseases and are characterized by a high rate of multiplication. It notes in particular varieties "Cinnamon Candy", "Californian Beauty" and "Aloha Orchid" from flower color foliage and shape.

2. Use especially for breeding the studied varieties substrates composed of peat+ perlite and peat + sand in 1:1 ratio substrates that led to the highest percentage of rooting. Perlite is not recommended for use with a small particle size because it is settle quickly and lead to asphyxiation cuttings.

3. Expansion into commercial crops the studied varieties to diversify the assortment of *Saintpaulia jonantha* in order to obtain substantial income for those who cultivate them.

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### UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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

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### A SURVEY OF THE GENETIC RESOURCES USED IN ROMANIAN PLUM BREEDING PROGRAM

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KEY WORDS: plum, germplasm, evaluation

#### ABSTRACT

In Romania, plum accounts for the first place in the national fruit patrimony as acreage and number of trees. One of the most important conditions for the objectives in the breeding program is availability of a rich germplasm. The National Plum Collection (NPC) located at the Research Institute for Fruit Growing Pitesti – Maracineni, has 560 accessions from which 9 species, 183 autochthonous plum cvs., old and new breeded, 318 foreign genotypes, as well as 50 promising hybrids.

This germplasm fund has been evaluated regarding tolerance to PPV, blooming period, fruit quality, yielding capacity, according to the numerical scale of IBPGR descriptors used in Genres Project 61 of "Prunus Data Base". Study shows that the numerous genotypes existing in the NPC offer enough initial material to achieve the objectives of Romanian plum breeding program.

# INTRODUCTION

One of the most important conditions for the objectives in the breeding program is availability of a rich germplasm. The success of any breeding program is conditioned by the availability of a large, diverse and valuable germplasm fund. Its usefulness depends on the proper complete identification of the genetic resources required in the breeding work. (Cociu, 1997).

The establishment of some methodical organized plum collections started in 1967 at RIFG Pitesti – Maracineni and 1985 at Strejesti, Fruit Research Station.

Presently, there are plum collections in two centers, respectively at RIFG Pitesti– Maracineni and Valcea, Fruit Research Station, summing up 812 genotypes of which 43 species, 323 autochthonous cultivars, 564 foreign cvs and promising selections. (Braniste and Butac, 2006; Botu et al., 2008).

The NPC, located at the RIFG Pitesti – Maracineni, was established in 1997, including 560 accessions.

The purpose of this study is the evaluation of some plum cultivars from the germplasm fund in Pitesti – Maracineni, for selecting some potential genitors to be used in the breeding programs.

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#### MATERIAL AND METHODS

The studies were performed on 229 accessions from the National Plum Collection at RIFG Pitesti – Maracineni.

The collection includes in all 560 accessions, with 9 wild species, 183 autochthonous cultivars, old and newly breeded, 318 foreign cvs., as well as 50 promising hybrids. The accessions are arranged according to the ripening season; they are grafted on mirobolan, spaced at 4/4 m. Each genotype is a variant and each variant has 3 replications (1 tree = 1 replication) (Butac and Budan, 2007, Butac et al., 2007).

The observations and measurements were done according to the descriptors for *Prunus* genus (Genres 61 Project of the Working Group *"Prunus* Data Base", IPGRI).

### List of descriptors for Prunus genus

Fruit use: 1. No use; 2. Table; 3. Dual or multipurpose use.

**Plant use:** 1. Clonal rootstock; 2. Clonal interstock; 3. Seedling rootstock; 4. Ornamental / Pollinator; 5. Dual or multipurpose use; 6. Botanical (wild) species; 7. Other; 8. Timber; 9. No use.

**Status of sample:** 1. Wild; 2. Primitive cultivar / Landrace; 3. Breeded cvs.; 4. Clones; 5. Clonal rootstock.

Virus disease status: 1. No virus; 2. No symptoms; 3. With virus.

**Season of flowering:** 1. Extremely early (Earlier than Ive); 2. Very early (Ive); 3. Early (Early Rivers, Prolific); 5. Intermediate (Blue de Belgoque, Reine Claude Verte); 7. Late (Pozegaca); 8. Very late (Quetsche Blanche de Letricourt); 9. Extremely late (Later than Quetsche Blanche de Letricourt).

**Harvest maturity:** 1. Extremely early (Earlier than Ruth Gerstetter); 2. Very early (Ruth Gerstetter); 3. Early (Ersinger Fruhzwetsche, Cacak Lepotica); 5. Mid-season (Agen, Tuleu gras); 7. Late (Pozegaca); 8. Very late (President); 9. Extremely late (Later than President).

**Fruit size:** 1. Very small - sub 10 g (Mirabelle de Metz); 3. Small - 11-25 g (Early Rivers, Bonne de Bry); 5. Medium - 26-40 g (Reine Claude Verte, Ruth Gerstetter); 7. Large - 41-55 g (California Blue, Reine Claude d'Oullins) ; 8. Very large - 56-70 g (Yakima, President); 9. Extremely large – peste 70 g (Record).

**Fruit shape:** 1. Flat; 2. Round (Reine Claude Verte); 3. Elliptic (Monsieur Hatif); 4. Elongated-elliptic (Pozegaca)); 5. Ovate (Victoria); 6. Heart shaped (Damas); 7. Drop shaped (Coe's Golden drop).

**Skin colour:** 1. Whitish (Transparent Gage); 2. Green (Reine Claude Verte); 3. Yellow / Green (Reine Claude d'Oullins); 5. Orange (Mirabelle de Nancy); 7. Purple / Red (Reine Claude d'Althan); 8. Violet / Blue (Anna Spath); 9. Dark blue (Pozegaca).

**Stone adherence to flesh:** 1. Freestone (President, Tuleu gras); 2. Semi-freestone (Frontier, Centenar); 3. Clingstone (Favorita del Sultano).

**Susceptibility to** *Monilia* **sp.**: 1. Resistant; 2. Very low susceptible (Pozegaca); 3. Low susceptible ; 5. Intermediate (Victoria); 7. Susceptible (President); 8. Very susceptible (Kirke's Plum); 9. Extremely susceptible.

Susceptibility to PPV: 1. Resistant; 2. Very low susceptible (Opal, Scolduş); 3. Low

susceptible (Anna Spath, Oteșani 8); 5. Intermediate (Tuleu gras); 7. Susceptible; 8. Very susceptible (Vinete românești, Diana, Debreceni); 9. Extremely susceptible.

The data were statistically calculated by the following statistical indices: average, standard deviation and variability coefficient).

#### **RESULTS AND DISCUSSIONS**

Studies on material in the collection have emphasized some parents with complementary traits which can be used in controlled hybridizations.

# Blooming time

Regarding the time of full bloom, the varieties made up 5 classes as follows: 39 very early blooming cultivars, 89 early blooming, 34 mid-season blooming, 60 late blooming and 7 very late blooming (Fig 1). Usually, late blooming is considered a positive characteristic to better avoid late spring frosts. From this point of view, the following cultivars are recommended as potential genitors: 'De Bistrita cl. VIII', 'Vinete romanesti 300', 'Bistritene de Hateg', 'Prune rosii', 'Busuioace de Geoagiu', 'Perje de toamna', 'Pozegaca GC1', 'Pozegaca 11/81', 'Agen 711', etc.

# Harvesting time

Normally in the southern hilly area of Romania, the plum ripening period expend for 3 month (July, August and September). Varieties were classified into 5 classes, namely: class 3 (early maturation) - 52 cultivars, class 5 (mid-season maturation) 66 cultivars, class 7 (late maturation) - 107 cultivars, class 8 (very late maturation) – 4 genotypes and class 9 (extremely late maturation) – one genotype (Fig. 2). Concerning the maturation are recommend as potential genitors for earliness, the following cvs: 'Popesti', 'Scoldus de vara', 'Stanigele', 'Boboloase', 'Grase', 'Krimska', 'Early Zorleans', 'Sugar', 'Fruhewetsche', 'Queenstal', 'Early Blue', H 6-29 B, H 6-22 B, H 13/18 B, etc. For lateness (ripening in September) are suggested cvs. like: 'Carandane', 'Gatlanos', 'Vinete romanesti 300', 'Poroabe II', 'Oneida', 'President', 'Stanley', 'Blue Bell', 'Verity', 'Sugar 2', 'Forli III', 'Anna Spath', H 13/5 B, H 6/81, etc.

<u>Yield capacity</u> in the 229 genotypes was done by weighing the production per each tree (kg/tree) at the optimum harvesting time. Were noted for high yields following genotypes: 'Pitestean', 'Uriase', 'Tuleu gras', '91/1 Iugoslavia', 'Zuchella', 'Stanley', 'Sakarka', 'Troian 3-13-41', 'Verity' (above 25 kg / tree), which can be used with successful in breeding work for transmission productivity character. The variability coefficient was very high (above 50%) (Table 1). The genotypes was evaluated by a scale performed in the Genetics and Breeding Department: 1 - very low production (less than 5 kg/tree), 3 - low production (5-10 kg/tree), 5 - medium production (10 - 15 kg/tree), 7 - productive (15 - 20 kg/tree), 8 - very productive (20 - 30 kg/tree), 9 - extremely productive (over 30 kg/tree). Thus, accessions were grouped into six classes (Fig. 3).

# Fruit characteristics.

Average fruit weight (AFW) was determined by weighing 25 fruit in each of 3 consecutive years. The cultivars were put into 6 classes: 2 cultivars with AFW under 10 g; 88 cultivars with AFW of 11 - 25 g; 79 cultivars with AFW of 26 - 40 g; 45 genotypes with AFW of 41 - 55 g; 12 cultivars with AFW of 56 - 70 g; and 2 cultivars with AFW over 70g. There are genotypes with high fruit (class 8) and very high (class 9), like: 'Roman', 'Lachi', 'Dambovita', 'Pond's Seedling', 'Jeferson', 'Vision', 'Edwards', 'Cacanska Najbolja', H 16/27, H 9/13, H 5/10 B, H 5/13, H 1/12 B. The variability coefficient of character "fruit weight" was very high (39.29%), indicating a high variability and, therefore, opportunities to use the potential genitors for breeding work (Table 1).

Regarding the *content of soluble dry weight*, genotypes studied were classified into five classes (Fig. 5). They noted a higher content of soluble dry weight (over 20%) following genotypes: 'Grase', 'Agen de Sirauti', 'Inalte', 'Lungulete', 'Bumbuleu mare', 'Vanat romanesc cu fruct mare', 'Lunguiete', 'Boambe de Leordeni', 'Tuleu gras', 'Sibiel', 'Poroabe II', 'Montfort', 'LU', Dry Seedling', 'Valor', 'Vision', 'Ente 707', 'Cochet Pere',

'Abbaye d'Arton', 'Giant plum', 'Condata', 'Grossa Suzucheratta', H 11/20 B, H 1/118 M. The variability coefficient of character "content of soluble dry weight" indicates a mid variability (20.50%) (Table 1).

The optimum *fruit shapes* (elliptic, elongated-elliptic and ovate) is found in 193 cultivars and 32 cultivars had round shape (fig. 6).

Dark blue skin colour is found in 162 cultivars, violet - blue in 11, purple - red in 46 and yellow - green in 10 genotypes (fig. 7). Predominant flesh colour is yellow (121 genotypes) and green (84 cultivars) (Fig. 8).

Regarding the fruit shape and skin colour, on the Romanian market are demanded elliptic and ovate shaped fruit ('Boambe de Leordeni', 'De Bistrita', 'Centenar', 'Pitestean', 'Flora', 'Lungulete', 'Tamaisoase de Bistrita', 'Vinete romanesti', 'Belle de Louvain', 'Blue Bell', 'Bluefree', 'Oneida', etc), with blue and dark blue colour ('Albatros', 'Busuioace de Geoagiu', 'Centenar', 'Carpatin', 'Dambovita', 'Grase de Pesteana', 'Grase de Becz', 'Negre de Bilcesti', 'Blue Bell', 'Bluefree', 'Brompton', 'Burton', 'Cardinal', 'Huttner', 'Kjustendilska', 'Montfort', 'Oneida', 'Pozegaca', 'Stanley', 'Valor', etc).

According to our observations, 149 varieties are completely freestone ('Gras ameliorat', 'Motroase de Mehedinti', 'Negre de Bilcesti', 'Vinete romanesti', 'Belle de Louvain', 'Blue Bell', 'Bluefree', 'Jefferson', 'OK', 'President', 'Valor'). A trace of flesh remains in the ventral suture zone in 38 others, and in 42 varieties, the flesh clings over the whole surface of the stone, causing losses when the stone is removed. (Fig. 9).

Susceptibility to Plum Pox Virus

Plum pox virus is a disease that can cause great losses to plum culture. Regarding the tolerance to Plum Pox Virus, the varieties made up 7 classes (Fig. 10.a, 10.b), as follows: no susceptible (on the fruit) - 207 cultivars, very low susceptible - 8 cultivars, low susceptible - 8 cultivars, mid susceptible - 1 cultivar, susceptible - 3 cultivars, very susceptible - 1 cultivar and extremely susceptible - 1 genotype. As a gene source for the resistance to Plum Pox Virus (Sharka) can be used – 'Popesti', 'Vieneze', 'Roman', 'Cisnadie', 'Lachi', 'Alina', 'Unguresti', 'Ciorasti de Prahova', 'Flora', 'Negre de Saru', 'Negre de Bilcesti', 'Krimska', 'Peche', 'Chabat', 'Huttner', 'Belle de Liege', 'Kirke', 'Marry Mather', 'Giant plum', 'Condata', H 6-29 B, H 13/18 B, etc. As a conclusion to that aspect, there is not always a positive correlation between the symptoms on leaves and fruit of the same cultivar, but the most severe damage is rather on leaves than fruit.

### CONCLUSIONS

Study shows that the numerous genotypes existing in the NPC offer enough initial material to achieve the objectives of Romanian plum breeding program:

- for tolerance to PPV - 'Grase de Becs', 'Grase de Pesteana', 'Ciorasti de Mehedinti', 'Belle de Louvain', 'Burton', 'Kirke', 'Giant Plum';

- for large fruit – 'Tita', 'Pitestean', 'Carpatin', 'Record', 'Roman', 'Cacanska Rodna', 'Cacanska Ranna', 'Valor', 'Vision';

- for high and constant productivity - 'Centenar', 'Grase de Becs', 'Grase de Pesteana', 'Vision', 'Verity', 'Oneida', 'Standard', 'Stanley', 'Sakarka';

- for late blooming - 'De Bistrita cl. VIII', 'Vinete romanesti 300', 'Bistritene de Hateg', 'Prune rosii', 'Busuioace de Geoagiu', 'Perje de toamna', 'Pozegaca GC1';

Farly Orleans', 'Sugar', 'Fruhewetsche', 'Queenstal', 'Early Blue';
for late ripening – 'Carandane', 'Vinete romanesti 300', 'Poroabe II', 'Oneida', 'President', 'Blue Bell', 'Verity', 'Forli III', 'Anna Spath'.

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Table 1

20.50

	Statistical indices determinate for some characteristics of genotypes						
	Statistical indices	Yield capacity (kg/tree)	Fruit weight (g)	Soluble dry weight (%)			
ſ	Average	9.81	35.37	16.73			
ſ	Standard deviation	6.12	13.15	3.41			

63.19

Statistical indices determinate for some characteristics of genotypes

39.29

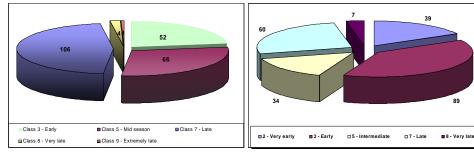
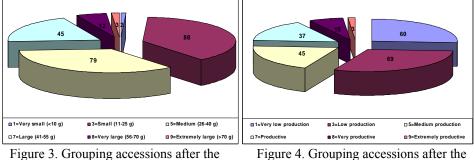


Figure 1. Grouping accessions after the blooming time

Variability coefficient

Figure 2. Grouping accessions after the harvesting time

fruit weight



yield capacity

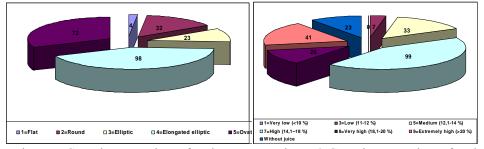


Figure 5. Grouping accessions after the content Figure 6. Grouping accessions after the of soluble dry weight fruit shape

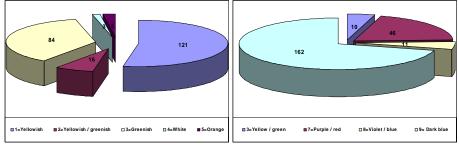


Figure 7. Grouping accessions after the skin colour

Figure 8. Grouping accessions after the flesh colour

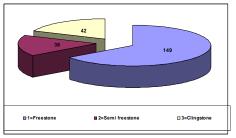


Figure 9. Grouping accessions after the stone adherence to flesh

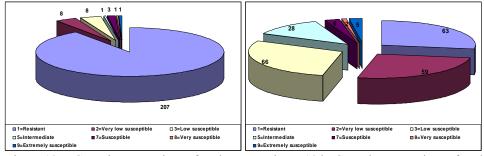


Figure 10.a. Grouping accessions after the susceptibility to PPV on the leaf

Figure 10.b. Grouping accessions after the susceptibility to PPV on the fruit

### UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

Seria:	🖌 Biologie
	✔ Horticultură
	<ul> <li>Tehnologia prelucrării</li> </ul>
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	🖌 Ingineria mediului

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#### DISPERSION OF SO<sub>2</sub> FOR SHORT AND LONG PERIODS OF TIME

Buzatu Gilda-Diana<sup>1</sup>\*

KEY WORDS: modeling, air pollutants, SO<sub>2</sub>, dispersion

### ABSTRACT

A major advantage of the use of modeling air pollutants in air quality management and evaluation is to improve capability to represent the spatial distribution of concentrations of pollutants, with effect from regional scale to local scale, even at the level of cities and streets. Furthermore, modeling will contribute to compliance or non-compliance to the quality objectives set by legislation while helping to identify areas where limits are exceeded.

In this view, in this paper we will attempt a study of modeling the dispersion of pollutants at local level which will focus on assessing the impact on air quality resulting from emissions sources associated with the activities of the S.E. Craiova II, and quantify the impact that some air pollutants are having on the viticultural agro-ecosystems from the Craiova Hills area by calculating the probabilities of exceeded maximum concentrations, allowable for 30 minute.

# INTRODUCTION

The European directives on ambient air quality enter for the first time in Europe the concept of modeling in air quality assessment and management.

Local scale dispersion study will take into account the regional background concentrations of pollutants from transport-induced mesoscale to regional scale. By its geographical position Craiova is affected by regional transport of pollutants, particularly sulfur dioxide emissions from large power plants in the Jiu Valley or from thermal plants in the county Halânga. This was demonstrated in nationally studies to evaluate the quality of air using mathematical modeling (Racoceanu and Popescu C., 2006, Ciolea, 2006).

Boix et al. have used vectorial model to assess the influence of local breeze and other meteorological parameters on ground level concentrations of  $SO_2$  in the urban area of Castellón - Spain. They have reported a decrease in the concentrations of  $SO_2$  in winter due to strong prevailing wind, while measured concentrations in summer were higher. A decrease in concentrations was noticed in hot days in winter, while no such phenomenon was observed in summer. Honaganahalli and Seiber have compared ISCST3 -a plume dispersion model with CALPUFF-a puff dispersion model.

Impact assessment of air quality objectives due to operation belonging to S.E. Craiova II was made by mathematical modeling, the results comply with the limits set by the Minister of Waters and Environment Protection Order no. 592/2002 for approving the

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Norms on the establishment of limit values, threshold values and criteria and methods of evaluation of sulfur dioxide, nitrogen dioxide and nitrogen oxides, particulate matter, lead, benzene, carbon monoxide and ozone in ambient air, complemented by Ministry of Environment and Water no. 27/2007.

Modeling study will examine the impact on air quality due to emission sources of pollutants from the Craiova Energy Complex by modeling the emissions data from 2008.

We opted for modeling the dispersion of 2008 because this year is representative, is the year for which emission limits imposed by the Order of the Ministry of Environment and Water Management no. 833/2005 approving the National Programme for reducing emissions of nitrogen oxides, sulfur dioxide and particulate emissions from large combustion plants will lead to lower emissions as in 2005, as a representative year of transition.

### MATERIAL AND METHODS

To calculate the dispersion of gaseous and particulate pollutants discharged into the atmosphere we used a specialized program called SIMGP v.4 developed under Visual Basic platform as a complete theory of the American model ISC3 (Sources Industrial Complex Models), computer algorithms presented in the guide of ISC3 model, Volume I and II, developed by the U.S. Environmental Protection Agency - Office of Air Quality-North Carolina, in September 1995 (ISC 3, Vol I and II, 1995).

Pollution dispersion simulation program SIMPG v.4 operating environment is compatible with Windows 9x, ME, 2000, XP, Vista or Windows 7. It is also compatible with all computer programs that use the ISC3 theory and the climatologic model. The program is particularly strong in calculating the likelihood of wind classes by classes of atmospheric stability on the 16 wind directions, also called triples, meteorological data for periods of up to 10 years. It can also calculate the average concentrations for time intervals from 30 minutes, hourly, daily, monthly, seasonal, annual or multiannual, the gaseous pollutants and particulate matter including PM10 discharged from a source or multiple sources (500 sources for gaseous emissions and 100 sources for particulate emissions).

The program also calculates the likelihood of exceeding the maximum allowable concentration for 30 minutes or one hour intervals of time considered for mediation of calculations, typically monthly average concentrations, seasonal, annual or multiannual. An important feature of the program is that is able to calculate the concentration for cases with thermal inversions or without thermal inversions.

Average concentrations are interpolated by a great performing graphic interpolator with presentation of spatial distributions of concentrations in two or three dimensions, color, or gray-scale, superimposed on maps or scale drawings constructed by the user using only a few points landmark.

Tested for 7 years to calculate average concentrations of 30 minutes, daily, monthly and annual in Bacău city as well as in cases of accidental pollution or due to strong thermal inversions (a well data from weather stations, automatic registration of 30 to 30 minutes and very accurate measurements of emission sources), resulted in a correlation coefficient between measured values and calculated field program of about 0.85.

Design software SIMGP v.4 received feedback from specialists in the Netherlands after the simulations study the impact of major projects emissions of pollutants for different urban areas and protected areas (Bacău, Tulcea, Galati, Baia Mare, Suceava, Delta-Sulina branch, etc.).

## **RESULTS AND DISCUSSIONS**

Dispersion of SO<sub>2</sub> for short period of time - hourly average

To calculate the hourly emissions of  $SO_2$  dispersion there were introduced the following input data in the calculation program:

- physical characteristics of the stack no. 1: stack height, 150 m, mouth diameter exhaust stack, 8.8 m, average rate of exhaust gas, 12.6 m, exhaust gas temperature, 138 °C;

- meteorological characteristics: average air temperature, 10°C, class of atmospheric stability, b (unstable), ground wind speed, 2.2 m/s

- area of dispersion characteristics: rural area with varied vegetation and buildings with average heights of 4 m,

- allowable hourly limit established by Order 592/2002 for SO<sub>2</sub> of 350  $\mu$ g/mc

- hourly SO<sub>2</sub> emission rate for the operation of only one boiler of 473 MW/t that releases into stack no.1 gas at a rate schedule of 1382822 Nm<sup>3</sup>/h is 1137.3 g/s.

In the following figure, there are shown the  $SO_2$  concentrations measured on wind axis (where concentrations are maximum), expressed in  $\mu g/mc$ . Concentration values thus calculated are valid for any wind direction considered, and hence, in the case when the wind blows from the direction of SSW-Source-S.C.D.A Şimnic, but also for the case when it blows from the NW-Source-S.D. Banu Mărăcine.

Table 1

SO<sub>2</sub> concentrations calculated on wind axis for the input, and for other weather states - averaging time 1 hour [µg/mc]

Distance [m]	Stability class	2	1	3	4	5	6
	Wind	2.2	1.5	3	3.5	2.5	2
	speed						
1000		2.78	446.30	0.00	0.00	0.00	0.00
2000	48	84.72	1325.68	8.74	0.00	0.00	0.00
3000	88	88.03	1039.02	135.56	0.00	0.00	0.00
4000	6.	34.79	765.50	255.87	0.00	0.00	0.00
5000	62	27.80	593.44	313.75	0.04	0.04	0.00
6000	51	13.20	520.89	365.99	0.22	0.14	0.00
7000	43	34.58	407.66	427.56	0.81	0.32	0.00
8000	3	95.39	411.19	375.18	1.93	0.56	0.00
9000	38	85.53	352.99	333.92	4.17	0.84	0.00
10000	32	27.59	293.80	301.60	6.46	1.14	0.00
Max.	9	06.20	1536.13	440.76	6.46	1.14	0.00
concentr							
ation							
X Max	29	947.0	1768.0	6785.0	10000.0	10000.0	10000
Н	-	500.0	500.0	500.0	500.0	287.5	252.9
Effective							

The analysis can be concluded that the calculated values for atmospheric instability type classes: 1 - strongly unstable - characterized by sunny days with weak winds and strong alternative (increased turbulence), 2 - weak unstable where turbulence is lower in intensity and wind has a higher speed and, 3 - neutral weak, characterized by low

turbulence, wind speed above 3 m/s (ground),  $SO_2$  concentrations exceed the permissible limit determined by Order 592/2002 amounting to 350 mg/m at distances between 1000 and 9000 m from the source, whatever the wind direction.

For other classes of atmospheric stability type: 4 - neutral, characterized by low turbulence and overcast, 5 - and 6 - stable, characterized by little wind without turbulence (occurring particularly at night, evening and early morning), and by the pollutant is almost parallel with the ground, these types of air, maximum concentrations are transported long distances over 10-25 km, depending on the source height and topography of the area.

Recommendations: Emission rate must be less than: 983.76 g/s or: Existing stack height increasing by: 70 m. Increasing stack value is approximate. Values are very good for heights between 5...30 m of the stack. At a greater height of the stack, diameter and both speed and gas temperature are changing.

To calculate annual average concentrations of  $SO_2$  data on physical characteristics of the source remain the same, average annual temperature is 9 °C and annual emission rate is 998 g/s relative to the total annual volume of gas discharged in 2008, 10629994  $Nm^3/year$ .

In the following graphic representation is apparent that the annual permissible limit of 20 mg/m<sup>3</sup> for SO<sub>2</sub> is exceeded in the study area SCDA Şimnic. Also, the SD Banu Maracine annual average concentrations of SO<sub>2</sub> have values between 20 to 30 mg/m<sup>3</sup>, higher than the permissible value. Most important values registered are in areas east and west of the source because of high frequency of these cardinal points, where the wind blows predominantly.

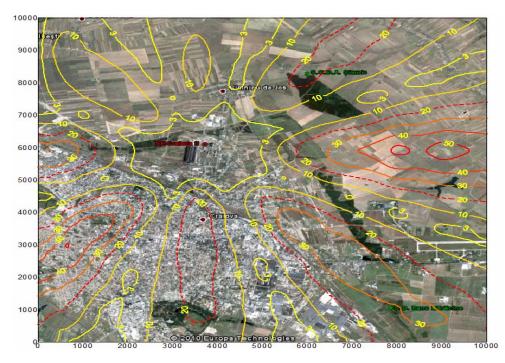


Figure 2: Annual SO<sub>2</sub> concentrations expressed in mg / m. Acceptable limit for SO<sub>2</sub> ecosystem is 20 mg/m

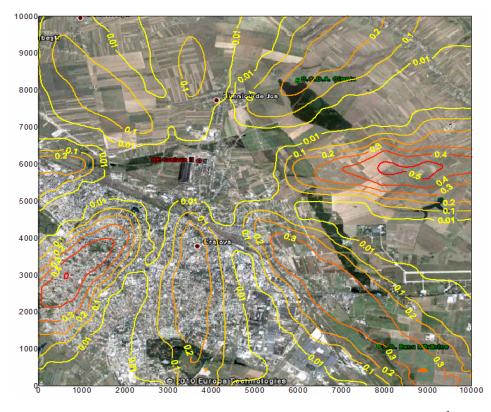


Figure 3: Frequencies (in %) to be exceeded allowable hourly 350µg/m<sup>3</sup> for SO<sub>2</sub> emissions during the year

Interpreting the results of calculations of the above representation is this: if a year has 8760 hours, representing 100% during a calendar year, then the value curve within 0.2%, for example SCDA Şimnic specific area, there is the probability P to exceed 350 mg/m<sup>3</sup> is approximately 18 times.

$$P = \frac{8760 \cdot 0.2}{100} \approx 18$$
 - Average hourly exceedances

Banu Maracine area is inside the curve of 0.3% resulting in a probability P of exceeding the allowable limit for SO<sub>2</sub> hourly by:

$$P = \frac{8760 \cdot 0.3}{100} \approx 26$$
 - Average hourly exceedances

After calculations of dispersion emissions of gaseous, one can draw the following conclusions:

- high values of hourly SO<sub>2</sub> concentrations in excess of one to three times the allowable limit  $350\mu g/m^3$  occur so SCDA Şimnic area where you can record about 18 exceeded the period of one year and for the SD Banu Maracine which are expected around 26 exceedances of the hourly limit for the period of a year

- the average annual values of  $SO_2$  concentrations exceeding the permissible limit of  $20\mu g/m^3$  are expected to occur in both areas of study.

So the most significant impact on areas of study - SCDA Şimnic and S.D. Banu Maracine emission of pollutants due to the SE Craiova II is given by hourly and annual average concentrations of SO<sub>2</sub>. Given the degree of phytotoxicity and wide territorial distribution, SO<sub>2</sub> is seen in many parts of Europe as one of the most important air pollutants on plants.

According to Ramadan et al. (2008), the computed annual SO<sub>2</sub> concentrations were always less than KEPA standards for all scenarios. The daily SO<sub>2</sub> concentrations were within KEPA standards for 1S% but violated KEPA standards for higher S%. In general, the concentrations obtained from the combined hourly and seasonal cycle were the lowest and those obtained from the no cycle case were the highest. The comparison between the results of the three cycles revealed that the violation times cannot be solely attributed to the increase in emissions and the meteorological conditions have to be taken into consideration.

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### UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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

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### THE MONITORING OF SOME STRELITZIA REGINAE'S VARIETIES IN ORDER TO PROMOTE IN CULTURE

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KEY WORDS: morphological characteristics, varieties, new assortment

### ABSTRACT

Strelitzia reginae Aiton is one of the most decorative plant that is being used as a cut flower. It is part of the genus Strelitzia, and Strelitziaceae family, the common name is Bird of Paradise Flower. The research had been made with the purpose of promoting in culture the most valuable varieties and for that three cultivars of Strelitzia reginae specie had been monitories: 'Miniature', 'Dwarf', 'Mandela Gold'. The behavior of the three cultivars of Strelitzia was studied in the period 2008-2009 at the Fruit Research Station Cluj (S.C.D.P. Cluj) and at the U.A.S M.V. Cluj, Floriculture's department. The observations and determinations where made at the main morphological characteristics and data where statistical analyzed. The results of the researches show that cultivar 'Dwarf' was appreciated more because of its rich leaves, and the best results were obtained by 'Mandela Gold' cultivar.

# **INTRODUCTION**

This must be one of the most well-known plants in the world. The fascinating blooms are sold as cut flowers by the million. *S. reginae* is very popular as an ornamental plant. It was first introduced to Europe in 1773, when it was grown at the Royal Botanic Gardens, Kew. Since then, it has been widely introduced around the world, including the Americas and Australia, growing well in any area that is sunny and warm. In the United States, Florida and California are the main areas of cultivation, due to their warm climate (http://en.wikipedia.org/wiki/Strelitzia reginae).

In Los Angeles strelitzias are so extensively planted that it is regarded as the emblem of the city. *Strelitzia reginae* is, however, indigenous to South Africa where it grows wild in the Eastern Cape. Here the strelitzias grow between other shrubs along the riverbanks and clearing in the coastal bush. Many other well known plants like *Plumbago*, *Tecomaria* and cycads come from the Eastern Cape which has a mild climate and rain distributed throughout the year (www.plantzafrica.com). Common names include Strelitzia, Crane Flower or Bird of Paradise, though these names are also collectively applied to other species in the genus *Strelitzia*.

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Bird of Paradise flowers are associated with liberty, magnificence, and good perspective.

It is propagated by division or from seeds, and is a low-maintenance plant that is easy to grow; it is fairly tolerant of soil conditions and needs little water once established. If cared for well, they will flower several times in a year.

They will thrive in rich loamy soil, especially when they get plenty of water throughout the year (Cantor and Pop, 2008). They do well in full sun to semi-shade and respond well to regular feeding with a controlled release fertilizer and compost.

They are sensitive to cold and need to be sheltered from frost, as it can damage the flowers and leaves. In Romania *Strelitzia* can be cultivating only in greenhouse and in the summer can be grown in different containers outside (Selaru, 1995). Bird-of-paradise is a real eyecatcher, in bloom or not. Whether in the landscape or as cut flowers making star appearances in arrangements, this plant is recognized by most and enjoyed by all. It is inexpensive and widely available, even in non-tropical areas where it does duty as a houseplant (Selaru, 2006).

Bird-of-paradise is an icon of the tropics, its image turning up on fabrics, wallpaper, tasteless resort apparel, and assorted works of art both crappy and sublime. As beautiful as some of these may be, none can compare to the real thing. Find a place in your home or garden so you can enjoy this flamboyant showoff (http://www.floridata.com/ref/s/stre reg.cfm).

#### MATERIALS AND METHODS

Biological material used in the experiments consisted of three varieties of *Strelitzia reginae*: ,Miniature', ,Dwarf' and ,Gold Mandela' were studied during 2008-2009 in the S.C.D.P. Cluj-Napoca and greenhouses Flowers Teaching Department of the University.

Variety 'Miniature' is higher than the 'Dwarf', stem reaching 1 m, but the flowers are smaller and leaves but thinner (Figure 1).

Variety 'Dwarf' is small 60-90 cm, flowers in 18 months after planting (Figure 2).



Figure 1. 'Miniature' cultivar



Figure 2. ,Dwarf' cultivar

Variety Gold Mandela reached 1.5 m, is a variety with yellow flowers. It is grown for many years in Europe, Australia, west coast of USA, Japan and some places in Africa.

Monofactorial experience was made in three repetitions; the observations and measurements were made on 15 plants taking averages. The results were interpreted statistically using variance analysis (Ardeleanu and Sestras, 1996).

The varieties studied were observed and measurements made on leaf, flower and plant. At the leaf were observed: petiole length, length of leaf, leaf width and the number of leaves per plant. Characters in the bushes were pursued: plant height and diameter of the plat. In flower were observed the following characters: number of inflorescences, careen length, sepal length, sepal width, petal length, petal width.

# **RESULTS AND DISCUSSIONS**

Leaf characters are interpreted in Tables 1, 2, 3 and 4.

Table 1

No.			Petiole length	Significance of				
Var.	Variety	Absolute (cm)	Relative (%)	±d (cm)	difference from the control			
1	Miniature	32.5	85.10	-5.7	0			
2	Dwarf	3.0	81.20	-7.2	0			
3	Mandela Gold	51.0	133.50	12.8	**			
4	Average (Control)	38.2	100.00	-	-			
	DL = 4.86 $DL = 3.92$ $DL = 19.77$							

### Petiole length - synthesis results

 $DL_{5\%}=4, 86$   $DL_{1\%}=8,92$   $DL_{0.1\%}=19,77$ 

The character length of petiole varies between 31cm (,Dwarf') and 51 cm (,Mandela Gold'). If we take as reference to the average 38.2 cm we can see that the varieties ,Dwarf, and ,Miniature' have a negative significant difference and the variety ,Mandela Gold' has a distinct significant positive difference comparing with the average, makes it most valuable in terms of this character.

Table 2

No.			Leaf length	Significance of			
Var.	Variety	Absolute (cm)	Relative (%)	±d (cm)	difference from the control		
1	Miniature	22.0	84.1	-4.15	00		
2	Dwarf	29.5	112.8	3.35	**		
3	Mandela Gold	26.5	101.3	0.35	-		
4	Average (Control)	26.15	100,00	-	-		
	$DL_{5\%} = 1,14$ $DL_{1\%} = 2,09$ $DL_{0.1\%} = 4,63$						

Leaf length - synthesis results

It was found from measurements and data processing method variance analysis in variety 'Miniature' that leaf length has a distinct negative significant difference compared with the average. At 'Dwarf' variety is found at a distinct difference significantly positive while 'Mandela Gold' variety has close to the average difference being insignificant.

Table	e 3
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No.			Leaf width	Significance of			
Var.	Variety	Absolute	Relative	±d (cm)	difference from the control		
		(cm)	(%)		control		
1	Miniature	24.00	92.8	-1.85	0		
2	Dwarf	28.00	108.3	2.15	**		
3	Mandela Gold	25.50	98.6	-0.35	-		
4	Average (Control)	25.85	100.0	-	-		
	$DL_{5\%}=1,06$ $DL_{1\%}=1,95$ $DL_{0.1\%}=4,32$						

Leaf width - synthesis results

Summary analysis shows that leaf width compared with the average control (25.85 niature' variety with a leaf width of 24 cm has a negative distinction to it. We can

cm) ,Miniature' variety with a leaf width of 24 cm has a negative distinction to it. We can not say the same about ,Mandela Gold' variety has approximately equaled neither to the average values nor as ,Dwarf' variety that has a distinct significant positive difference to the average distinct showing an amount equal to 28 cm.

Table 4

# Number of leaves per plant - synthesis results

No.	Number of leaves/ plant				Significance		
Var.	Variety	Absolute (number)	Relative (%)	±d (number)	of difference from the control		
1	Miniature	8.50	95.00	-0.95	-		
2	Dwarf	8.00	89.40	-0.95	-		
3	Mandela Gold	10.50	117.30	1.55	-		
4	Average (Control)	8.95	100.00	-	-		
	$DL_{5\%}=2,8$ $DL_{1\%}=5,15$ $DL_{0.1\%}=11,41$						

Synthesis analysis of the results we see that the average is 8.95 per plant leaves and the difference between the three varieties is insignificant comparatively with average. Interpreted values of the characters of plant are presented in Tables 5 and 6.

# Table 5

Plant height - synthesis results

No.			Significance		
Var.	Variety	Absolute (cm)	Relative (%)	±d (cm)	of difference from the control
1	Miniature	99.00	94.00	-6.3	0
2	Dwarf	100.00	95.00	-5.3	0
3	Mandela Gold	117.00	111.10	11.7	**
4	Average (Control)	105.30	100.00	-	-
	<b>B</b> \ /		7 70 DL=	17.26	

 $DL_{5\%}=4,24$   $DL_{1\%}=7,79$   $DL_{0.1\%}=17,26$ 

Plant height from the variance method of data analysis shows that ,Miniature' and ,Dwarf' varieties have negative significant differences compared with the average (105.3 cm) and observe the variety ,Mandela Gold' presented distinct positive significantly difference from the average, this variety is highest of those studied.

Table 6

No.	_	Plant diameter					
Var.	Variety	Absolute	Relative	±d (cm)	of difference		
		(cm)	(%)		from the		
		× /	× /		control		
1	Miniature	79.5	81.7	-17.8	00		
2	Dwarf	96.5	99.2	-0.8	-		
3	Mandela Gold	116.0	119.2	18.7	**		
4	Average (Control)	97.3	100.0	-	-		
L	$DL_{5\%}=5.9$ $DL_{1\%}=10.84$ $DL_{0.1\%}=24.02$						

Plant diameter - synthesis results

Concerning the diameter of the bushes (Table 6) in the three varieties we can say that: ,Miniature' variety has a value of 79.5 cm in diameter which is significantly distinct negative from the control 97.3 cm, the ,Dwarf' variety mean difference is worth giving a significant negative 96.5 cm, is slightly below average. ,Mandela Gold' variety compared with the control has a significantly distinct positive difference.

Analyzed characters were in flower number inflorescences (Table 7), careen length (Table 8), sepal length (Table 9), sepal width (Table 10), petal length (Table 11) and leaf width (Table 12).

Number of inflorescences - synthesis results

Table 7

No.			Significance					
Var.	Variety	Absolute (number)	Relative (%)	±d (number)	of difference from the control			
1	Miniature	8.00	96.00	-0.33	0			
2	Dwarf	11.00	132.10	2.67	***			
3	Mandela Gold	6.00	72.00	-2.33	00			
4	Average (Control)	8.33	100.0	-	-			
	$DI_{12} = 0.14$ $DI_{12} = 1.74$ $DI_{12} = 2.5$							

 $DL_{5\%} = 0,14$   $DL_{1\%} = 1,74$   $DL_{0.1\%} = 2,5$ 

The number of inflorescence is very important in economic point of view, such as the varieties that are looking to offer more inflorescences flower as a commercial matter. After synthesis of the results we see that we have equal average value of 8,33 pieces, the "Mandela Gold" and "Miniature" varieties have values below this average, the difference is significantly negative at "Miniature", at "Mandela Gold" the difference distinct significant negative and "Dwarf" varieties have a very significant positive difference comparing with the average of experience.

Table 8

No.			Significance					
Var.	Variety	Absolute (cm)	Relative (%)	±d (cm)	of difference from the control			
1	Miniature	14,75	87,0	-2,2	00			
2	Dwarf	17,50	103,2	0,55	-			
3	Mandela Gold	18,75	110,6	1,8	**			
4	Average (Control)	16,95	100,0	-	-			
	DI = 14 $DI = 256$ $DI = 571$							

#### Length of careen - synthesis results

 $DL_{5\%}=1,4$   $DL_{1\%}=2,56$   $DL_{0.1\%}=5,71$ 

Is not decorative careen must give special attention to because it is one that protects inflorescences and has an important role in pollination. It was found that after synthesis ,Dwarf' variety have similar values mean difference being insignificant ,Miniature' variety differs significantly negative from the average, and variety ,Mandela Gold' (18.75 cm) show a distinct significantly positive difference from the average control (16.9 cm).

Table 9

No.			Significance				
Var.	Variety	Absolute (cm)	Relative (%)	±d (cm)	of difference from the control		
1	Miniature	11,80	91,8	-1,05	-		
2	Dwarf	12,40	96,5	-0,45	-		
3	Mandela Gold	14,40	112,1	1,55	**		
4	Average (Control)	12,85	100,0	-	-		
	$DL_{5\%}=1,48$ $DL_{1\%}=2,73$ $DL_{0.1\%}=6,04$						

Sepal length - synthesis results

Sepal length in terms of data analysis have values between 11.8 and 14.4 cm. ,Miniature' variety (11.8 cm) and ,Dwarf' (12.4 cm) have values below the average 12.85 cm as compared with the average, the difference being insignificant negative. At ,Mandela Gold' variety in terms of morphological character, we can say that sepal length has a

significant distinct positive difference to separate media.

Sepal width - synthesis results

Table 10

No.			Significance		
Var.	Variety	Absolute (cm)	Relative (%)	±d (cm)	of difference from the
					control
1	Miniature	3.3	102,6	0,09	-
2	Dwarf	2,5	77,8	-0,72	00
3	Mandela Gold	3,85	119,8	0,63	**
4	Average (Control)	3.22	100,00	-	-

DL<sub>5%</sub>=0,38 DL<sub>1%</sub>=0.7 DL<sub>0.1%</sub>=1,56

Analyzing the sepal width of Miniature variety in table 10 observe insignificant difference, at 'Dwarf' variety see a distinct significant negative from the control. A distinct difference can be observed in significant positive 'Mandela Gold' variety, which has a value of 3.85 cm.

#### Table 11

No.	_	]	Significance			
Var.	Variety	Absolute (cm)	Relative (%)	±d (cm)	of difference from the control	
1	Miniature	11,40	93,10	-,.85	0	
2	Dwarf	11,80	96,30	-0,45	-	
3	Mandela Gold	13,55	110,6	1,3	**	
4	Average (Control)	12,25	100,00	-	-	
$DL_{5\%}=0,66 DL_{1\%}=1,22 DL_{0.1\%}=2,69$						

Length of petals - synthesis results

Length of petals in 'Miniature' variety differs significantly negative. 'Dwarf' varieties have a slight negative difference, and the third kind studied 'Mandela Gold' has significant distinct positive difference.

Table 12

No.			Significance			
Var.	Variety	Absolute	Relative	±d	of difference	
		(cm)	(%)	(cm)	from the	
					control	
1	Miniature	2,3	83,6	-0,45	00	
2	Dwarf	2,45	89,1	-0,30	0	
3	Mandela Gold	3,5	127,3	0,75	***	
4	Average (Control)	2,75	100,00	-	-	
$DI_{roy} = 0.18$ $DI_{roy} = 0.34$ $DI_{roy} = 0.75$						

Petal width - synthesis results

 $DL_{5\%} = 0.18$   $DL_{1\%} = 0.34$   $DL_{0.1\%} = 0.75$ 

Analyzing the table 12 we see that variety 'Miniature' is a distinct difference significantly negative. 'Dwarf' variety is found in slight negative difference and the 'Gold Mandela' we able to talk about a very distinct difference significantly positive at a value of 3.5 cm from the average 2.75 cm. Following the analysis of this data we can say that 'Mandela Gold' variety has petals widest.

# CONCLUSIONS AND RECOMMANDATIONS

After the results of the three varieties of Strelitzia reginae which were analyzed at SCDP Cluj - Napoca and greenhouse of U.A.S.M.V Cluj-Napocay can assert that: - 'Miniature' variety does not show morpho-ornamental characters of paramount importance;

- 'Dwarf' variety from the experience of presented morphological characters and decorative foliage and habit of plant representative is superior to average in terms of these characters;

- 'Mandela Gold' variety presents important characters in both leaf and flower;

- 'Mandela Gold' and 'Dwarf' variety can be recommended to be used as genitors in the works of breeding;

- Variety 'Miniature' being smaller may be grown in containers and used for indoor and garden design in summer.

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### UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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

Vol. XV ( XLXI ) - 2010

# THE STUDY OF THE FENOLIC MATURATION POTENTIAL IN SOME VARIETIES OF GRAPES FOR GOOD QUALITY RED WINES IN THE CONDITIONS OF THE WINE-GROWING AREA OF SEGARCEA

Căpruciu F. D.<sup>1</sup>, Popa A.<sup>2</sup>, Căpruciu Ramona<sup>2,\*</sup>, Costea D. C.<sup>2</sup>, Cichi Daniela Doloris<sup>2</sup>

KEY WORDS: phenolic compounds, kind, insolation, precipitations

# SUMMARY

In the last two decades, there has been an increase of interest in the scientific research of viticulture and winemaking for those chemical constituents of grapes that define different types of wine.

Between 2008 - 2009 the evolution of parameters in the Fetească neagră varieties was observed, Pinot Noir (enshrined in the wine-growing area of Segarcea) and Syrah, Marselan (both Mediterranean), newly introduced to the culture to determine their fenolic maturation potential. In this respect, the main climatic parameters were monitored with direct involvement in the degree of staining substance (sunlight and rainfall), it was determined the amount of skin as a consequence of physical and mechanical analyses to establish a relationship between it and the amount of polyphenols, and to determine the quantity of total polyphenols.

### **INTRODUCTION**

The exceptional features of collection wines extracted at Segarcea prove the oenoclimatic possibilities of this wine-growing centre and recommend it for the usage of a quality viticulture (Popa, 2005). To obtain a certain type of wine, with a normal biochemical composition and pleasant organoleptic features to the winegrower and also to the oenologist must first study carefully the raw material and the phenomenon of maturation of grapes (Popa, 2008).

The polyphenols represented primarily by anthocyanins and tannins play an important part in the metabolism and development of the wine, equal to sugar, acids and proteins and decisively influence chemical and organoleptic characteristics of red wines (Gheorghită *et al.*, 2006; Kennedy, 2008).

Specialized works show that grapes pigmentation is much better as the duration of sunshine is longer and of course (Cortell and Kennedy, 2006), when it's associated with an increased heat treatment. The phenolic compounds present in grapes and wine have

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beneficial health effects as in anti-cancer effects, protection against cardiovascular diseases and when it comes to red wines, they are responsible for lowering the cholesterol.

The influence of the climate on the composition of grapes and hence on the production obtained has been studied by many researchers (Costea *et al.*, 2008). Due to the change in weather conditions in the past several years, rigorous monitoring is required in order to ascertain the degree of water stress and heat that the vine is subjected to with consequences on the potential quality.

# MATERIAL AND METHOD

The monitoring of the climatic data was performed using the weather station positioned in the wine-growing are of Segarcea.

The experiments were carried out in the wine-growing regions of Segarcea in plots located in the same orographic conditions (plateau) after randomized block method.

To carry out the lab analyses variety grapes as Fetească neagră, Pinot noir, Syrah and Marselan were used as biological material ,these varieties being harvested from early August to late September.

The extraction of coloring matter was made spectrophotometrically using the method of Puissant A. and Huguette Leon, 1967. The dynamic of *phenolic compounds* was monitored for total polyphenols starting August 1<sup>st</sup> until September 29<sup>th</sup> every five days.

The Physico-mechanical analysis was carried out of 100 grains during the study period on the varieties mentioned above in order to determine the amount of skin to achieve a correlation between it and the pholyphenol contained of the varieties studied. Weighing the total weight and quantity of each variety of studied skin was done in the laboratory with an analytical scale with four decimals. The results were listed in a register especially created for this purpose.

# **OBSERVATIONS AND DETERMINATIONS**

The climate monitoring was performed in order to asses the impact of climatic indicators on the qualitative behavior of the varieties analysed. To this effect, the illumination resources were evaluated as well as precipitation under known thermal conditions.

The duration of sunlight variates every day, the requirements of the vine are different depending on the species, variety, age and stage of vegetation. This is expressed by the annual total sum of hours of sunlight ( $\Sigma$ ia potential) and the amount of hours of actual brightness (actual  $\Sigma$ ir). In terms of  $\Sigma$ ir following the calculations there is a higher favorability in 2009 (1827,9 hours 1684,6 hours to 2008 hours), and data regarding  $\Sigma$ ia indicates the presence of light in abundance, especially in ripening period (2438,7 hours 2397,7 hours in 2008 and 2009) - Table 1. We can notice from figure 1 small amounts of precipitation throughout the years of study (especially during the ripening of grapes). These aspects are extremely important, the quantity of grapes analyzed in dye content depend on the quantity of insolation. By analyzing Table 2 we can see that both 2008 and 2009 show a variation in rainfall during the growing season, low values standing out during the maturation of grapes (2,4 mm/m<sup>2</sup> in 2008 and 6,6 in mm/m<sup>2</sup> 2009), Figure 2, Table 2.

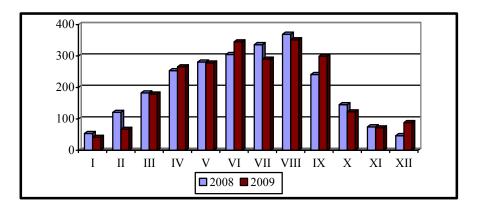


Figure 1 The insolations registred in the years of study in the wine-growing district Segarcea

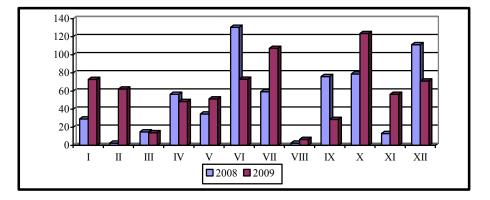


Figure 2 The precipitations nivel registred in the years of study in the wine-growing district Segarcea

In terms of coloring material to extract a red wine with sanogenic and olfactory properties in line with EU requirements, it is necessary to harvest the grapes at the right moment. This way we ought to follow the curves of maturation as well as the phenolic analyses at intervals rigorously established. The lack of rainfall during maturation, correlated with a period of intense sunshine and higher temperatures are defining factors for the biosynthesis of the coloring matter. Thus, we can notice from Figure 3 and 4 an increase of the value of total polyphenols with a constant maximum academic year in late August 2008 of Fetească neagră varieties (5106 mg/kg) and Marselan (5532 mg/kg) and early September the varieties of Pinot Noir and Syrah (4851 respectively 5223 mg/kg) - Figure 3. The year 2009, is remarkable for slower and delayed accumulation of polyphenols (eg Feteasca neagră variety reaches its top in early September when it accumulates 5002 mg/kg total polyphenols) –Figure 4. The sort with the maximum potential in accumulating dye material during the years of study proved to be Marselan with a maximum of 5532 mg/kg in 2008. As noted, the biosynthesis of the coloring material is performed according to climatic conditions (light, temperature, precipitation) but also variety.

Regarding the variety, the quantity of skin from physical and mechanical tests during ripening varieties analyzed (Table 3) has is very important in determining the quantity of staining substance. The mechanical analysis on the skin of the varieties studied is extremely important given that the skin is the place synthesize and form red anthocyanin pigments which together with the sugars and acidity lead to the final step in obtaining the best quality in red wines. The amount of skin resulted from 100 grapes recorded large differences from one variety to another within the same kind but also within the same kind in different years of study.

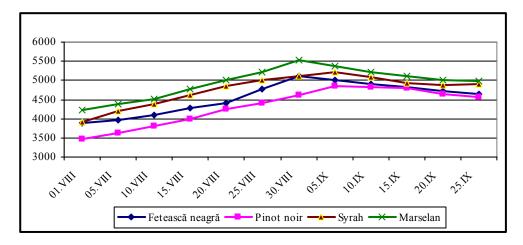


Figure 3 The dynamic of total polyphenols (mg/Kg) by the grapes variety studied in the wine-growing district Segarcea -2008

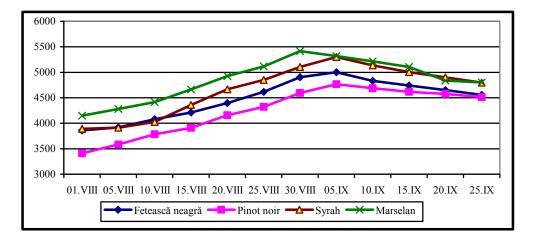


Figure 4 The dynamic of total polyphenols (mg/Kg) by the grapes variety studied in the wine-growing district Segarcea – 2009

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∑ir (ore)		1684,6	1827,9	1756,2
Žia	∑ia (ore)		2397,7	2418,2
	¥	46,8	88,4	67,6
	X	74,8	71,5	73,1
	Х	145,4	122,6	134,0
	ы	241,2	298,8	300,8
	ΠΛ	369,0	351,2	360,1
ı (hours)	ΠΛ	335,9	289,9	312,9
Insolation (hours)	М	304,7	344,6	324,6
	Δ	280,6	277,8	279,2
	N	253,2	265,6	259,4
	日	182,6	178,8	180,7
	н	120,7	66,99	93,8
	н	53,8	41,6	47,7
Year study		2008	2009	Med. 2008-2009

Table 2

The hygroscopicity registred in the wine-growing district Segarcea (2008-2009)

Insolation (hours)

608,4 715,0 661,7

315,0

56,4 34,7

123,6 101,2

28,6 52,3

4,5 6,6

101,7 73,0

32,3 62,2

Media 2008-2009

72,8 51,0

2009 2008

337

 $\overset{(mm)}{\succeq}^a$ 

 $\sum_{\mathbf{p}.\mathbf{v}.}$ 359,0

> 111,2 71,0 91,1

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X 78,8

M N N

VⅢ 2,4

<u>VП</u> 59,2 107,2 83,2

VI 130,4

34,6 51,2 42,9

56,4 48,4 52,4

日4,8 14,0 14,4

1<sup>2,4</sup>

29,2

Year study

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The insolations registred in the wine-growing district Segarcea (2008-2009)

Table 3 shows that the largest amount of skin were made in 2008 out of Marselan kind (19,75 g) followed by Syrah kind (19,02 g) and the lowest percentage out of Pinot noir 12,42 %, 2009 stand for a good amount of skin collected but below the level calculated in 2008. Significant differences from year to year have been registered in the Fetească neagră kind 17,16 g in 2008 and 16,11 g in 2009.

The mechanical analysis on the varieties studied in the wine-growing district Segarcea (2008-2009)

		× ×	,		Table 3	
Sorts						
Parameters	U.M.	Fetească	Pinot			
	(g,%)	neagră	noir	Marselan	Syrah	
			100 grapes			
Total weight	g	120,71	124,72	117,04	143,02	
Skins 2008	g	17,16	15,50	19,75	17,20	
	%	14,21	12,42	16,87	12,02	
Total weight	g	118,53	121,14	104,50	138,51	
Skins 2009	g	16,11	15,77	19,02	17,78	
	%	13,59	13,01	18,20	12,83	

### CONCLUSIONS

Because of the drought at the end of the vegetation period (both 2008 and 2009) in conjunction with the temperature and the duration of sunshine, increased during ripening in grape varieties it covers a good dynamic of the total pholiphenols with a high phenolic potential of the Marselan kind followed by the Feteasca neagra and Syrah. Pinot Noir was characterized by a decreased phenolic potential during the years of study.

At the same time, we can see after the physical and mechanical analysis that the variety with the highest percentage of skin (Marselan) has accumulated the highest amount of total polyphenols.

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Vol. XV ( XLXI ) - 2010

# THE ACCLIMATIZATION OF GRAPES OF MEDITERRANEAN ORIGIN TO THE WEATHER CONDITIONS OF THE WINE-GROWING REGIONS OF SEGARCEA

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**KEY WORDS**: the grape varieties, climatic conditions

#### SUMMARY

This study was carried out during 2008-2009 on plots belonging to the wine-growing areas of Segarcea, famous wine areas in point of extracting red wine with remarkable qualities in the south of the country. The significance of the work consists in improving the useful climate of the wi ne area of Segarcea by introducing into the culture beside black grapes varieties, renowned varieties like Fetească neagră, Merlot, Cabernet Sauvignon, Pinot noir, other grape varieties of Mediteranean origin, such as Syrah and Marselan.

The aim is to rigorously monitor the weather conditions with a direct impact on the soils with a production potential, observing the dynamics of its establishment and maturation index for each variety during the period studied.

#### **INTRODUCTION**

Thorough knowledge of environmental factors as well as the opportunities of taking control over them by means of cultivation technologies, it is an essential condition to obtain high yields of grapes, top quality, in terms of increased economic efficiency (Barbeau *et al.*, 2003; Tonietto *et al.*, 2004; Deloire *et al.*, 2005; Dejeu , 2010).

Because of the change in weather conditions over the last years, strict monitoring is required in order to ascertain the degree of water stress and heat the wine is subjected to with potential repercussions on the quality (Cichi Daniela Doloris *et al.*, 2008; Popa, 2005, 2008).

To decide which is the best moment for harvesting the grapes, several parameters and subscripts of maturity are used (Abbal *et al.*, 1992; Carreno *et al.*, 1995).

# MATERIAL AND METHOD

The climate data was supervised with the help of the weather station settled in the vineyard of Segarcea. The biological material used for analysis consisted of grape varieties belonging to the newly introduced Mediterranean origin (fourth year after planting), Syrah

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and Marselan. The test was done by using the method of randomized block with four variants, representing the varieties studied, constitutes studied varieties grown under identical adaphic and orographic (plateau) conditions.

The qualitative analysis of the varieties already mentioned was carried out every five days during the process of maturation and included: establishing the weight of 100 grains using an laboratory automatic analytical scale, determining the sugar by using the refractive method (Abbe refractometer), determining the total acidity by using the process of maturity of the varieties of Mediterranean origin which was traced by determining the glucose-acidimetric index rendered in the formula: IGA = G / TA, where G stands for sugars (carbohydrates) and At for total acidity of the soil.

# **OBSERVATIONS AND DETERMINATIONS**

Climatic factors were monitored in order to asses restrictive climatic indicators with an impact on the bioproductive an qualitative behavior of the soils being analyzed, the data being processed and analyzed in light of the general climatic indicators (unicriterial).

Data regarding the assessment of heat (absolute minimum and maximum temperatures, average temperatures), the assessment of light resources, the quantity of precipitation as well as the relative humidity in the air are climate factors which were processed and analysed.

The thermal resources constitute the permanent element that, being variable, causes the most important manifestation of the vine. It is also known the lower threshold that triggers the biological processes of the vine at 10°C. From the data given in Table 1 it is noted that this threshold was reached both in 2008 as well as in late spring of 2009 in Segarcea, when the average monthly temperatures were  $12,2^{\circ}C$  13,5° correlated with absolute minimum temperatures 2,1°C 3,1°C. A criteria to asses the condition for the quality of grapes is indicated by the average temperature of the warmest month (July and August), which in Romania is a minimum of 18,5°C and maximum of 23,2°C. We noticed that in 2008 the temperatures exceeded the maximum monthly average (24,5°C), the year 2009 being optimal in this concern (22,8°C)- Table 1. Exceeding the maximum threshold allowed for our country (in 2008), is closely related to accumulating compounds that give black grapes and later the red wine the quality required.

The absolute maximum temperature in August is a restrictive factor concerning the quality of grapes when the temperatures exceed 42°C. When air temperature reaches this value the process of photosynthesis is blocked leading to increased respiration and evapotranspiration, processes leading to lower levels of organic acids (malic acid), sugars and aromatic substances.

This phenomenon was not recorded during the study analyzed, the maximum of temperature reached was of 38°C in August 2008 and 37,2°C in 2009. Average maximum temperature in August is a restrictive factor when it exceeds 34°C. During the reported period the value of this factor was 31,5°C in 2008 and 29,6°C in 2009 (Table 1).

The requirements for the soils analyzed for moisture content were estimated by precipitation and relative humidity (hygroscopicity). Overall, the years studied, represented in terms of hygroscopicity a normal year for an optimal growth of vines (values ranged from 50% in July and August to 82-93% in the rest of 2008).

We can also observe that the average recorded UR/2008 72,9% is different from 2009 (78,1%). During the vegetation RH (%) it had optimal values (64,0% in 2008 and 68,6% in 2009) - Table 2.

We can say, in terms of rainfall, looking at the data given in Table 2, the period studied was characterized by fluid deficit which required the provision of a fluid through the irrigation system. We notice from this view restrictive August rainfall was losing 2,4 mm (2008) and 6,6 mm in 2009-Table 2.

Table 1

Climatic parameters (temperature) registred in the year 2008 and 2009, in the wine-growing				
district Segarcea				

	Air temperature (°C)						
Month	Absolute	Absolute maximum		Month medium		Absolute minimum	
	2008	2009	2008	2009	2008	2009	
Ι	9,8	12,4	-3,6	-0,9	-17,2	-13,0	
II	18,5	16,4	3,2	1,2	-9,7	-11,6	
III	21,2	18,5	8,5	9,2	-4,9	-2,0	
IV	24,7	25,3	12,2	13,3	2,1	3,1	
V	34,0	34,2	16,8	17,3	4,2	4,9	
VI	35,2	34,3	21,3	20,1	9,1	6,5	
VII	36,2	37,1	23,8	23,3	11,1	11,1	
VIII	38	37,2	24,5	22,8	12,6	13,2	
IX	36,2	34,6	16,7	20,1	4	7,3	
Х	24,4	27,9	12,5	11,6	0,6	-1,1	
XI	22,6	18,7	6,1	7,4	-7,1	-0,9	
XII	15,5	11,0	2,0	-0,5	-12,1	-19,4	

The light varies daily, the requirements of the vine varieties are different as for variety, age and stage of vegetation. This is expressed by the total amount of annual hours of sunlight (potential) and the amount of actual brightness (actual).

As for  $\Sigma$ ir, following the estimation there is a higher favorability in 2009 (1827,9, 1684,6 hours to 2008 hours), and data regarding  $\Sigma$ ia indicates the presence of light in abundance, especially in ripening period (2438,7 hours 2397,7 hours in 2008 and 2009) - Table 2. These issues are extremely important, dependent insolation quantities of grapes analyzed in dye content.

In the last two decades, there has been an increasing interest in the scientific research in viticulture and winemaking for those chemical constituents of grapes which define different types of wine. The research on the potential quality of grapes is significant .In this respect, the curve of aging was tracked by determining the sugar ,acidity and weight of 100 grains) for each species examined at intervals rigorously established.

Due to small amount of rainfall recorded during the growing and maturation processes, a steady increase in grain weight was recorded, without significant changes. Among the Mediterranean soils studied, Syrah comes forward ,covering a weight at harvest of 141,0 per 100 grains per 100 grains in 2008 and 140,1 in 2009, while Marselan variety, although it had a uniform growth, not managed to accumulate more than 115,3 g/100 grains in 2008, 114,1 g/100 grains in 2009 (Table 3).

Due to the draught during the maturation phase (2,4 and 6,6 mm/m<sup>2</sup> mm/m<sup>2</sup> in 2008 in 2009) there are noticeable rapid decreases in acidity in all species analyzed until the first half of September. In point of acidity, both varieties recorded pH values approximately equal when they reached full maturation (Syrah 4,6 g/l and Marselan 4,5 g/l in 2008). The year 2009 is remarkable for a more increased acidity in ripening grapes (Syrah 4,8 g/l and

Marselan 4,7 g/l) - table quantitative 3. The progressive accumulation, more or less spectacular, lead to obtaining a superior level of sugars in maturation, different in varieties, with a maximum of the variety Marselan (277 g/l) and a lower quantity of the variety Syrah (minimum 248 g/l), in 2008. By paying attention to the dynamic of the maturation index in Figure 1, we notice that during the period studied, with climatic conditions already established , all the varieties meet the conditions necessary for obtaining high quality red wines, the best ripening being made by Marselan, closely followed by Syran .

Table 2
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Month	Precipitati	ons (mm)	A relative 1	noisture			solations (hours)	
			(%	<b>o</b> )	Σ	]1	∑ia	∑ir
	2008	2009	2008	2009	2008	2009	2008/ 2009	2008/ 2009
Ι	30,4	72,8	91	92	53,8	41,6		
II	2,4	62,2	77	86	120,7	66,9		
III	14,8	14,0	63	81	182,6	178,8		
IV	56,4	48,4	76	77	253,2	265,6		
V	34,6	51,2	71	72	280,6	277,8		
VI	130,4	73,0	70	75	304,7	344,6	2438,7/	1684,6/
VII	59,2	107,2	50	66	335,9	289,9	2397,7	1827,9
VIII	2,4	6,6	50	59	369,0	351,2		
IX	76	28,6	67	63	241,2	298,8		
Х	78,8	123,6	82	84	145,4	122,6		
XI	13	56,4	85	90	74,8	71,5		
XII	111,2	71,0	93	93	46,8	88,4		

The hygroscopicity and insolations registred in the wine-growing district Segarcea

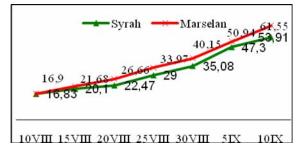


Figure 1 The dynamic of the maturation index by Marselan and Syrah registred in the winegrowing district Segarcea

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Parametrii	Soiuri	III A O I	111	11V21	III	207	20 V I II	25VIII	III	30 V III	III	31	SIX	10:	10IX
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Greutatea a	Syrah	85,2	89,2	98,6	99,4	107,6	108,2	119,7	120,3	128,3	127,5	137,4	156,2	141,0	140,0
	Marselan	68,3	70,1	73,9	74,2	80,3	6'08	87,8	89,2	97,4	98,3	107,9	106,1	115,3	114,1
Aciditate	Syrah	10,1	9,8	9,3	8,7	8,5	8,1	7,0	7,3	6,1	6,5	5,2	5,4	4,6	4,8
(g/1H2SO4)	Marselan	11,0	10,8	9,5	9,6	8,7	∞ ∞	7,3	7,5	6,6	6,8	5,3	5,7	4,5	4,7
Glucide (g/l)	Syrah	170	173	187	182	191	190	203	209	214	220	246	243	248	249
	Marselan	186	185	206	200	232	229	248	240	265	258	270	269	277	275

Tabel 3

## CONCLUSIONS

Both 2008 and 2009 were, in terms of temperature and RH (%), average years for best conditions of growth of the vine.

We can notice a steady increase in grain weight of the varieties analyzed by the end of August, after which, due to the precipitations in September 2008, compared to the same period in 2009, there was a change in the ratio of weight without any significant differences being registered in the harvest. It is the same with determining the amount of acid content.

In terms of accumulation of carbohydrates, we can notice that the variety Syrah shows a smaller amount when compared to Marselan all the way until the harvest.

After determining the quality parameters of the Mediterranean varieties studied, we can say that both Syrah and Marselan were adapted to the climate of wine-growing region of Segarcea which brings together all the necessary conditions to obtain high quality red wines.

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# UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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

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# ARTHROPODS BIODIVERSITY IN APPLE ORCHARDS UNDER INFLUENCE OF CHEMICAL PEST CONTROL TREATMENTS: ASPECTS OF SOME ECOLOGICAL PARAMETERS

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KEY WORDS: biodiversity, pest and beneficial arthropods, ecological parameters, chemical control, apple orchards

## ABSTRACT

Investigations concerning biodiversity of arthropods, using the analytical ecology indexes (abundance, dominance, constancy and the ecology significance index), were performed on two apple orchards in 2007. In each apple orchard were designed two plots of 0.5 ha where two different chemical pest control programs have been applied (a standard treatment program and the other with low toxicity products treatments), resulting four experimental plots. The beating and sticky yellow traps methods were used to sample the arthropods. The results have shown the presence of a higher number of arthropod species collected on the low toxicity products than on the standard program plots. The most abundant pests belonging to Aphididae, Cicadellidae, Thripidae, Phlaeothripidae, Latridiidae, Brachycera taxons have shown to be eudominant and euconstant groups. Cecidomyidae, Nematocera, Chalcididae, Formicidae, Coccinellidae, Diptera and Aranea taxons were emphasized as beneficial arthropods.

# INTRODUCTION

Biodiversity is the main element enable assuring the balance, stability and support capacity of orchard systems. The apple orchard systems having a perennial characteristic favor the development of an abundant arthropods community, in the same time offering the opportunities for different ecological management scenario (Brown and Adler, 1989). The inappropriate entropic activities, such as the use of inadequate doses of pesticide applications, will enhanced the orchard biocenose vulnerability favoring crucial changes in its structure and composition (Blommers, 1994; Brown and Welker, 1992; Miliczky et al., 2000; Suckling et al., 1999).

The purpose of this study is to present a comparative sinecological analyze of the arthropods collected from two apple orchards, where two different chemical phytoprotection systems have been conducted: one with low toxicity and another with standard products. It has been determined the species composition and ecological parameters of the pest and beneficial arthropods.

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## MATERIALS AND METHODS

The observations has been carried out on two apple orchards, one placed in hilly area (Baicoi-Prahova) and the other in plain field conditions (Baneasa-Bucharest). In each orchard were designed two experimental plots of 0.5 ha, where have been used separately two chemical pest control programs. One program has been applied low toxicity products (Calypso 480SC-0,02 %, Rimon 10EC-0,06%, Mitestar-1,5%, Demitan 200SC-0,07%) and the other program has been applied standard chemical products (Agrozim 40EC-0,075%, Sinoratox 35EC-0,15%, Karate 2,5EC-0,1%, Demitan 200SC-0,07%). A number of four test plots have resulted, where the arthropods have been collected. The methods for the arthropods collection were branch beating and sticky yellow traps. Twice a month, 50 young branches have been beaten and two sticky yellow traps were placed on each test plot and changed at every two weeks. The arthropods have been separated in two main communities: pest and beneficial arthropods. In order to determine ecological parameters of the collected arthropods, separately by location and treatment programs, abundance, dominance, constancy and ecological significance index have been used (Stan, 1994).

# RESULTS

The arthropods collected on the observed orchards summed a number of 2973 specimens, out of which 1551 in Baneasa and 1422 in Baicoi orchard respectively (table 1).

#### Table 1

	Ban	easa	Bai	coi
	Low toxicity	Standard	Low toxicity	Standard
Pest arthopods	449	394	400	443
Yellow sticky traps	249	184	268	325
Branch beating	200	210	132	118
Beneficial arthropods	359	349	285	294
Yellow sticky traps	137	149	165	190
Branch beating	222	200	120	104
Total	808	743	685	737

Total number of arthropods specimens collected in the apple orchards chemical treated, distinct on the experimental plots and the sampling methods, 2007

The pests recorded a percentage of 53.71% and beneficial 43.29% out of arthropods collected over all plots during the study period. The approximately similar percentage of arthropods reported on both apple orchards as well as on both type of the experimental plots (low toxicity and standard products).

The quantitative analysis of total arthropods showed that on the low toxicity experimental plots has been collected a higher individuals number in Baneasa (808 specimens) and a lower individuals number in Baicoi orchard (685 specimens), compared to the standard treatment plots (743 and 737 specimens, respectively). The greater numerical difference recorded at the pest arthropods collected on colored traps and the beneficial arthropods collected by branch beating method.

In the tables 2, 3, 4 and 5, the specific composition of arthropod taxons collected on the two apple orchards are presented, separately on the two types of chemical pest control program applied and the collection methods implied. Our results showed a rather different situation regarding the qualitative composition of the arthropod community recorded on the two orchard locations. The arthropods caught on the yellow sticky traps (table 2 and 4) have shown a greater number of species compared to branch beating method (tables 3 and 5) in case of both apple orchards. The specific composition of pest arthropods showed a greater number of taxons (23-20 taxons) in Baneasa apple orchard compared to Baicoi apple orchard (9-24 taxons) by the two collection methods. In Baneasa apple orchard, pest arthropods on the yellow sticky traps belonging to 13 taxons on the low toxicity plots and 11 taxons on the standard plots. In Baicoi orchard, the number of taxons on the both variant plots has showed to be equal (12 taxons). The beating samples counted a less taxons number, 11 taxons at Baneasa and 4 taxons at Baicoi on the low toxicity plots, compared to the standard plots, 12 taxons and 5 taxons respectively.

The specific composition of beneficial arthropods showed to be slightly higher than pests, except in Baneasa orchard yellow sticky traps captures where taxons number of pest and beneficial arthropods showed to be approximately equal. The beneficial as well as pests showed a greater number of taxons in Baneasa (13–19 taxons) in opposition to Baicoi orchard (8–16 taxons). This situation might be the consequence of the two orchards treatments history applied before starting this study, when in the Baneasa orchard were applied less aggressive treatments together with some biotechnical means such as pheromones traps against Lepidoptera pests. Moreover, Baneasa orchard has been advantaged by the genetically resistance characteristics of apple cultivars against scab and powdery mildew. The beneficial caught on the yellow sticky traps (table 4) recorded an equal number of taxons in both chemical systems in Baneasa orchard, and a less number on the low toxicity plots than standard plots in Baicoi orchard. The beneficial collected by the beating method on the low toxicity plots (table 5) showed a greater number of taxons than on standard plots in both localities.

Aiming acknowledge the structural particularities, as well as ecological characteristics of arthropod communities, both pest and beneficial, the sinecological analysis have been chosen. The comparative analyse was carried out between the four experimental plots established in Baneasa and Baicoi orchards and was expressed in values of the basal ecological parameters – abundance, dominance, constancy and the ecology significance index. The observed values of the arthropods ecological parameters are given in tables 2-5.

The pest arthropods collected on yellow sticky traps (table 2) showed the greatest abundance of Homoptera (Aphididae, Cicadellidae), Thysanoptera (Thripidae, Phlaeothripidae), Diptera (Brachycera) groups, these are known to be especially optically attracted by yellow sticky traps and which gained eudominant and euconstant character in the test plots of both chemical control systems and orchard locations. The higher abundance also showed the mites, reaching an eudominant character in standard plots of both locations. On the constancy point of view, mites showed a constant and accessory character in Baicoi orchards low toxicity plots. Coleopterans have shown an eudominant and euconstant-dominant character in Baicoi orchard plots. The pest arthropods collected by branch beating (table 3) has shown a euconstant-dominant ecological character for the following taxons: Homoptera (Aphididae/Aphis fabae Scop) and Diptera (Brachycera) in both test plots of Baneasa orchard. These taxons reached also the highest level of ecological signification value.

Among the beneficial arthropods collected on yellow sticky traps (table 4), Hymenoptera (Chalcididae, Braconidae, Formicidae), Coleoptera (Coccinelidae) and Diptera have shown eudominant-euconstant characters, Coccinellidae, Coccinella 7punctata L. and Chilocorus bipustulatus L., have been constant-dominant in standard plots on the same orchard. The eudominant-euconstant character in the Baicoi orchard plots has been reached by Cecydomyidae and Chironomidae taxons. All these taxons reached the highest ecological signification values, proofing a good adaptation to the ecological factors created on experimental plots. Aranea and Hymenoptera taxons (four families all-together) collected by branch beating (table 5) showed eudominant-euconstant character in the samples of both orchards and types of plots, and showed the highest ecological significance value. In Baneasa orchard, predators belonging to Heteroptera and Coleoptera have gained an eudominant-euconstant character. For the Heteroptera order, this character has been gained by Deraeocoris lutescens Schilling mirid, well-known specie in the apple orchards of Baneasa area (Balan et al., 2001). Coccinellidae (Adalia bipunctata L. and Chilocorus *bipustulatus* L.) have been better represented in both types of plots. The two species showed a subdominant character in low toxicity plots and recedent species character in standard plots. In addition, these species have shown an accessory character, but Chilocorus bipustulatus L. has shown the constant character in low toxicity plots. In the Baicoi apple orchard the eudominant-euconstant character have been reached by Dipterans on both experimental plots.

#### CONCLUSIONS

The analysis of the result data during 2007 in the Baneasa and Baicoi apple orchards managed by different chemical pest control systems might lead to the following conclusions:

The chemical pest control system intensity in the apple orchards have caused changes in abundance, spectrum and composition of arthropod species associated to apple orchards.

The pest and beneficial arthropods have counted a greater number of taxons with higher number of individuals in Baneasa apple orchard, where has been applied an integrated pest control system in the years prior to this study, opposing to Baicoi orchard where the protection system applied has been chemically intensive.

The pest arthropods, Aphididae, Cicadellidae, Thripidae, Phlaeothripidae, Brachycera collected on yellow sticky traps, on both control chemical system in both location have shown eudominant and euconstant ecological character as well as Aphididae and Brachycera collected by branch beaten in Baicoi orchard and Latridiidae (*Corticarina gibbossa*) in Baneasa orchard on both experimental plots. These have also reached the highest values of ecological significance index.

The beneficial arthropods, Chalcididae, Braconidae, Formicidae, Coccinelidae (*Coccinella 7 punctata* and *Chilocorus bipustulatus*) and Diptera on yellow sticky traps as well as Aranea and Hymenoptera in branch beating samples, have shown the eudominant and euconstant character and also have reached the highest values of ecological significance index on the low toxicity test plots.

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				Bai	Baneasa							Bai	Baicoi			
Taxons		Low-to	Low-toxicity plot	lot		Stand	Standard plot			Low-tox	Low-toxicity plot	Ħ		Stand	Standard plot	
	A	Ω	ບ	M	Υ	Ω	ບ	M	Α	D	ບ	W	Α	Ω	ບ	W
ACARINA/Tetranychidae	24	9.7	25	2.4	19	10.3	25	2.6	46	17.2	66.6	11.4	34	10.5	50	5.3
THYSANOPTERA	51	20.5	100	10.2	65	35.3	50	17.7	52	19.4	100	19.4	60	18.5	100	18.5
Thripidae/Thrips validus Vzel.	26	10.5	75	7.9	32	17.4	20	8.7	52	19.4	100	19.4	60	18.5	100	18.5
Phlaeothripidae/Haplothrips niger Osb.	25	10.0	87.5	00 00	8	17.9	50	∞ ∞								
utato		0.4	12.5	0.1												
HOMOPTERA	82	32.9	12	32.9	5	31.0	101	31.0	81	30.2	100	30.2	92	28.3	100	28.3
Aphididae	46	18.5	10	18.5	33	19.0	100	19.0	32	11.9	83.3	9.9	34	10.5	83.3	8.7
Aphis pomi De Geer									32	11.9	83.3	9.9	34	10.5	83.3	8.7
Aphis fabae Scop.	46	18.5	10	18.5	8	19.0	100	19.0								
Psyllidae/Psylla mali. Schm.	9	2.4	12.5	0.3					15	5.6	50	2.8	17	5.2	50	2.6
Cicadellidae	8	12.0	10	12.0	5	12.0	100	12.0	34	12.7	100	12.7	41	12.6	100	12.6
Empoasca solani Curtis	27	10.8	100	10.8	21	11.4	75	8.6	33	12.3	100	12.3	40	12.3	100	12.3
Macrosteles laevis Ribaut	3	1.2	12.5	0.2		0.6	12.5	<0.1	1	0.4	16.6	<0.1	1	0.3	16.6	<0.1
HYMENOPTERA/Tenthredinidae/	12	4.8	25	1.2	m	1.6	12.5	0.2	Ĺ	2.6	16.6	0.4	15	4.6	33.3	1.5
Hopiocampa testuainea Kuug.	-	1		1	+				;				1			
COLEOPTERA	25	10.0	75	7.5	£	7.1	20	3.5	65	24.3	100	24.3	98	30.1	100	30.1
Apionidae/Apion ervi Kirbi	ω.	1.20	12.5	0.2	~	1.1	12.5	0.1								
Nitidulidae/Meligethes aeneus F.	18	7.2	75	5.4	Ξ	6.0	62.5	3.7	30	11.2	83.3	9.3	40	12.3	66.6	8.2
Buprestidae/Agrilus simatus 01.	4	1.6	12.5	0.2					3	1.1	33.3	0.4	6	2.8	20	1.4
Elateridae/Agriotes ustulatus L.									m	1.1	33.3	0.4	m	0.0	16.6	0.1
Bruchidae/Spermophagus sericeus Geoffr.									29	10.9	50	5.5	46	14.1	66.6	9.4
LEPIDOPTERA	2	4.0	20	50	4	2.2	25	9.0								
Lyon etida e/Leu coptera scitella Zell.	5	2.0	37.5	0.8 0	4	2.2	25	0.0								
Gracilariidae/Omix guteea Hw.	7	8.0 8.0	12.5	0.1												
Yponomeutidae/Yponomeuta malinelus Zell.	m	1.2	12.5	0.4												
DIPTERA/Brachycera	44	17.7	10	17.7	53	12.5	100	12.5	17	6.3	100	6.3	26	8.0	100	8.0
		-														

Table 2 Specific composition and ecological parameters of pest arthropods collected on yellow sticky traps in the apple orchards chemical treated, 2007

Abundance (A), dominance (D), constancy (C) and the ecology significance index (W)

				Baneasa	asa							Bai	Baicoi			
Taxons	Г	0W-toX	Low-toxicity plot	t		Standa	Standard plot		Г	ow-tox	Low-toxicity plot	ot		Stand	Standard plot	+
	Υ	D	υ	Μ	Α	D	C	M	Υ	Ω	C	Μ	Υ	Ω	Ð	Μ
THYSANOPTERA Thripidae/Thrips fuscipennis Holl.	52	26.0	75	19.5	48	22.8	62.5	14.3								
HOMOPTERA	4	2.0	25	0.5	26	12.4	75	9.3	2	71.2	100	71.2	82	69.5	8	69.5
Aphididae/Aphis fabae Scop.					16	7.6	50	∞. M	2	71.2	100	71.2	74	62.7	8	62.7
Psyllidae/Psylla mali Schm.													œ	6.8	16.6	1.1
Delphacidae/Javesella pelucida F	4	2.0	25	0.5	2	4.8	50	2.4								
COLEOPTERA	116	58.0	100	58.0	108	51.4	100	51.4	10	7.6	33.3	2.5	12	10.2	33.3	3.4
Nitidulidae/Meligethes aeneus F.	34	17.0	50	8.5	∞	3.8	25	0.9								
Bruchidae/Spermophagus sericeus Geoffr.									10	7.6	33.3	2.5	12	10.2	33.3	3.4
Latridiidae/Corticarina gibbossa Hrbst.	40	20.0	100	20.0	56	26.7	100	26.7								
Chrysomelidae	42	21.0	50	10.5	44	20.9	50	10.5								
Chaetocnema tibialis Illig.	10	5.0	25	1.3	14	6.6	25	1.7								
Haltica tamarius Schrauk.	2	1.0	12.5	0.13	6	2.9	25	0.7								
Haltica oleracea	~	4.0	37.5	1.5	<sup>∞</sup>	3.8	25	0.9								
Aphthona cyfarissiae Kock	12	6.0	25	1.5	ø	3.8	25	0.9								
Aphthona euphorbiae Schn.	10	5.0	37.5	1.9	×	3.8	25	0.9								
LEPIDOPTERA	14	7.0	37.5	2.6	9	2.9	25	0.7	2	1.5	16.6	0.2				
Geometridae	14	7.0	37.5	2.6	9	2.9	25	0.7								
Tortricidae/Adoxophyes veticul ana Hb.									2	1.5	16.6	0.2				
DIPTERA/Brachycera	14	7.0	37.5	2.6	22	10.5	37.5	3.9	26	19.7	100	19.7	24	20.3	100	20.3

Specific composition and ecological parameters of pest arthropods collected by branch beating in the two apple orchards chemical treated, 2007

Abundance (A), dominance (D), constancy (C) and the ecology significance index  $\left(W\right)$ 

Table 3

				Baneasa	asa							Ba	Baicoi			
Taxons		Low-to:	Low-toxicity plot	r T		Stand	Standard plot			Low-to	ow-toxicity plot	lot		Stand	Standard plot	
	A	Ω	ບ	M	A	Ω	ບ	M	A	Ω	ບ	M	Α	Ω	ບ	M
ARANEAE	9	6.5	62.5	4.1	5	3.3	50	1.7	14	9.0	33.3	3.0	ο	4.7	33.3	1.6
NEUROPTERA/Chrysopidae/ Chrysoperla camea Steph									2	1.3	33.3	0.4				
HYMENOPTERA	58	42.3	100	42.3	45	30.2	100	30.2	51	32.7	100	32.7	89	35.8	100	35.8
Ichneumonidae									2	1.3	33.3	0.4	4	2.1	33.3	0.7
Chalcididae	8	21.9	100	21.9	22	14.8	100	14.8	29	18.6	100	18.6	37	19.5	100	19.5
Braconidae	15	10.9	100	10.9	13	8.7	100	8.7	10	6.4	83.3	5.3	22	11.6	83.3	9.7
Formicidae	13	9.5	100	9.5	2	6.7	100	6.7	10	6.4	83.3	5.3	ŝ	2.6	66.6	1.7
COLEOPTERA	33	24.2	100	24.2	78	18.9	100	18.9	17	10.9	83.3	9.1	28	14.8	83.3	12.3
Coccinelidae	26	19.0	87.5	16.6	22	14.7	50	7.4	12	7.6	83.3	6.3	22	11.6	66.6	7.7
Coccinella 7punctata L	7	5.1	87.5	4.5	12	8.0	50	4.0	3	1.9	33.3	0.6	7	3.7	50	1.9
Halyzia 14 punctata L.									9	3.8	50	1.9	10	5.3	60.6	3.5
Chilocorus bipustulatus L.	19	13.9	87.5	12.1	10	6.7	50	3.4	3	1.9	50	< 0.1	ŝ	2.6	20	1.3
Cleridae	5	3.7	12.5	0.5	m	2.1	12.5	0.3	4	2.6	33.3	0.9	5	2.6	33.3	0.9
Trichodes ircutensis Lax.	2	1.5	12.5	0.2		0.7	12.5	< 0.1					2	1.0	16.6	0.2
Trichodes favarius III.		0.7	12.5	< 0.1		0.7	12.5	< 0.1	3	1.9	33.3	0.6	2	1.0	16.6	0.2
Necrobia violacea L.	2	1.5	12.5	0.2		0.7	12.5	< 0.1		0.7	16.6	0.1	-	9.0	16.6	< 0.1
Cantharidae/Cantharis annul arisMen.	2	1.5	12.5	0.2	e	2.1	12.5	0.3		0.7	16.6	0.1		0.0	16.6	< 0.1
DIPTERA	37	27.0	100	27.0	71	47.6	100	47.6	72	46.1	100	46.1	85	44.7	100	44.7
Cecydomyidae	21	15.3	75	11.5	27	18.1	75	13.6	41	26.3	100	26.3	46	24.2	83.3	20.2
Chironomidae	5	3.7	50	1.9	33	22.1	75	16.6	22	14.1	100	14.1	23	12.1	83.3	10.1
Nematocera	1	8.0	75	6.0	11	74	50	37	6	57	833	47	16	84	9 Y Y	02

Specific composition and ecological parameters of beneficial arthropods collected on yellow sticky traps in the apple orchards chemical treated, 2007

Abundance (A), dominance (D), constancy (C) and the ecology significance index (W)

Table 4

				Ban	Baneasa				Baneasa Baicoi			Ba	Baicoi			
Taxons		Low-to)	Low-toxicity plot	Ŧ		Stand	Standard plot			Low-to:	Low-toxicity plot	ы ы		Standa	Standard plot	
	A	Ω	ບ	M	A	Ω	ບ	M	A	<u>_</u>	ບ	Μ	A	Ω	ບ	M
ACARINA/Trombididae Trombidium holocericeum L.	18	8.1	37.5	3.0	10	5.0	25	1.3	<sup>©</sup>	6.7	33.3	2.2	12	11.4	20	5.7
ARANEAE	52	23.4	8	23.4	44	22.0	100	22.0	24	20.0	9	20.0	30	28.9	100	28.9
DERMAPTERA	4	1.8	25	0.5	7	1.0	12.5	0.1								
Forficulidae/Forficula auricularia L.	7	6.0	12.5	0.1												
Labiduridae/Apterigida media L.	7	6.0	25	0.2	7	1.0	12.5	0.1								
NEUROPTERA/Chrysopidae/ Chrysoperla camea Steph	9	2.7	37.5	1.0	1	5.0	37.5	1.9	4	3.3	33.3	1.1	2	1.9	16.6	0.3
HETEROPTERA	42	18.9	8	18.9	58	29.0	100	29.0								
Miridae	36	16.2	8	16.2	34	17.0	100	17.0								
Atractotomus mali Mey.D	~	3.6	25	0.9	∞	4.0	25	1.0								
Der aeo coris lutescens Schilling.	28	12.6	87.5	11.0	26	13.0	100	13.0								
Beritydae/Metacantus elegans Curt.	9	2.7	25	0.7	12	6.0	37.5	2.3								
HYMENOPTERA	99 89	30.7	100	30.7	46	23.0	100	23.0	33	31.7	83.3	26.4	30	28.9	83.3	24.1
Ichneumonidae	9	2.7	25	0.7	4	2.0	25	0.5	4	3.3	33.3	1.1	2	1.9	16.6	0.3
Chalcididae	22	9.9	<u>1</u>	9.9	18	9.0	100	9.0	12	10.0	50	5.0	9	5.8	33.3	1.9
Braconidae	~	3.6	20	1.8	9	3.0	25	0.8	9	5.0	33.3	1.7	4	80 100 100	16.6	0.6
Formicidae	32	14.5	100	17.2	18	9.0	100	9.0	16	13.4	66.6	8.9	18	17.4	50	8.7
COLEOPTERA	23	10.4	100	10.4	18	9.0	100	9.0	4	3.3	33.3	1.1				
Coccinelidae	19	8.6	100	8.6	12	6.0	50	3.0								
Adalia bipunctata L.	9	2.7	50	1.4	4	2.0	25	0.5								
Coccinella 7 punctata L.	3	1.4	37.5	0.5	2	1.0	12.5	0.1								
Halyzia 14 punctata L.	7	0.0	25	0.2	7	1.0	12.5	0.1								
Chilocorus bipustulatus L.	~	3.6	75	2.7	4	2.0	25	0.5								
Cantharidae/Cantharis annul aris Men	7	0.0	12.5	0.1	9	3.0	25	0.8 0								
Staphylinidae	7	0.0	25	0.2												
Scarabaeida e/C'etonia aurata L.									4	3.3	33.3	1.1				
TATTED A Alexandrocen	-		0	•		0 7	0	0	ç	0	100	0 40	c c	000	001	000

Table 5

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# THE QUALITY POTENTIAL OF VARIETY GRAPES FOR WINES WITH OLTEȚUL INFERIOR AREA: IANCU JIANU – SARULUI FOREST

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KEY WORDS: varieties, grapes, glucides, acidity, flavors, polyphenols

## ABSTRACT

On the hills and slopes within Oltețul inferior area, from the south - eastern part of Drăgășani vineyard, general ecologic conditions that are very favourable for viticulture, allow the obtaining of all wines categories: white, aromatic, red. The quality parameters of grapes, at the stage of technological maturity, represented by the contents of: glucides, acidity, flavours, anthocyans, fully confirm these possibilities.

# **INTRODUCTION**

The high degree of favourability for viticulture of Oltețului Inferior area, where the well-known Iancu Jianu viticultural centre, is very well presented in the reference scientific work Romania's Oenoclimate - Oenoclimatul României (Teodorescu *et al.*, 1987). Remarkable contributions in defining the viticulture and wine production potential of Iancu Jianu area and of the quality of wines obtained in the past, have brought other important specialists (Oprean, 1975; Olteanu *et al.*, 2002; Condei, 2004).

Under the present circumstances there has been imposed the necessity of developing a complex study, with the scope of viticulture and wine production "conduct" of the varieties from the new types taken into consideration for the entire Drăgășani vineyard.

# MATERIAL AND METHOD

According to official methodologies recommended by ICVV, for all existing varieties in Iancu Jianu – Sarului Forest within Olt county, in the wine-growing years 2008 and 2009 has been followed the maturation of grapes. In the case of technological maturity, there have been determined the contents of: glucides, acidity, total polyphenols, tannin. In the case of grapes belonging to aromatic and semiaromatic varieties, there have been presented the contents in total flavours, and within them, of the free ones and of those linked in precursors. In the case of black grapes, there have been determined the contents in anthocyans as well.

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#### **RESULTS AND DISCUSSION**

■ The main characteristics of composition of grapes for white wines, at the moment of technological maturity or of harvest, are quantified within table 1.

The climatic conditions from the 2 wine-growing years with some discrepances, especially regarding the sun shining and rain classification in the entire period of active vegetation, have determined also differences between the contents of grapes, for the same ripening phenophasis.

Although, the harvesting has taken place in 2009, from a calendaristic point of view, later than in 2008, the contents in glucides, total polyphenols, and tannin have been lower, and the acidity higher, for all varieties.

Table 1

Varieties	Years	Glucides	Acidity g/l	Total	Tannin
		g/l	$H_2SO_4$	polyphenols g/kg	g/kg b.
		-		b.	
Crâmpoșie	2008	192	3,54	1,69	1,57
selecționată	2009	188	3,65	1,66	1,53
	Average	190	3,60	1,68	1,55
Riesling italian	2008	214	4,11	1,59	1,50
	2009	211	4,05	1,52	1,47
	Average	212,5	4,08	1,56	1,49
Pinot gris	2008	231	3,11	1,43	1,36
	2009	226	3,18	1,38	1,29
	Average	228,5	3,15	1,41	1,33
Fetească albă	2008	219	3,75	1,82	1,71
	2009	216	3,89	1,71	1,68
	Average	217,5	3,81	1,76	1,69

The main quality and	composition c	haracteristics (	of white v	vines
(a	t technological	maturity)		

Concerning the entire variety and the 2 years, the lowest content in glucides has been registered in the case of Crâmpoșie variety grapes in 2009 (188 g/l), and the highest in the case of Pinot gris variety, in 2008 (231 g/l).

In the case of glucides parameter, the average of the 2 years situate in the first place the Pinot gris variety, with 228,5 g/l and the last place is occupied by Crâmpoșie selecționată variety, with 190 g/l. For the other varieties, the averages of glucides have been situated to the levels of 212,5 g/l (to Riesling italian) and respectively 217,5 g/l (to Fetească albă).

In the case of the acidity, on the ensemble of wine-growing years and varieties, the lowest content has been registered to Pinot noir, in 2008 (3,11 g/l), and the highest content to Riesling italian, in 2008 (4,11 g/l). The acidity – as average classify the varieties, in a descrescent order, in the followin manner: Riesling italian (4,08 g/l); Fetească albă (3,81 g/l); Crâmpoșie selecționată (3,60 g/l); Pinot gris (3,15 g/l).

In the case of total polyphenols, during the two years the lowest cotents have been registered to the grapes belonging to the Pinot gris variety, and the highest in the case of Fetească albă grapes. The averages to this measure have been comprised between 1,41 g/kg beans (to Pinot gris) and 1,76 g/kg beans (to Fetească albă). In the case of Crâmpoșia selecționată the average has registered a value of 1,68 g/kg beans, and to Riesling italian the level of 1,56 g/kg beans.

In the case of tannin, the average contents have been comprised between 1,38 g/kg beans (la Pinot gris) și 1,69 g/kg beans (la Fetească albă). There has been noticed that, between the total polyphenols contents and tannin there is a direct relationship, that determines a similar classification of varieties, within the variety for neutral white wines.

■ For the grapes of aromatic and semiaromatic varieties, the main composition parameters – during technological maturity - are written as values within table 2.

Concerning the glucides contents, as opposed to the varieties for white wines, in the case of Fetească regală and Muscat Ottonel, in 2009, their levels have been very high, but not significantly different.

Table 2

Varieties	Years	Glucides	Acidity	Total	Те	rpenic fla	vour
v arrettes	i cuis	g/l	g/l	polyphenols	Total	Free	Linked in
			$H_2SO_4$	g/kg b.	µg/kg b.	%	precursors
							%
Fetească	2008	209	4,78	1,69	809	38,0	62,0
regală	2009	211	4,26	1,78	760	40,6	59,4
	Average	210,0	4,52	1,74	784	39,3	60,7
Sauvignon	2008	230	3,66	1,82	1785	73,5	26,5
	2009	227	3,72	1,77	1510	73,3	26,7
	Average	228,5	3,69	1,80	1648	73,4	26,6
Muscat	2008	228	3,08	1,82	9890	38,9	61,1
Ottonel	2009	235	2,63	1,68	9570	36,6	63,4
	Average	231,5	2,86	1,75	9730	37,8	62,3
Tămâioasă	2008	226	4,12	1,84	11560	26,8	73,2
românească	2009	220	3,96	1,71	11130	25,3	74,7
	Average	223,0	4,04	1,78	11345	26,0	74,0

The main composition and quality parameters of semiaromatic and aromatic wines (at technological maturity)

On the ensemble of the variety and of the wine-growing years, the minimum content of glucides has been situated to 209 g/l to Fetească regală, in 2008, and the maximum one to Muscat Ottonel, of 235 g/l, in 2009.

The averages of the glucides contents have registered levels comprised between 210,0 g/l – to Fetească regală and 231,5 g/l – to Muscat Ottonel.

High averages of glucides contents have been present also in the case of the variety grapes such as Sauvignon (228,5 g/l) and Tămâioasă românească (223,0 g/l), that could determine alcoholic degrees of over în 13,0 %vol. By applying appropriate primary vinification biotechnology, according to the glucides contents during technological maturity, from the grapes of Sauvignon, Muscat Ottonel and Tămâioasă românească varieties, there can be obtained demidry and even demisweet natural wines, without an alcoholic degree below 11,5 - 12,0 %vol.

The acidity from grapes has presented lower contents in 2009 in the case of Fetească regală, Muscat Ottonel and Tămâioasă românească. The lowest acidity content has been registered to Muscat Ottonel (2,63 g/l – in 2009), and the highest to Fetească regală (4,78 g/l – in 2008).

Taking into account the averages of acidity there has been noticed an important discrepancy in the case of Muscat Ottonel (2,86 g/l) grapes, but in a high degree also in the case of Sauvignon (3,69 g/l). In the case of Fetească regală grapes, the acidity has presented very good levels (4,78 g/l and 4,26 g/l), and the grapes belonging to Tămâioasă românească

variety, in certain situations, there are imposed slow corrections with tartric acid, in the must phase, when the level becomes lower than 4,0 g/l.

In the case of total polyphenols, taking into consideration the averages of the two years, with a minimum level of 1,74 g/kg beans (to Fetească regală) and the maximum level of 1,80 g/kg beans (Sauvignon), there appear insignificat discrepances between the varieties.

Total flavours of terpenic type have presented high levels, for all varieties in 2008, which has benefited from the sun more than 2009. These constituents, of real quality for semiaromatic and aromatic wines have presented total contents, as averages, situated between 784  $\mu$ g/kg beans – to Fetească regală and 11345  $\mu$ g/kg beans – to Tămâioasă românească. In the case of Sauvignon, the flavours, as an average level, has been comprised between 1648  $\mu$ g/kg beans, and in the case of Muscat Ottonel 9730  $\mu$ g/kg beans.

Differences between varieties have been registered in the case of proportions of free flavours and linked in precursors, within the total terpenic aromatic background. Thus, the varieties of Fetească regală, Muscat Ottonel and Tămâioasă românească the flavours linked in precursors (TLP) are dominant (comprised between 60,7 % - to Fetească regală and 74,0 % - to Tămâioasă românească). In the case of Sauvignon variety grapes, during technological maturity, free flavors (TVL) represent a dominant proportion (73,4 %).

■ For black grapes varieties, the main composition contents in grapes, during technological maturity, are quantified in table 3.

Table 3

(at technological maturity)								
Varieties	Years	Glucides	Acidity	Total	Anthocyans	Chromatic structure		ture of
		g/l	g/l	polyphenols	mg/kg b.	anthocyans		5
			$H_2SO_4$	g/kg b.		Pigm.	Pigm.	Pigm.
						Yellow	Red	blue
						%	%	%
Cabernet	2008	219	4,88	3,49	1501	29,6	61,8	8,6
Sauvignon	2009	214	4,53	3,63	1406	28,1	63,0	8,9
	Average	216,5	4,71	3,56	1453,5	28,9	62,4	8,75
Merlot	2008	214	4,43	3,25	1335	30,1	61,0	8,9
	2009	218	4,31	3,58	1289	29,8	61,6	8,6
	Average	216,0	4,37	3,42	1312	29,95	61,3	8,75
Pinot noir	2008	236	3,70	3,06	692	33,4	59,3	7,3
	2009	230	3,75	2,83	602	33,7	59,4	6,9
	Average	233	3,72	2,95	647	33,6	59,4	7,1
Fetească	2008	221	4,28	3,46	1331	26,6	66,5	6,9
neagră	2009	219	4,24	3,30	1296	26,9	66,4	6,7
	Average	220	4,26	3,38	1314	26,8	66,5	6,8
Novac	2008	217	4,38	3,83	1460	27,2	64,5	8,3
	2009	215	4,41	3,79	1405	27,8	64,7	7,6
	Average	216	4,40	3,81	1433	27,5	64,6	7,9
Negru de	2008	214	4,29	3,92	1471	28,0	64,4	7,6
Drăgășani	2009	217	4,36	3,81	1402	28,2	64,6	7,2
	Average	215,5	4,33	3,86	1437	28,1	64,5	7,4

The main quality and composition parameters of high-quality red wines

Regarding the glucides, on the ensemble of the variety and of the wine-growing years, the lowest contained level has been observed in the case of grapes belonging to:

Cabernet Sauvignon (in 2009), Merlot (in 2008), Negru de Drăgășani (in 2008) meaning 214 g/l. The highest contained level has been registered to Pinot noir (in 2008), of 236 g/l. Except for Merlot and Negru de Drăgășani, in the case of the other varieties, the glucides contents have been lower in 2009. The averages, in the case of glucides, situate on the first place, the Pinot noir variety (with 233 g/l), followed, in a descrescent order by: Fetească neagră (220 g/l), Cabernet Sauvignon (216,5 g/l), Merlot and Novac (216 g/l), Negru de Drăgășani (215,5 g/l).

The acidity of grapes belonging to the entire variety and to the period of the study presents the lowest level in the case of Pinot noir (3,70 g/l in 2008), and the highest in the case of Cabernet Sauvignon (4,88 g/l in 2008). The acidity potential of the varieties for red wines brings, as averages of the 2 years, on the first place the variety Cabernet Sauvignon (with 4,71 g/l), on the last place being the variety Pinot noir (with 3,72 g/l). In the grapes of the other 3 varieties, the acidity has reached similar levels, comprised between 4,25 g/l and 4,40 g/l ( $n H_2SO_4$ ), being considered as extremely favourable for a normal composition of future wines.

The polyphenolic components of the grapes, with a defining role for basic parameters of red wines, on the same area and in the same period of time, represent genetic attributes of variaties. Under these circumstances, the total polyphenols situate on the first place the variety Negru de Drăgășani (3,86 g/kg beans) closely followed by Novac (3,81 g/kg beans) and at a small difference by Cabernet Sauvignon (3,56 g/kg beans). With the lowest level of total polyphenols, there has been evidenced Pinot noir (2,95 g/kg beans), while Merlot and Fetească neagră varieties present relatively appropriate contents (3,42 g/kg beans, respectively 3,38 g/kg beans).

The anthocyanic potential of the varieties, together with the specific genetic influence has been accompanied by the climatic conditions of wine-growing years. For all varieties, during the year 2008, with a sun shining larger duration, the contents of anthocyanic colouring material from grapes have been higher than those in 2009. With the highest anthocyanic potential – as average, there has been classified the variety Cabernet Sauvignon (with1453 mg/kg beans) closely followed by Negru de Drăgășani and Novac (with 1437 mg/kg beans, respectively 1433 mg/kg beans). The closeness between Merlot and Fetească neagră varieties has been developed by the anthocyans contents (1312 mg/kg beans, respectively 1314 mg/kg beans). As in the case of other areas, the Pinot noir occupies, among anthocyans, the last place in the variety (with 647 mg/kg beans).

Within the anthocyanic complex of grapes: the yellow component occupies the highest proportion to Pinot noir variety (33,6%), and the lowest to Novac variety (27,5%); the red component occupies the highest proportion to Fetească neagră variety (66,5%), and the lowest to Pinot noir variety (59,4%); the blue component has registered the highest proportion to Cabernet Sauvignon and Merlot varieties (8,75%), and the lowest to Pinot noir (6,8%).

The proportions of the three categories of pigments, found in optical densities at 420, 520 and 620 nm represent the basis of establishing the values of chromatic characteristics, represented by colouring intensity (Ic) and by the quality indicators of anthocyanic background – the colour tonality (Tc) and flavilium cathions (dA%).

## CONCLUSIONS

When the technological maturity takes place:

- the varieties for white wines realize comprise: contents of glucides – as average comprised between 190 g/l – to Crâmpoșie selecționată and 229 g/l – to Pinot gris, and

contents in acidity comprised between 3,60 g/l – to Crâmpoșie and 4,08 g/l – to Riesling italian;

- the varieties for aromatic and semiaromatic wines comprise: glucides contents comprised between 210 g/l – to Fetească regală and 232 g/l – to Muscat Ottonel, acidity situated between 2,86 g/l – to Muscat Ottonel and 4,52 g/l – to Fetească regală and total flavours comprised between 784 µg/kg beans – to Fetească regală and 11345 µg/kg beans – to Tămâioasă românească.

- the varieties for red wines realize glucides contents comprised between 215 g/l – to Negru de Drăgășani and 233 g/l – to Pinot noir, acidity situated between 3,72 g/l – to Pinot noir and 4,71 g/l – to Cabernet Sauvignon, anthocyans comprised between 647 mg/kg beans – to Pinot noir and 1454 mg/kg beans – to Cabernet Sauvignon.

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# THE CONTENT AND QUALITY OF WINES OBTAINED WITHIN THE AREA OF THE HILLS AND SLOPES BELONGING TO THE INFERIOR OLTEŢ RIVER: IANCU JIANU – SARULUI FOREST

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KEY WORDS: wine, alcohol, acidity, glycerol, extract, anthocyans

# ABSTRACT

Corresponding to quality parameters of grapes at harvest maturity, the obtained wines, by applying carefully controlled technologies, by their composition and their organoleptic characteristics, cover a large range of types and categories. Generally, by judicial establishment of the right moment of harvesting the grapes, the wines may fulfill, on a regular basis, the imposed conditions for superior quality wines and wines with name of origin, white "neutral", white semiaromatic, aromatic and red, taking into consideration the contents of: alcohol, acidity, extract, ash, flavors, anthocyans.

# INTRODUCTION

For a long time, the wine - growing center Iancu Jianu, with hills and slopes along the Oltet river, towards Balş – Sarului Forest and east towards Cârlogani is well known for the quality of wines that have been produced and continue to be produced, within the areas that are part of it. Although positive appreciations regarding Jianu wines have been transmitted from generation to generation, specialized literature mentions few data on their composition and their quality, taking into consideration the fact that the research is not consistent.

Some mentions about Iancu Jianu wines are found in older works (Coltescu, 1943; Bernaz et al., 1962). New works contain very favourable appreciations regarding Sauvignon and Tămâioasă românească Iancu Jianu wines (Teodorescu et al., 1987) and there are scientific arguments regarding the area, that is proper for obtaing wines with controlled appellation of origin and superior wines (Condei quoted by Nicolaescu, 2007).

In order to revigorate the wine - growing area Iancu Jianu – Sarului Forest, at the same time with the establishment of the most appropriate types and the definition of wine - growing production defining, during the last years, there have been made adequate investigations, some of their results representing the object of the present work.

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# MATERIAL AND METHOD

During the years 2008 and 2009, from the grapes that were technological maturity, belonging to more white varieties, semiaromatic, aromatic and black, by applying biotechnological measures of primary vinification, there have been obtained the wines that, after clearance and a sufficient stabilization, have been submitted to physico-chemical and sensory analysis, according to OIV methods and adopted by ICVV Valea Călugărească.

# **RESULTS AND DISCUSSION**

■ Table 1 mentions the levels of the main composition characteristics of Crâmpoşie selecționată, Riesling italian, Pinot gris and Fetească albă wines .

Table	1

The main composition and quarty parameters of white whies								
Varieties	Years	Alcohol	Acidity	Glycerol	Un-	Ash	% glycerol	% ash
		%vol.	g/l	g/l	reduced	g/l	with	with
			$H_2SO_4$	-	extract	-	alcohol	extract
					g/l			
Crâmpoșie	2008	11,02	3,60	7,05	17,8	1,60	8,10	8,99
selecționată	2009	10,90	3,81	6,95	17,9	1,58	8,08	8,83
	Average	10,96	3,71	7,00	17,9	1,59	8,09	8,91
Riesling	2008	12,36	4,32	9,01	20,1	1,92	9,24	9,55
italian	2009	12,16	4,22	9,20	19,8	1,81	9,58	9,14
	Average	12,26	4,27	9,11	20,0	1,86	9,41	9,35
Pinot gris	2008	13,40	3,14	10,20	21,1	2,01	9,62	9,53
	2009	13,06	3,19	10,16	20,7	1,98	9,68	9,57
	Average	13,23	3,16	10,18	20,9	2,00	9,65	9,55
Fetească albă	2008	12,74	3,81	9,31	20,4	1,97	9,25	9,66
	2009	12,50	4,02	9,25	20,1	1,85	9,37	9,20
	Average	12,62	3,92	9,28	20,3	1,91	9,31	9,43

The main composition and quality parameters of white wines

Corresponding to the glucides contents in grapes at the moment of the harvest and vinification, alcoholic degrees, applying to the entire variety and to the two viticultural years, there have been situated values comprised between the minimum level of 10,90 %vol – in the case of Crâmpoșie selecționată in 2009 and 13,40 %vol – in the case of Pinot gris in 2008. The average values of alcohol contents place as first the Pinot gris wines (with 13,23 %vol), while on the last place there can be found Crâmpoșie selecționată wines (with 10,96 %vol).

According to the alcoholic degree – the main parameter taken into consideration when classifying the wines – except those obtained from Crâmpoșia selecționată, in all the other cases, is considered as being superior. The alcoholic degree from Pinot gris wines, suggests the possibility that from this variety's grapes will be obtained natural real quality semidry wines.

The little inappropriate acidity of Pinot gris and Crâmpoșie wines (3,16 g/l and respectively 3,71 g/l - as average) is appropriate in the case of Riesling italian wines (4,23 g/l) and in certain years even Fetească albă wines.

Glycerol contents, as a result of Neuberg glycero-pyruvic fermentation, have demonstrated high values in the case of Riesling italian, Pinot gris and Fetească albă wines. Under these circumstances, the glycerol has not become lower under 9,0 g/l, but has reached 10,20 g/l, representing an important factor of rising taste characteristics. Annual

values of glycerol contents in wines are comprised as averages between 7,0 g/l - to Crâmpoșie selecționată and 10,18 g/l – to Pinot gris.

Extractivity, a parameter that confers in a significant measure the feature of "consistency", is good or very good in the case of Riesling italian, Pinot gris and Fetească albă wines, comprised between 20,0 g/l and 21,0 g/l. Just below 18 g/l in the case of unreduced extract to Crâmpoșie selecționată wine.

The natural character of the wines as well as the large framework of obtaining them can be observed form the proportions of glycerol as opposed to alcohol and ash proportions as opposed to un-reduced extract. Both reports, under 9 in the case of Crâmpoșie selecționată wine, that reach the superior limit (considered 10) in the case of wines from other varieties, with a special mention for Pinot gris.

• The main composition parameters of semiaromatic and aromatic wines obtained from within the area of Oltetul inferior river, are quantified in table 2.

The highly favourable environment for the vines culture, the genetic nature of the four varieties and the correct measures of primary vinification are reflected in wine compositions.

The alcoholic degree has not lowered under 12,0 %vol (Fetească regală) to any variety and any year, but has reached 13,65 %vol (Muscat Ottonel).

The alcohol contents - as average, order the varieties, in a descending order as it follows: Muscat Ottonel (13,38 %vol); Sauvignon (13,01 %vol); Tămâioasă românească (12,91 %vol); Fetească regală (12,20 %vol). Except for Feteasca regală, from the grapes of other varieties, by rationally applied technologies ther can be obtained, demidry wines, and through a very careful establishment of harvesting moments, even natural demisweet wines.

The main composition and quality parameters of semiaromatic and aromatic wines							
Varieties	Years	Alcohol	Acidity Glyce		Un-	Ash	% ash
		%vol.	g/l H <sub>2</sub> SO <sub>4</sub>	g/l	reduced	g/l	with
					extract g/l		extract
Fetească	2008	12,09	4,93	8,96	19,8	1,87	9,44
regală	2009	12,30	4,26	9,31	20,3	1,90	9,36
	Average	12,20	4,60	9,14	20,0	1,89	9,40
Sauvignon	2008	13,02	4,78	10,21	21,8	2,09	9,59
	2009	13,00	3,82	10,02	22,1	2,12	9,60
	Average	13,01	4,30	10,12	22,0	2,11	9,60
Muscat	2008	13,11	3,04	10,30	22,7	2,19	9,65
Ottonel	2009	13,65	2,71	10,39	22,6	2,20	9,73
	Average	13,38	2,88	10,35	22,7	2,20	9,69
Tămâioasă	2008	13,02	4,16	10,03	22,8	2,17	9,52
românească	2009	12,80	4,06	9,97	22,9	2,25	9,83
	Average	12,91	4,11	10,0	22,9	2,21	9,68

Table 2

The reduced acidity in the case of Muscat Ottonel (under 3,0 g/l - as an average), is being observed in very good contents in the case of wines belonging to other varieties, comprised as individual values between 3,82 g/l (Sauvignon) and 4,93 g/l (Fetească regală), situated as averages within 4,11 g/l (to Tămâioasă românească) and 4,60 g/l (to Fetească regală).

The quality of wines is significantly higher by the considerable contents of glycerol. Thus, the single case of a lowered glycerol just below 9.0 g/l (Fetească regală –

2008), but many times has exceeded the limit of 10 g/l, especially in the case of Sauvignon, Muscat Ottonel and Tămâioasă românească wines.

Obtained by pelicular maceration or by classic maceration, the wines have presented a favourable extractivity under taste report, together with high glycerol contents with clearly organoleptic positive effects.

The average contents of un-reduced extract, comprised between 20,0 g/l (Fetească regală) and 30,0 g/l (Tămâioasă românească), sustain the above information.

In the un-reduced extract contents, the mineral substances (the ash) has registered low contents, but not much under 1,87 g/l (Fetească regală – 2008), but with the superior limit of 2,25 g/l (Tămâioasă românească – 2009). These ash proportions confirm the high quality level of aromatic and semi-aromatic wines obtained from within the area Iancu Jianu – Pădurea Sarului.

■ The basic composition of red wines, obtained from the grapes if the 6 black grapes varieties is presented in table 3.

The climatic conditions and the soils in total accordance with the vines demands, as well as flowing waters together with forests form the vecinity of plantations find positive influences in the exceptional quality of red wines.

Alcohol contents, taking into consideration the genetic nature of varieties and glucides contents at the moment of vinification, in the case of the entire variety, and through the entire period of the study, have presented levels comprised between 12,32 %vol (Cabernet Sauvignon – 2009) and 13,45 %vol (Pinot noir – 2009), totally corresponding to the highest quality stages.

The wines have presented favourable contents as well as concerning the acidity, with individual annual values, in the ensemble of the variety, comprised between 3,86 g/l - to Pinot noir 2009 and 4,99 g/l - to Cabernet Sauvignon 2008. Acidity contents – as averages, place as first Cabernet Sauvignon wines (4,67 g/l), while the last place is occupied by Pinot noir wines (3,94 g/l).

The high content alcohol levels correspond to glycerol considerable contents, with a variability, regarding the total of wines, relatively low, comprised between 9,58 g/l (to Novac -2008) and 10,65 g/l (to Pinot noir -2009). Glycerol contents - as averages, restrain the difference between varieties, being situated between 9,60 g/l (to Novac) and 10,57 g/l (to Pinot noir).

To the characteristics mentioned, with appreciative quotas, is added a considerable extractivity, in contents that have reached 27 g/l or even exceeded this limit and have not gone below 25,7 g/l (Merlot – 2008). Except for Merlot variety wines, in the case of which extractivity - as average has lowered just below 26 g/l, in the case of all wines obtained from the other 5 varieties, the extractivity has exceeded substancially the value of 26 g/l (with variability between 26,36 g/l – to Novac and 26,83 g/l – to Pinot noir).

The ash with contents (as average) not much under 2,54 g/l (to Merlot and Novac), but with a superior level of 2,68 g/l (to Pinot noir), represents within un-reduced extract proportions comprised between 9,63 % - to Novac and 9,97 % - to Pinot noir, very close to the ideal limit, with a stable quota of 10.

1

The main quarty and composition parameters of red wines									
Varieties	Years	Alcohol %vol.	Acidity g/l H <sub>2</sub> SO <sub>4</sub>	Glycerol g/l	Un-reduced extract g/l	Ash g/I	Total polyphenols g/l	Anthocyans mg/l	% ash with extract
Cabernet	2008	12,68	4,99	9,88	26,30	2,59	2,41	682	9,85
Sauvignon	2009	12,32	4,36	9,60	26,85	2,63	3,12	798	9,80
	Average	12,50	4,67	9,74	26,58	2,61	2,77	740	9,82
Merlot	2008	12,39	4,65	9,63	25,70	2,53	2,36	607	9,84
	2009	12,55	4,28	9,73	26,12	2,54	2,89	721	9,72
	Average	12,47	4,47	9,68	25,91	2,54	2,63	664	9,78
Pinot noir	2008	13,30	4,02	10,49	26,60	2,65	2,00	447	9,96
	2009	13,45	3,86	10,65	27,05	2,70	2,66	396	9,98
	Average	13,38	3,94	10,57	26,83	2,68	2,33	422	9,97
Fetească	2008	12,75	4,36	9,84	26,68	2,62	3,01	704	9,82
neagră	2009	12,60	4,32	9,80	26,40	2,60	2,91	730	9,83
	Average	12,68	4,34	9,82	26,54	2,61	2,96	717	9,83
Novac	2008	12,51	4,46	9,58	26,62	2,58	2,96	720	9,69
	2009	12,44	4,56	9,62	26,10	2,50	3,02	716	9,58
	Average	12,47	4,51	9,60	26,36	2,54	2,99	718	9,63
Negru de	2008	12,40	4,38	9,71	27,01	2,67	3,11	709	9,87
Drăgăşani	2009	12,53	4,52	9,66	26,22	2,52	3,07	723	9,62
	Average	12,47	4,45	9,69	26,62	2,60	3,09	716	9,75

The main quality and composition parameters of red wines

Phenolic constituents (those that confer in a decisive manner the particularities of red wines) are well represented. The total polyphenols, compounds with a major influence over chemical content and of taste characteristics, have presented variable contents – as average, comprised between 2,33 g/l – to Pinot noir and 3,09 g/l – to Negru de Drăgășani.

The thermal regime and glowing durations of the sun as well as elaboration biotechnologies are found within anthocyans contents quite good in the case of Pinot noir and Merlot wines (422 mg/l and respectively 664 mg/l) and exceptionally, over 700 mg/l in the case of the wines from the other varieties.

# CONCLUSIONS

Riesling italian, Pinot gris and Fetească albă white wines fulfill all predicted conditions for high superior quality wines. Crâmpoșie selecționată wines follow under the superior wines category presenting a balanced composition and sensorial qualities favourably appreciated.

Aromatic and semi-aromatic wines present a well defined aromatic profile, specific to varieties, with obvious expressivity and high contents in the case of all physicochemical parameters, except acidity in the case of Muscat Ottonel wines.

Well coloured red wines, that incorporate important quantities of anthocyans (except Pinot noir, but sufficient). They are presented in live colours, glowing colours, by the complete dominance of the red component in chromatic structures. They are extractive, rich in alcohol and glycerol, and with important contents in mineral elements.

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# IMPORTANCE OF THE GENITAL SCREENING IN INFECTIOUS FACTORS DETECTION WITH IMPACT IN HUMAN HEALTH

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KEY WORDS: Screening, genital infection, human papilloma viruses, Trichomonas vaginalis, Candida albicans

#### ABSTRACT

Screening consists of looking for cancer before symptoms appear. It can detect cancer in an early stage. When tumor cells are detected early, cancer is easier to treat. At the time symptoms appear it is possible that malignant cells is already widespread. Scientists are trying to better understand the propensity of people to certain types of cancer.

The results of this study show the implication in the etiology of the infectious factor viral and bacterial. Laboratory investigations have a role in clinical diagnosis, the stage of evolution and early implementation of therapeutic conduct to achieve desired results.

## INTRODUCTION

Doctors can not always explain why some people get cancer and others do not. Scientists have studied general patterns of people with cancer to see which of the things and people's actions increase the risk of developing cancer (Bosch et al., 2003). Any factor that increases a person's chance of developing a disease is called a risk factor, any factor that decreases the chances of developing a disease is called a protective factor. Some risk factors can be avoided but not many (Robbins et al., 1995).

The uterine cervical infections are a particular problem in gynecological and obstetrical practice, because the cervix is the most important reservoir for pathogens, especially those with sexual transmission (Schiffman et al., 2003).

Update on the carcinogenesis studies support the idea that the emergence and development of malignant tumors would be the consequence of events that weaken or exceed the immunological competence of the body, allowing a critical moment, the emergence of atypical cells is induced by an infectious agent, physical or chemical (Lowy and Howley, 2001).

Among the risk factors are the genital infections, especially herpes virus and human papilloma virus, which are sexually transmitted diseases, the multiplicity of sexual partners, sexual precocity, the fact that the patient had more than one child (Gillison and Shah, 2003).

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## MATERIAL AND METHODS

The study was conducted on a sample of 148 patients aged 20 to 70 years in evidence at the Family Planning Clinic County, Craiova with genital problems, from 01/01/2008 to 05/01/2009 before start of treatment.

All patients were performed the following tests:

1. Subjective and objective clinical examination

2. Examination of vaginal secretions: Microbiology and Parasitology.

3. Exam citotumoral - Babes Papanicolaou

4. Virological and serological investigations (RFC and IF) to detect HSV-2 infection, HPV.

# Methodological principles virological and immunological diagnosis for the viral infections

a) Direct microscopic examination

b) Isolation of viruses

c) Serological diagnosis

# Laboratory diagnosis of infection with herpes simplex virus type 2 1. Collecting of pathological products:

-Fluid from the vesicle;

- Material collected from the broken blisters from skin lesions;

- Material represented by vaginal exudates;

- Infected cells in the mucosa scraped with a curette;

- Sperm.

# 2. Direct examination

A) Scanning Electron Microscopy, X-Ray Diffraction;

B) Optical Microscopy

# Laboratory diagnosis of genital bacterial infections

Secretion vaginal secretion and other lesions of the cervix were examined under the optical microscope after Giemsa stained smears and methylene blue.

In gonococcal infection were found kidney-shaped cocci with the concavity in front, Gram-negative intra-and extracellular located.

It was also noted the inflammatory reaction present through numerous polymorphonuclear in the infections caused by Gram-positive cocci, as well there were observed staphylococci (Gram positive specific cocci placed in piles) or streptococci (Gram-positive cocci, spherical placed in short chains).

The enterobacteria were represented by *E. coli* and *Proteus*, Gram negative bacilli with varying sizes and thicknesses.

Isolation of bacteria was done on liquid medium (glucose broth, peptone water) and solid media simple or complex taking into account the biological needs of the bacteria (plain agar, agar-blood, Istrate Meitert, Mac Conkey).

# Laboratory diagnosis of vaginal trichommonasis

Cervico-vaginal secretion was examined under the optical microscope after Giemsa staining preparations.

# Laboratory diagnosis of vaginal candidomicosis

Usually is performed by direct microscopic examination of vaginal secretion after staining with methylene blue.

# **RESULTS AND DISCUSSIONS**

The results of this study show the implication in the etiology of the infectious factor viral and bacterial. Laboratory investigations have a role in clinical diagnosis, the stage of evolution and early implementation of therapeutic conduct to achieve desired results.

Even though most statistics show that there are significant differences in the incidence of dysplastic lesions and genital infections in terms of environment of origin of the affected women, this study shows that the incidence is higher in urban areas.

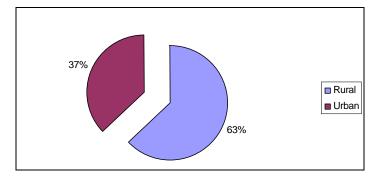


Figure 1. Genital infection frequency in rural and urban area first trimester 2008

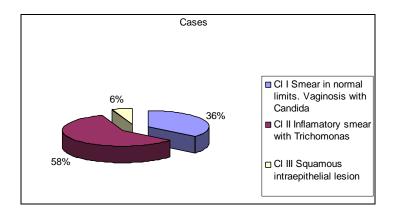


Figure 2. Genital infection frequency on risk classes first trimester 2008

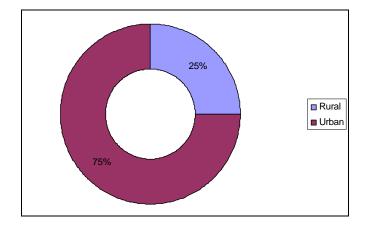


Figure 3. Genital infection frequency in rural and urban area second trimester 2008

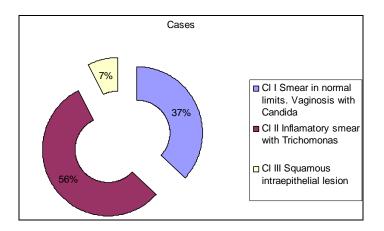


Figure 4. Genital infection frequency on risk classes second trimester 2008

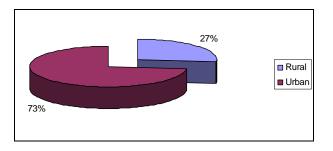


Figure 5. Genital infection frequency in rural and urban area third trimester 2008

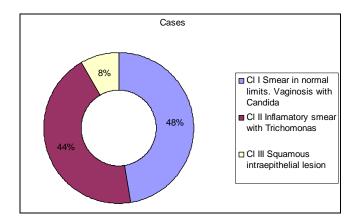


Figure 6. Genital infection frequency on risk classes third trimester 2008

Studying pathological personal antecedents of these persons, it was found that a considerable number were suffering genital problems ignored for a long time.

Some treatments have been empirical, others were only partial treatment recommended by the gynecologist, if requested.

To these can be added for presentation in the low-smear testing and nefmalizarea citotumoral Babes, largely through no-show, the cases that have raised suspicions

The average age at which women were diagnosed with dysplastic lesions was 29 years.

This table shows the need to focus efforts on prevention and detection of the disease in the period of 25-40 years, as well for the ones past 60 years.

Depending on the causative infectious agent, we noticed the increased incidence of viral infections with human papilloma viruses and herpes virus type 2 as shown in Table 1.

Table 1.

Etiologic agents	Dysplastic lesions
HPV	24%
HSV-2	52%
Trichomonas vaginalis	18%
Candida albicans	2%
Bacterian agents	4%

Incidence of the infectious agents

## CONCLUSIONS

In pathogenesis of dysplastic lesions and cervical cancer, the main event is the sexual act, transmitting a carcinogen, the most likely viral or other nature.

Identification of viral infections with high percentage of papilloma-virus type 14 and 18 and herpes type 2 virus in people with cervical cancer and cervical dysplastic lesions, demonstrating the possible role of these viruses in oncogenesis.

The process of cervical carcinogenesis is a multifactorial process, these factors enter and condition each other. To detect the disease in early stages is necessary to enlarge the range of paraclinical diagnosis, effective treatment for the adoption of behavior and preventive measures are appropriate

Unfortunately a proper education is poor or even nonexistent, in contrast is sex education in schools which encourages young people start sexual life early, with handy methods of contraception "safe" (contraceptives and condoms) and free. In this way do not give them a warning but just correct the risk factors for genital diseases, including those of the cervix, the occurrence of unwanted pregnancies (resulting in abortion).

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# POLYMERIZATION OF METHYL METHACRYLATE OBTAINED BY THERMAL DEGRADATION OF POLY (METHYL METHACRYLATE)

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**KEY WORDS:** Poly(methyl methacrylate), methyl methacrylate, polymerization, degree of conversion

# ABSTRACT

The paper presents a study related to radical polymerization of methyl methacrylate (MMA) using benzoyl peroxide as initiator, for two samples: a commercial MMA and a sample of MMA obtained by thermal degradation. The reaction was studied using FT-IR for polymerization degree calculation.

The MMA obtained by thermal degradation of PMMA waste contains till 8 % impurities that reduced polymerization rate in the stage of propagation. In the final stage of polymerization the both samples reach the same polymerization degree, leading to the conclusion that the impurities from the samples influenced the mechanism of polymerization but did not influenced the degree of polymerization of the final product.

# INTRODUCTION

There is an increasing concern regarding environment, taking into consideration sustainable development. A major aspect is related to raw materials saving and environment protection by applying as much as possible recycling methods. In the case of plastic materials, the great diversity of materials implies a great diversity of recycling methods. The recycling methods depend on the type of polymers (thermoplastic, thermosets or elastomers). Thermoplastic materials can be mechanically recycled. For some thermoplastics, thermosets and elastomers chemical recycled by thermal degradation or pyrolysis leads to fuels or raw material production. Incineration for energy recovery is the method for mixed and contaminated plastic materials recycling.

PMMA is a relatively expensive polymer, and a series of studies were focussed on PMMA recycling.

Martin *et al.* (2008) studied the effect of morphology on optical transparency of recycled PMMA/clay nanocomposites with several organically modified clays and different clay contents. Transparency increased with the dispersion of particles. Thermal properties had no improvement. Tensile modulus was increased with the clay content. They concluded that it is possible to recycle PMMA for preparing nanocomposites with interesting properties.

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Charmondusita and Seeluangsawat (2009) used an industrial waste of PMMA, resulted during the casting process as a co-monomer with polystyrene (PS) for obtaining styrene - MMA copolymers (S-MMA) They concluded that the use of styrene-substituted MMA monomer can reduce raw material cost, and improve the recycling of PMMA scrap with a decrease in plastic waste that is produced from the PMMA casting process. The influence of PMMA scrap recycle concentration did not affect the mechanical and physical properties of the S–MMA copolymer cast sheet. Slight decreases in the impact strength and heat resistance were observed at higher concentrations of styrene.

Laverty made a study related to mechanical recycling of mixtures of automotive thermoplastics containing polycarbonate (PC), PMMA and acrylonitrile–butadiene–styrene (ABS) showing that the presence of PMMA at a relatively high content determined inferior performance for the resulted polymer blends (Ellis *et al.*, 1996).

Rybnicek *et al.* (2005) made a study on rear lights parts containing recycled PMMA and they concluded that the transition from ductile elasto-plastic behaviour to brittle linear elastic fracture behaviour takes places for the PC/ABS/PMMA blend at 10% PMMA.

Selective dissolution of PMMA from PMMA decorative sheets (Papaspyrideset *et al.*, 1994) or PMMA moulding compounds (Gouli *et al.*, 1994) was studied by Gouli *et al.* in order to recover PMMA.

Thermal degradation of PMMA waste leads to high yields in MMA, leading to the conclusion that chemical recycling of PMMA can be performed in an economical way (Grause *et al.*, 2006; Xi *et al.*, 2005).

Molten metal baths (Grause *et al.*, 2006; Newborough *et al.*, 2002) were used in order to ensure a good heat transfer, but unwanted toxic organo-metallic compounds were found in the gaseous phase and in the recovered monomer.

Other studies implied the use of sand in fluidized bed installations in order to ensure a good heat transfer and an uniform temperature in the reaction chamber (Kaminsky and Frank, 1991; Sasse and Emig, 1998; Kaminsky and Eger, 2001; Smolders and Baeyens, 2004). Catalysts based on various sulphates allowed the decrease of degradation temperature (Xi *et al.*, 2005).

The flash pyrolysis of PMMA granules in  $N_2$  and 2H-heptafluoropropane (HFP) atmosphere were carried out by Tianfang Wang et al., at 700 °C. The results indicate that the liquid and tar products mainly consist of some aromatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs), and the distributions of pyrolysis products in HFP atmosphere are rather different from the one obtained in  $N_2$  atmosphere. The presence of HFP decreased the formation of MMA (Tianfang *et al.*, 2009).

Bo-Sung Kang (2008) studied a process of pyrolysis of the virgin copolymer and the PMMA waste in a fluidized bed reactor to recover the monomer. The reaction temperatures ranged from 450 to 500°C. They obtained a recovery rate of oil over 97%, and the content of the MMA in the oils was up to 98 wt%.

Even if, as seen before, a series of studies were focused on PMMA recycling by de-polymerization of PMMA, the polymerization of recovered MMA was rarely approached.

Achilias (2007) studied the polymerization reaction of MMA obtained by thermal degradation using 0.03 M AIBN (azo-bis-isobutyronitrile) as initiator. Polymerization was investigated using the DSC, Pyris 1 (from Perkin–Elmer) equipped with the Pyris software for windows. The reaction exothermicity was considered proportional to the polymerization rate. The polymerization rate of recovered MMA was lower than the polymerization of pure MMA.

Our previous studies have shown that recovered MMA can be polymerized using a Doctor Hölne Sol 2 ultraviolet lamp (Popescu *et al.*, 2009). The polymerization took places also in the absence of initiators by exposing the monomers to visible light at temperatures higher than 40°C, for a long time.

We studied the polymerization reaction of MMA obtained by PMMA thermal degradation from a 20 year old Plexiglas panel, in order to evaluate the polymerization process as compared to the polymerization of a commercial MMA.

# **EXPERIMENTAL DETAILS**

A batch laboratory scale installation for PMMA thermal degradation has been used for the recovery of MMA at 450  $^{\circ}$ C starting from pieces of more than 20 years old used PMMA from a PMMA panel (Popescu *et al.*, 2009).

Thermal degradation of PMMA leaded to about 97 wt% liquid products that contain more than 90% MMA when waste PMMA was degraded. The other degradation products of PMMA consist of MMA dimmers and MMA derivatives.

For bulk polymerization of MMA obtained from waste (MMA-waste) we chose benzoyle peroxide (BPO) as initiator. We worked at 70°C, because at 60°C the reaction rate was to low (we reached a 6 % degree of conversion after more than 2 hours), and at 80°C some samples polymerized after only 15 minutes. Different samples behave differently even if they were obtained in the same conditions. Probable the oxygen acted as an inhibitor in some cases.

We evaluated the degree of conversion (DC) during polymerization. For this purpose we prepared 2 samples of MMA (one from commercial MMA (MMA-COM), and the other one from recovered MMA (MMA-Waste). We weighted 30 mg of BPO in Ependorf tubes, and 0.8 ml of MMA was added. After homogenization the samples were analysed using a Spectrum BX FTIR Spectrometer from Perkin Elmer. Than the samples were placed in a heated oven at 70°C. We took samples for Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy analysis after 30 and 15 minutes respectively.

The DC (%) of monomer-to-polymer was calculated by comparison of the absorbance ratio using a standard baseline technique of the C = C peak from the methacrylate group at 1640 cm<sup>-1</sup> to that of the unchanging C = O peak from the ester group at 1720 cm<sup>-1</sup>, the last was used as a reference peak, before (monomer) and after polymerization (group C) (Popescu *et al.*, 2009; Urbana *et al.*, 2007). By taking the ratio between the two absorbances, the fraction of unreacted double bonds could be calculated from the formula:

 $DC = \begin{bmatrix} \left(\frac{Abs(C=C)}{Abs(C=O)}\right)_t \\ \hline \left(\frac{Abs(C=C)}{Abs(C=O)}\right)_o \end{bmatrix} \cdot 100$ (1)

# **RESULTS AND DISCUSSION**

Figure 1 presents the variation of DC (%) for PMMA obtained from MMA-COM (PMMA-COM) and from MMA-Waste (PMMA-Waste). We noticed that in the first 60 minutes the reaction takes place with almost the same rate in both cases. Than, the reaction became slower for MMA-Waste, the impurities acting as inhibitors for the polymerization

reaction. After 165 minutes the polymerization reaction seems to progress in the same manner for the both samples.

The materials obtained from MMA-COM are fragile and became brittle sooner than the material obtained from MMA – Waste. In the first stage of the reaction, when MMA was liquid, sampling was easy. As the reaction advanced the sampling and determinations using ATR was increasingly more difficult.

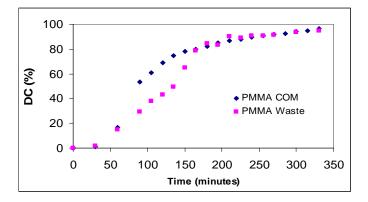


Figure 1. The variation of degree of conversion (DC %) for MMA-COM and MMA – Waste.

Figure 2 present a part of FTIR absorption spectra of MMA-COM in the presence of BOP at  $70^{\circ}$ C, (T = reaction time).

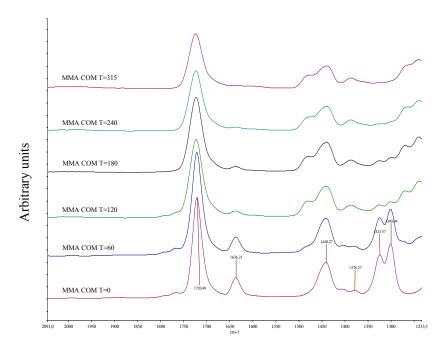
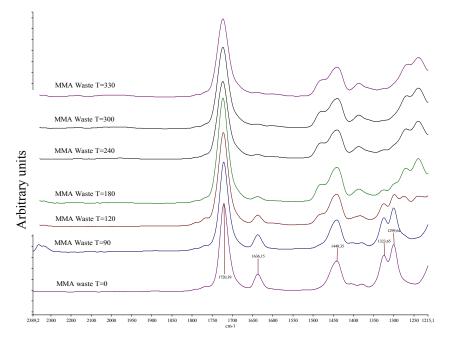


Figure 2. FTIR spectra of PMMA obtained from a commercial MMA (MMA-COM)



Figures 3 present FTIR absorption spectra of MMA – Waste polymerized in the same conditions.

Figure 3. FTIR spectra of PMMA obtained from a commercial MMA (MMA-COM)

One can notice the reduction of the absorption peak corresponding to C=C double bond, around 1640  $\text{cm}^{-1}$ .

## CONCLUSIONS

The impurities embedded in the MMA obtained by thermal degradation of PMMA waste influence the polymerization rate mainly in the propagation stage. Approximately a 80 % conversion degree was attained after 160 minutes both for MMA com and for MMA obtained from PMMA waste by thermal degradation.

We showed that even if the polymerization took place differently in the case of the two polymers, in the final the same conversion degree was obtained.

We showed that the polymerization of MMA is possible without any purification stage both for commercial and recovered MMA.

These impurities in recovered MMA retarded the polymerization compared with pure MMA. The total time for polymerization was the same for the both samples, even if the reaction rate differs in propagation stage of polymerization.

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## EMBRIOLOGIC AND CYTOLOGIC STUDIES ON FRUIT SETTING IN WALNUT TREE (Juglans regia L.)

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KEY WORDS: walnut, apomixis, fruit, structure

## ABSTRACT

Apomixis is an important characteristic of walnut. Fruit-setting through apomixis presents theoretical and practical importance; the embryo is homozygous and it loyally transmits the characters of mother plant. Research on apomictic formation of the embryo in walnut tree have been carried out in several countries; the mechanism of apomixis in walnut has been reported as adventitious embryony, apospory or diplospory. The paper aims at following-up the embryo development throughout its different stages, cell structure and development in walnut (fruits obtained by apomixis and by free pollination).

#### **INTRODUCTION**

Apomixis is an important feature of walnut, particularly in the northern cultivation areas (Loiko, 1990). Research carried out in walnut on fruit-setting have shown that some cultivars can generate fruits even without fecundation, through parthenocarpy or apomixis; but it was found out that fruit-setting is feeble, almost zero in some cases, while apomictic fruit-setting cannot be expected (Zhang et al., 2000;. Mu et al., 2001; Şan and Dumanoğlu, 2006; Guoliang et al., 2007, 2010). Rate of apomictic fruits differs from year to year and depends on climate and variety (Asadian et al., 2005).

Possibility of fruit-setting without pollination in walnut, under usual conditions, has raised special interest to researchers on flowering biology and seed formation. Sartorius and Stösser (1997) made the investigations aimed to explain the development of apomictic embryos by histological methods. Badalov (1989) also reported that apomictic embryos have developed mainly from the egg cell with subsequent doubling of chromosome numbers, leading to homozygosity. The mechanism of apomixis in walnut has been reported as adventitious embryony (Valdiviesso, 1990), apospory (Terziiski and Stefanova, 1990), or diplospory (Sartorius and Stosser, 1991; Pintea, 2004). Ultrastructure of the fleshy pericarp and seed coat cells was systematically investigated by using transmission electron microscopy, and this study makes a significant contribution to our understanding of ultracellular events in developing walnut fruit (Wu et al., 2009). In Romania there were

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not found out any cultivars or hybrids to possess valuable characteristic of apomixis (Cociu et al., 2003).

The aim of this study was to determine embryo development thougout different stages, structure and development of cells in walnut (fruits obtained by apomixis and by free pollination).

# MATERIALS AND METHODS

Cultivars were used by including all types of dichogamy: protandry, protoginy, homogamy. To obtain apomictic fruits, flowers isolation with pergamin paper pocket was carried out before stigmata bifurcation; and pockets opening was carried out when stigmata were fully dried (Cociu et al. 1989). For cross sections of apomictic fruit and normally developed fruit, fruits were collected (Figure 1-3), then fixated in FAA fixator (80% ethanol: 10% glacial acetic acid: 10% formol) and then kept in the refrigerator. Cross and longitudinal sections were visualized by microscope and photographed.



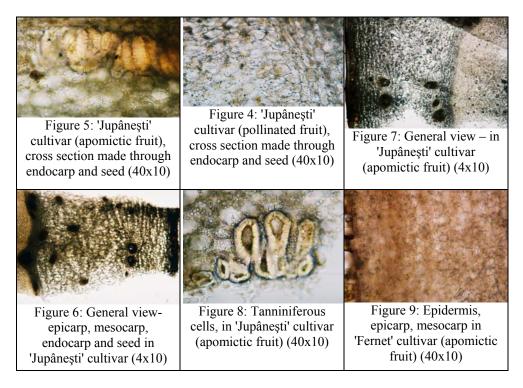
Figure 1-3: Fruits obtained by free pollination and apomictic fruits

# **RESULTS AND DISCUSSIONS**

From cross sections made in apomictic fruits and freely polinated fruits, the following facts were found out:

In 'Jupâneşti' cultivar (Figure 4), the cross section made through endocarp and seed -a fruit that is obtained by free pollination- showed that the unilayered skin lies at the exterior, equipped with a thick cuticle. Epicarp is made of 4-5 layers of cells that have colenchimatized walls. From place to place, at the limit between epicarp and mesocarp there are isolated cells or groups of large tanniniferous cells. Mesocarp is well developed; it is made of large spheroidal or ovoidal cells that contain numerous chloroplasts. In the mesocarp there are many conducive wood-free fascicles that are disorderly arranged and getting to the endocarp. Endocarp is made of small, parenchymatic cells that are tangentially elongated. There is no sclerification process in cells; the seed has thin tegument, made of 3-4 layers of small, parenchymatic cells that are slightly tangentially elongated. For the rest, the seed is made of spheroidal or ovoidal cells that are slightly larger than those in the tegument, but they do not have reserve substance at the interior. These cells have small intercellular spaces.

In apomictic fruit (Figure 5), 'Jupânești' cultivar, epidermis is unilayered and equipped with thick cuticle. Tanniniferous cells go inside the mesocarp. Epicarp is made of 2-3 layers of cells with slightly colenchimatized walls, and underneath there are numerous tanniniferous cells, some of them large and radially elongated that are goign inside the mesocarp. Mesocarp is made of spheroidal and ovoidal cells, with small spaces between them, and numerous chloroplasts at the interior. In the mesocarp there are conducive woodfree cells that are smaller than in pollinated form; and they are located somehow orderly on two circles; one is located under the epicarp, while the other is located at the exterior of endocarp; and in other areas they are disorderly arranged. Endocarp is made of 2-3 layers of cells with thin walls, slightly elongated tangentially. There is no sclerification process observed in cell walls. Seminal tegument cannot be seen in the seed; the cells located underneath the endocarp -that belong to the seed- do not contain any stored reserve substance at the interior.



General view (Figure 6) – epicarp, mesocarp, endocarp and seed in 'Jupânești' cultivar; fruit is obtained by natural pollination; and general view (Figure 7) – epicarp, mesocarp, endocarp and seed in 'Jupânești' cultivar, with apomictic fruit. In apomictic fruit there are tanniniferous cells; they are radially elongated, of spherodial and ovoidal shape, which go deeply into mesocarp (Figure 8).

In 'Fernet' cultivar, the cross section through epidermis, epicarp and part of mesocarp through apomictic fruit (Figure 9), has uni-layered epidermis equipped with very thin cuticle, and numerous pluricellular hairs that are elongated and club-shaped. The fruit is during the period right after fecundation (black-coloured stigmata). During this stage of development, the epicarp is poorly differentiated, made of 4-5 layers of cells with thin walls; the cells are slightly elongated tangentially. Mesocarp is made of spheroidal and ovoidal cells, without chloroplasts at the interior. Conducive fascicles are under differentiation stage, and they are disorderly arranged. Endocarp is not differentiated and one cannot see the difference between mesocarp, endocarp and seed. In some areas the internal epidermis of the ovar can be observed.

In conclusion, embriologic and cytologic studies are outlining the different stages in the embryo's development. Further precise studies are required to outline morphological differences between apomictic and pollinated fruits.

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# POSSIBILITIES FOR IMPROVING THE DEVELOPMENT OF TĂMÂIOASĂ ROMANIAN AND SAUVIGNON WINES BIOTECHNOLOGIES IN DRĂGĂȘANI VINEYARD

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KEY WORDS: variety, potential, yeasts, maceration, composition, flavor

## ABSTRACT

High technological potential of Sauvignon and Romanian Tămâioasă grapes varieties can be best transferred in the finial products through the application of appropriate and rigorously controlled primary wine biotechnologies. Using specific selected yeast together with pectolytic enzymes, in terms of rational maceration times are sure possibilities to improve the composition and quality of wines. Among the mentioned factors effects are: the reduction of fermentation ratio and of the volatile acidity and the content increase of glycerol, nereduced extract, ash and terpene-type flavors.

### INTRODUCTION

On the technological potential of Sauvignon and Romanian Tămâioasă varieties and on the compositional and organoleptic parameters of wines produced from them, according to traditional procedures, some researches were carried out, both in the past and even recently.

Concerning Sauvignon variety the researches targeted: defining the most appropriate type of wine from Sauvignon (Butănescu, 1963); primary technology issues (Butănescu, 1966); the basic characteristics of type I semidry and type II semisweet Dragasani Sauvignon wines (Butănescu, 1969); oenological potential in different areas of Dragasani vineyard (Nicolaescu, 2007; Cocos, 2010).

Romanian Tămâioasă variety from Dragasani vineyard was studied concerning the following aspects: the oenological behavior of the variety in different soil conditions (Puiu and Butănescu, 1970); the designation of origin award of Romanian Tămâioasă wines (Macici *et al.* 1975); the composition and quality of wines from various parts of the vineyard (Nicolaescu, 2007; Cocos, 2010).

## MATERIAL AND METHOD

During the wine years 2007 - 2009 several experiments were carried out aiming the optimizing of the production biotechnologies of both grapes varieties wines. In the

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experiment whose results will be presented in this paper it were used: indigenous yeasts (Li) and induced yeasts (selected yeasts), as singular factors and also in combination with specific pectolytic enzymes. Both as singular factors and in combination with enzymes, the yeasts have been conjugated with different contact time between phases, on equal terms concerning: the temperature of maceration, the sulfitation, the regime of mixing the phases.

## **RESULTS AND DISCUSSIONS**

In Sauvignon grape must, with high glucides content the compositional effects of the two types of yeasts (domestic and industrial) are different (Table 1). Differences, some quite important both in the case of yeasts action in the must separated from the solid fraction immediately after processing the grapes, and also in conditions of involving the maceration process.

If the case of action without maceration, the fermentation superiority of "specialized" selected yeasts is clearly outlined. Due to the achievement of a fermentation ratio below 17 (16,86) and to the high capacity of carbohydrates metabolisation, the alcohol level of 13,42 %vol. was with 1,17% vol. bigger than the one that was achieved by indigenous yeasts (12,25 %vol). These explain also the very different levels of residual sugar content (9,7 g/l for selected yeast activity compared with 25.0 g/l for indigenous yeasts situation).

Table 1

	composition of Sauvignon wines - Dragasani									
Biotechno- logy	Macera- tion period (hour)	Alcohol %vol.	Volatil Acidity g/l H <sub>2</sub> SO <sub>4</sub>	Ferm. ratio	Glycerol g/l	Unred. Extract g/l	Residual sugar g/l	Ferm. Priod (days)		
Ferm. with Indigenous yeasts	_	12,25	0,45	17,23	8,12	19,84	25,0	17		
Ferm. with selected yeasts	_	13,42	0,44	16,86	10,31	19,84	9,7	11		
Indigenous	12	12,25	0,46	17,23	8,14	21,10	25,0			
U	24	12,28	0,46	17,20	8,15	22,02	24,8	16		
yeasts	36	12,30	0,48	17,18	8,15	22,98	24,7			
Selected	12	13,44	0,36	16,89	10,34	21,16	9,0			
yeasts	24	13,49	0,38	16,80	10,41	22,20	9,4	10		
yeasts	36	13,56	0,39	16,79	10,41	23,01	8,3			

Combined effect of yeasts type, period of maceration and enzymes on the basic physicochemical composition of Sauvignon wines - Dragasani

Experimental conditions: ferm glucides 236 g/l; selected yeasts 6,5 mil.cell/ml; enzymes 2,5 g/hl; macer. Temp. 17-18<sup>0</sup>C

Significantly higher content of glycerol and the much reduced fermentation periods in the case of fermentation with selected yeasts are other safe reasons for the use of induced yeasts. For both types of yeasts, corresponding to their fermentative capacity, insignificant increase in alcohol content with the increasing of maceration period, as also the volatile acidity and glycerol content. Major increases are recorded at unreduced extract, in the same direction with the extend of the contact period between must phases.

For the same period of maceration, fermentation and compositional parameters are clearly in the favor of induced yeasts.

Polyphenolic compounds evolution during maceration for the two types of yeasts in combination with pectolytic enzymes is presented in Table 2.

All four polyphenolic constituents increase the quantity with the increasing of the maceration period, both in the case of indigenous yeasts and also when it were uesd selected yeast in association with pectolytic enzymes. For the same period of maceration, the polyphenol content is higher due to the action of yeast selected with pectolytic enzymes. After 36 hours the increases are bigger with: 15,4% for total polyphenols; 20,8% for tannin; 28,0% for flavonoid polyphenols; 30,2% for unflavonoids polyphenols.

Table 2

Combined influence of the yeasts type, pectolytic enzymes and period of maceration on phenolic
composition of Sauvignon wines

Biotech.	Maceration period (hours)	Total polyphenols (g/l)	Tanin (g/l)	Polyphenol flavonoids µg/l catechina	Polyphenol unflavonoids µg/l Cafeic acid
Indigenous yeasts (Li)	-	0,274	0,254	85,2	39,6
Selected yeasts (Ls)	-	0,275	0,254	86,4	39,6
Li +	12	0,372	0,302	131,2	47,3
pectolytic	24	0,489	0,456	147,0	54,7
enzymes	36	0,624	0,491	181,3	65,3
Ls +	12	0,410	0,371	168,8	54,7
pectolytic	24	0,525	0,512	206,3	64,1
enzymes	36	0,720	0,593	232,0	85,2

Given that in certain contents, polyphenolic compounds may affect the taste characteristics of Sauvignon wines, maceration periods will not exceed 36 hours for indigenous yeasts with pectolytic enzymes and will not be greater than 24 - 25 hours for application selected yeasts with pectolytic enzymes.

To obtain Romanian Tămâioasă aromatic wines, the effects of compositional order due to the activity of indigenous and selected yeasts in combination with equal doses of SO<sub>2</sub> and pectolytic enzymes, during different maceration periods, are quantified in Table 3.

Table 3

	and chemical composition of Romanian Tămâioasă wines - Dragasani											
Biotech.	Macera- tion period (hours)	Un- reduced extract g/l	Ash g/l	Ash x100 /extract	Total polyphe -nol g/l	Tanin g/l	Poly- phenol Flavon. µg/l	Total Nitrog mg/l				
Li+SO <sub>2</sub> (Mt1)	-	20,43	1,76	8,61	0,372	0,226	67,3	288				
Ls+SO <sub>2</sub> (Mt2)	-	20,43	1,76	8,61	0,372	0,226	67,3	288				
Li+SO <sub>2</sub> +	12	20,89	1,84	8,83	0,396	0,351	72,2	345				
pectolytic	24	21,20	1,93	9,11	0,431	0,402	77,5	379				
enzymes	36	22,10	2,05	9,26	0,563	0,436	80,4	402				
chizyilles	48	22,76	2,12	9,32	0,701	0,587	85,4	435				

Combined influence of the yeasts type, pectolytic enzymes and maceration period on basic physical

Ls+SO <sub>2</sub> + pectolytic	12	22,93	2,12	9,23	0,446	0,411	85,2	355
	24	23,14	2,18	9,41	0,520	0,493	89,4	412
	36	23,82	2,26	9,50	0,669	0,578	90,2	456
enzymes	48	24,47	2,34	9,58	0,816	0,707	91,1	488

Experimental conditions: ferm glucides 231 g/l; flavor: TVL 4050 µg/kg b., TLP 8110 µg/kg b.; Lallzyme enzymes 3 g/hl; SO<sub>2</sub> 80 mg/l; macer. temp. 17-18<sup>0</sup>C; phases mixing 6 times/24h

For both biotechnological groups, the composition parameters increase with the increase of the contact period between must phases. Thus, between 0 and 48 hours maceration period, unreduced extract increases by 2,33 g/l using indigenous yeasts and adjuvants and by 4,04 g/l using selected yeast with adjuvant; the ash increases by 0,36 g/l respectively 0,58 g/l. In these situations, ratio ash x 100 / extract increased from 8,61% to 9,32% in the first group and from 8,61% to 9,58% in the situation of using selected yeasts +  $SO_2$  + pectolytic enzymes.

Substantial increases are recorded, in direct connection with maceration period for polyphenolic compounds and total nitrogen. After 48 hours of maceration, the contents of these have reached: 0,701 g/l respectively 0,816 g/l for total polyphenols; 0,587 g/l respectively 0,707 g/l for tannin, 435 mg/l respectively 488 mg/l for total nitrogen.

In relation to these data, maceration periods longer than 48 hours for indigenous yeasts and longer than 36 hours for selected yeasts (in both cases in combination with  $SO_2$  + pectolytic enzymes) may lead to organoleptic difficulties.

Terpene flavor profile of Romanian Tămâioasă wines is shown in Table 4. Different pattern of fermentation in the two types of yeasts, combined with the participation of  $SO_2$  and enzymes have influenced the dynamics of terpene compounds extraction from solid phase of grapes must. In both biotechnology cases the terpene extraction is continuing with the increase of maceration period. For the same period of maceration, however, total aroma contents are higher for selected yeasts +  $SO_2$  + pectolytic enzymes. Concerning flavored fund components, both free terpenes (TVL) and the linked ones (TLP) are increasing with longer maceration periods, for both biotechnologies. During all periods of maceration, the proportions of free flavors are greater than those precursors linked.

Table 4

composition of the Romanian Tămâioasă wine- Dragasani									
		Tornoni	%	%		Increase of	flavor %		
Biotech.	Maceration	Terpeni c flavor	TVL	TLP	Т	VL	TLP		
	period (hours)	in wine μg/l	extr- acted	extr- acted	Comp. to Mt1	Comp. to Mt2	Comp. to Mt1	Comp. to Mt2	
Li+SO <sub>2</sub> (Mt1)	-	6051	51,20	49,05	-	-	-	_	
Ls+SO <sub>2</sub> (Mt2)	-	6066	51,35	49,15	-	-	-	-	
	12	7553	66,10	60,12	29,1	28,6	22,6	22,3	
Li+SO <sub>2</sub> +	24	7975	68,16	64,30	33,1	32,7	31,1	30,8	
pectolytic enzymes	36	8369	72,90	66,80	42,4	42,0	36,2	35,9	
enzymes	48	8522	75,05	67,60	46,6	46,2	37,8	37,5	
La SO I	12	7697	67,90	61,00	32,6	32,2	24,4	24,1	
Ls+SO <sub>2</sub> + pectolytic enzymes	24	8180	71,60	65,12	39,8	29,4	32,8	32,5	
	36	8544	76,80	67,00	50,0	49,6	36,6	36,3	
	48	8514	75,75	67,16	48,0	47,5	36,9	36,6	

Combined influence of the yeasts type, pectolytic enzymes and period of maceration on the flavor composition of the Romanian Tămâioasă wine- Dragasani

TVL - free flavors; TLP -precursors linked flavors

Applying indigenous yeasts + SO<sub>2</sub> + pectolytic enzymes, the maceration periods can reach up to 48 hours and the use of selected yeasts + SO<sub>2</sub> + pectolytic enzymes, the contact periods between phases shall not exceed 36 hours to obtain the most representative flavors profile.

#### CONCLUSIONS

For obtaining semi-flavored Sauvignon wines and flavored Romanian Tămâioasă wines, it is necessary to consider the following:

- for the same raw material, the resultes of compositional and organoleptic order are different depending on the type of yeasts, as single factor or in combination with adjuvants;

- for the same type of yeasts (indigenous and selected) as a single factor and in combination, results vary in relation to the period of maceration;

- using the singular or in combination selected yeasts, the advantages are related to the reduction of: fermentation ratio, the volatile acidity, length of maceration period, the residual sugar and the increasing of: alcohol, glycerol, extract, ash, polyphenols and total nitrogen, terpenic flavors;

- adopting the process with selected yeasts +  $SO_2$  + pectolytic enzymes, maceration period will not exceed 24 - 25 hours for Sauvignon wine and not more than 36 hours to obtain Romanian Tămâioasă wine.

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## EFFECTS OF ORGANOLEPTIC AND COMPOSITION ORDER OF APPLYING PELLICULAR MACERATION IN THE BIOTECHNOLOGY OF "NEUTRAL" WHITE WINES OBTAINING IN DRAGASANI VINEYARD

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KEYWORDS: grapes, pellicular maceration, enzymes, composition

#### ABSTRACT

To improve the composition and quality of 'neutral' white wines from Dragasani vineyard, pellicular maceration, in biotechnology of primary winemaking, can be a real possibility. By appropriate choice of the main biotechnological factors that are specific to this modern technique, several technological and composition parameters can be improved: grapes output, the alcohol content, extract, ash. Alcoholic fermentation of pellicular maceration musts is potentiated due to higher proportions of nutritive constituents for yeast, extracted from the solid phase.

## INTRODUCTION

The beneficial oenological effects of pellicular maceration and pellicular criomaceration were clearly evidenced by the results of several studies conducted, particularly in "the new world for viticulture and wine" (Australia, New Zeeland, South Africa), but also in some major European wine countries, on Chardonnay variety grapes winemaking, but also on those semiflavored and flavored varieties. The main beneficial effects of pellicular maceration and criomaceration are primarily related to: better extraction of flavored compounds of Vitis vinifera variety (Gunata, 1985; Sapis, 1995); the increasing of free flavors potential of wine (Cannal, 1993); the increasing of products extractivity (Le Fur, 1992); the favoring of alcoholic fermentation (Aldave, 1992) and of malolactic fermentation (Meurques, 1996).

Researches on pellicular maceration that were done in our country have confirmed all the results obtained abroad (Tică, 2009).

## MATERIAL AND METHOD

The study was done in the years 2008 and 2009 using Italian Riesling and Feteasca alba varieties grapes, perfectly healthy and in an advanced stage of maturation. The objective was to increase the quality of the final product - wines.

Following the principle set out by Le Für (1992) concerning the pellicular maceration application, the experiments included the following aspects: different periods of

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contact between must phases in relation to temperature at fixed level, variable maceration temperature in relation to fixed maceration period, different maceration period in relation to different maceration temperature for each period of maceration.

At winemaking for each variety we used the same raw material and uniform biotechnological factors were involved, for all variants and sub-variants of the same variety. The different levels of wine primary factors will be mentioned with the results.

## **RESULTS AND DISCUSSIONS**

With a fixed level of maceration temperature, the periods of contact between must phases decisively influence the development of various constituents of wines and the level of tasting notes (Table 1).

Table 1

Influence of variable pellicular maceration period and of fixed maceration temperature on some compositional and organoleptic characteristics of white wines from Italian Riesling – Dragasani

Maceration Period hours	Maceration temp.⁰C	Volatile acidity g/l H <sub>2</sub> SO <sub>4</sub>	Unred. Extract g/l	Ash g/l	Total poly- phenols g/l	Tannin g/l	Tasting Note S. 1 – 20
0	18 - 20	0,34	17,12	1,67	1,08	0,99	18,55
6	18 - 20	0,34	17,65	1,68	1,12	1,05	18,60
12	18 - 20	0,36	18,09	1,71	1,26	1,22	18,75
18	18 - 20	0,36	18,55	1,79	1,42	1,36	18,75
24	18 - 20	0,36	18,78	1,80	1,54	1,49	18,60
30	18 - 20	0,38	19,06	1,82	1,78	1,61	18,20
36	18 - 20	0,43	19,15	1,89	1,99	1,79	18,00

As the longer pellicular maceration period is, the volatile acidity, unreduced extract, ash and phenolic compounds increase. Between the versions without maceration and with 36 hours of contact between the phases increases are: from 0,34 g/l to 0,43 g/l (with 26,5 %) for volatile acidity; from 17,12 g/l to 19,15 g/l (with 11,9 %) for unreduced extract; from 1,67 g/l to 1,89 g/l (with 13,2 %) for ash; from 1,08 g/l to 1,99 g/l (with 84,3 %) for total polyphenols; from 0,99 g/l to 1,79 g/l (with 80,8 %) for tanin.

Linking the developments of various composition constituents, with the tasting notes it comes out that, in conditions of  $18 - 20^{\circ}$ C temperatures, the periods of 12 to 48 hours between phases attract the most favorable results, at Italian Riesling winemaking. In terms of fix maceration periods, the pellicular maceration temperature exercises decisive action on the development of various constituents and on the tasting notes (Table 2).

Increasings of different physico-chemical composition constituents are directly related to rising levels of pellicular maceration temperatures. Between steps  $10 - 12^{\circ}$ C and  $30 - 32^{\circ}$ C increases were: with 58,1% for volatile acidity; with 7,4% for unreduced extract; with 8,3% for ash; with 67,2% for total polyphenols; with 75,5% for tannin.

It comes out that, at very high maceration temperature levels, the extract and ash contents are modest. Instead there are attracted considerable proportions of polyphenolic compounds that seriously affect the taste characteristics. In addition to these specific varietal flavor profile is affected, and this is reflected by the tasting notes.

Analyzing in connection the composition parameters with the organoleptic characteristics it comes out that in terms of 18 hours maceration period, the temperature around  $16 - 17^{0}$ C attracted clear benefits in line with the intended purpose.

# Table 2

Influence of variable pellicular maceration temperature and fixed length of maceration period on the composition and organoleptic characteristics of white wines from Italian Riesling – Dragasani

Maceration Period hours	Maceration temp. <sup>0</sup> C	Volatile acidity g/l H <sub>2</sub> SO <sub>4</sub>	Unred. Extract g/l	Ash g/l	Total poly- phenols g/l	Tannin g/l	Tasting Note S. 1 – 20
10 - 12	18	0,31	17,98	1,69	1,16	1,02	18,05
14 – 16	18	0,32	18,20	1,73	1,18	1,07	18,80
19 - 20	18	0,32	18,33	1,75	1,21	1,10	18,75
22 - 24	18	0,31	18,45	1,79	1,34	1,21	18,06
26 - 28	18	0,41	18,83	1,80	1,61	1,47	17,30
30 - 32	18	0,49	19,31	1,83	1,94	1,79	17,05

Combined influences of pellicular maceration periods and temperatures were also followed at winemaking using this technique of Feteasca alba grapes, without the contribution of enzymes (Table 3) and with the contribution of pectolytic enzymes (Table 4).

Table 3

Combined influence of maceration period and of maceration temperature on the composition constituents and organoleptic characteristics of Feteasca alba wines – Dragasani

Macera	Macera	Must	Un-	Ash	% ash	Total	Tanni	Residu	Tastin
-tion	-tion	output	reduced	g/l	comp.	poly-	n g/l	-al	g note
period	temp.	1/100	extract	-	to	phenols	-	sugar	
hours	<sup>0</sup> C	kg	g/l		extract	g/l		g/l	
		grapes							
0 (Mt)	-	68,6	17,9	1,66	9,27	0,875	0,712	3,44	17,60
	9 – 11	68,6	18,1	1,69	9,36	0,902	0,744	3,40	18,05
6	17 – 19	68,9	18,4	1,73	9,41	0,921	0,799	3,26	18,15
	25 - 26	69,0	18,5	1,74	9,43	0,988	0,861	3,11	18,00
	9 – 11	68,9	18,3	1,72	9,41	0,951	0,805	3,02	18,35
12	17 – 19	69,1	18,8	1,78	9,49	0,992	0,901	3,03	18,85
	25 - 26	69,2	19,0	1,81	9,51	1,226	1,007	3,10	18,10
	9 – 11	69,3	18,5	1,75	9,48	1,016	0,986	3,26	18,40
24	17 –19	69,6	19,1	1,82	9,52	1,218	1,132	3,06	18,95
	25 – 26	69,8	19,3	1,84	9,54	1,502	1,395	3,02	17,80

The overall analysis of results reveals the following:

- For the same period of maceration, the must output and the composition parameters increase concomitantly with increasing of maceration temperature;

- For the same level of temperature, the levels of oenological parameters increase with the increase of the pellicular maceration period;

- The lowest must outputs and contents of composition constituents were recorded during the maceration period of 6 hours and temperature between  $9-11^{\circ}$ C, and the highest at maceration period of 24 hours and temperature of 25 -  $26^{\circ}$ C;

- Concerning organoleptic aspect, the most favorable results are obtained when applying maceration period between 12- 24 hours, at temperatures not less than  $17^{\circ}$ C and not higer than  $20^{\circ}$ C.

Table 4

Combined influence of maceration period and of maceration temperature on the
composition constituents and organoleptic characteristics of Feteasca alba wines -

Dragasani

Maceration period hours	Maceration temp. <sup>0</sup> C	Must output I/100 kg grapes	Unreduced Extract g/l	Ash g/l	% ash comp. to extract	Total poly- phenols g/l	Tannin g/l	Residual sugar g/l	Tasting note
0 (Mt)	-	68,6	17,9	1,66	9,27	0,875	0,712	3,44	17,60
	9 – 11	69,1	18,4	1,73	9,40	0,935	0,791	3,09	18,15
6	17 – 19	70,4	18,7	1,77	9,46	0,976	0,822	3,07	18,45
	25 - 26	70,6	19,1	1,81	9,47	1,055	0,915	3,08	18,25
	9 – 11	69,8	19,2	1,83	9,51	0,988	0,891	2,83	18,55
12	17 – 19	70,3	20,1	1,93	9,60	1,110	0,996	2,26	18,90
	25 - 26	70,5	20,3	1,95	9,63	1,340	1,166	2,24	18,05
	9 – 11	70,0	20,4	1,94	9,52	1,162	1,096	2,91	18,90
24	17 – 19	71,2	20,6	1,98	9,60	1,402	1,275	2,73	18,60
	25 – 26	71,4	20,7	2,00	9,64	1,605	1,488	2,25	17,90

Using the same raw material, additing pectolytic enzymes in must at all times and levels of maceration temperature, the must output and the physico-chemical composition parameters increase (Table 4).

Adding enzymes to Feteasca alba must, correlating the composition parameters with the tasting notes, the most favorable results can be obtained from 12 hours maceration period and  $17 - 19^{\circ}$ C temperature and 24 hours maceration period and the temperature of 9 -  $11^{\circ}$ C.

In comparison with the results from the two variants without enzyme, the enzymes contributions were: 1,7 % for must output, 6,9 % for unreduced extract, 8,4 % for ash, 11,9% for total polyphenols, in the case of combination : 24 hours maceration period and the temperature of  $17 - 19^{\circ}$ C.

## CONCLUSIONS

The composition and quality of Italian Riesling and Feteasca alba white wines from Dragasani vineyard can be improved by applying to the primary winemaking the prefermentative technique of pellicular maceration.

Winemaking without adding enzymes to the two varieties of grapes, the period of contact between phases will not fall below 12 hours and will not exceed 24 hours at temperatures of  $16 - 19^{\circ}$ C.

By adding enzymes to Feteasca alba must increase: must output, extract, ash, polyphenols.

At bigger periods and temperatures than those specified, the composition and the specific varietal aroma profile are seriously affected.

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## CONTRIBUTIONS TO THE KNOWLEDGE REGARDING THE HERPETOFAUNA FROM VĂLIȘOARA GORGE NATURAL RESERVATION, ALBA COUNTY, ROMANIA

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KEY WORDS: herpetofauna, distribution, anthropogenic impact, western Romania

## ABSTRACT

We identified 7 amphibian species (Lissotriton vulgaris, Triturus cristatus, Bombina variegata, Hyla arborea, Bufo bufo, Rana dalmatina, Pelophylax ridibundus) and 5 reptile species (Lacerta viridis, Podarcis muralis, Anguis fragilis, Natrix natrix și Coronella austriaca) at the level of the Vălișoara Gorge Natural Reservation from Alba County. The composition of the herpetofauna is typical of the sectors with average altitudes from the western part of Romania. Most of the identified species' populations are large-sized. The anthropogenic impact that affects the herpetofauna from the reserve is represented by road mortality, depositing of domestic wastes, as well as by the presence of concrete basins, with high walls, that are presently abandoned and constitute traps for several species belonging to the herpetofauna.

#### INTRODUCTION

The herpetofauna from different protected areas from Romania has started to be included in various research programs in the past years (Covaciu-Marcov et al., 2008, 2009a,b,c; Strugariu and Gherghel, 2008). However, there are presently many areas in the country that have been briefly studied regarding this point of view. The knowledge concerning the composition and the geographical distribution of Romania's herpetofauna earlier than the years 2000 represented only 5-10 % of the real situation observed on the field (Ghira et al., 2002). Currently, it is an important challenge for the conservationist biology to understand the environmental factors responsible for the distribution and the persistence of the organisms in the habitats (Hartel and Öllerer, 2009). Understanding this relation is a key step in successfully preserving the habitats (Manenti et al., 2009). These data are even more necessary in protected areas, where the habitats that are most important to different species should benefit from a special attention and status. Although the number and surface of the protected areas has grown in the past years in Romania, there are few complete studies regarding at least one group of animals from these levels. In this context, the herpetofauna is a distinct group that comprises many rare species, included in the Romanian and European conservation legislation (Iftime 2005, O.U.G. 57/2007). However,

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studies regarding the Romanian herpetofauna are presently scarce. Therefore, our study presents preliminary results concerning the composition of the herpetofauna from the Vălişoara Gorge Natural Reservation from Alba County.

## MATERIAL AND METHODS

Our study was realised at the end of May, 2010. The analysed area is represented by the Vălişoara Gorge Natural Reservation, as well as by the neighbouring sectors. The reservation is situated in Alba County, at the southern limit of Trascău depression, in Trascău Mountains, belonging to the Apuseni Mountains (Posea and Badea, 1984). The altitude of the studied region varies between 425 and 729 m. The area is covered with deciduous forests, respectively beech and oak, but the slopes have been deforested in many places, being covered by degraded meadowlands and pastures, or even nude rocky regions have been left behind.

The transect method was used on the field study (Cogălniceanu, 1997), the most representative habitats from the area being investigated. The animals were captured through different methods depending on their origin group. Thus, the aquatic species and the ones that were found in the water during the reproduction period were captured using different nets mounted at the level of long metallic rods. The terrestrial species, as well as all of the reptile species were captured directly by hand. The captured animals were released in the habitats in all of the cases. We also identified the animals that were killed by locals or by vehicles.

## **RESULTS AND DISCUSSIONS**

During our study we identified 12 herpetofauna species in the Vălişoara Gorge Natural Reservation from Alba County. From these, 7 were amphibian species (*Lissotriton vulgaris, Triturus cristatus, Bombina variegata, Hyla arborea, Bufo bufo, Rana dalmatina, Pelophylax ridibundus*) and 5 were reptile species (*Lacerta viridis, Podarcis muralis, Anguis fragilis, Natrix natrix* and *Coronella austriaca*). Following the fact that neither the Vălişoara Gorge nor the two neighbouring localities have been mentioned in the most recent synthesis upon the herpetofauna from Transylvania (Ghira et al., 2002), all of the recorded species were identified in a premier for the studied region.

*Lissotriton vulgaris* is a common species for the Vălişoara Gorge Natural Reservation, being a generally well represented form in Transylvania (Ghira et al., 2002). It reproduces in the low areas of the reservation, where there are present greater water accumulations, which lack from the higher and steeper areas. It can be encountered in ditches and canals, but also in the bogging areas formed at the level of some streams. These reproducing habitats are also characteristic to the species from other areas of Romania (Sos et al., 2008; Strugariu et al., 2008).

*Triturus cristatus* is also well represented, being present in the same sectors and habitats as the previous species. In Romania the crested newt is at the southern limit of its distribution area, therefore it is a species connected to higher areas (Arntzen and Borkin, 1997), its presence in the reservation being thus expectable. However, the number of observed individuals was lower than in the case of the previous species. This fact does not indicate more difficult conditions for the species, being only a consequence of its larger body size. The smaller size of the *Triturus cristatus* populations compared to the ones of *Lissotriton vulgaris* has been previously signalled (Cicort-Lucaciu et al., 2009, Dobre et al., 2009).

*Bombina variegata* is the most well represented amphibian species from the studied region. The fact is a normal one, generally being a well represented species in the higher areas from western Romania (Covaciu-Marcov et al., 2003, 2005, 2006, 2007, 2008). It is present both in the higher areas neighbouring the gorge, at its level, as well as in the streams from the slopes. Moreover, *Bombina variegata* also populates artificial habitats such as canals from the road margin, or basins with concrete walls. It can be even observed in the sectors that are used for the watering of the cattle.

*Hyla arborea* is harder to be observed than the previous one, due to its way of life. However, the species is well represented in the Vălişoara Gorge Natural Reservation, being advantaged by the large surface of woods and by the wet areas from the reservation.

*Bufo bufo* is also a well represented species in the reservation, being especially present in its lower areas, where the wide wet and relatively permanent surfaces used for reproduction are found. Relatively numerous individuals fall victim to the road traffic on the county road that crosses the reservation, both inside of it and in the neighbouring area. The very bad state of the road, where the speeding rate is about 20-30 km / h, suggests that the drivers intentionally cross over the frogs. In addition, this frog frequently falls victim to road traffic in other parts of its area (Elżanowski et al., 2009; Hartel et al., 2009).

*Rana dalmatina* is a common species in the Välişoara Gorge Natural Reservation, being encountered in all of its forested areas. It is present both in the lower areas neighbouring the river, in the humid areas that surround them and at the level of the forested slopes. Exceptionally, there were identified individuals that had fallen in two basins with concrete walls, which were used in the past as fish-breeding places, but are now abandoned. The respective basins are true traps for frogs, which cannot escape them, being identified in an advanced state of malnutrition. The two basins should be covered, or at least their walls should be partially broken down, which would enable the fallen frogs to escape.

*Pelophylax ridibundus* is spread in a more localized manner in comparison to the previous ones, following its ecological requirements, being a form connected to permanent waters, usually of large sizes (Cogălniceanu et al., 2000). Thus, the species is limited to the lower areas of the gorge, being present at the level of the main course of the river that crosses the gorge as well as at the springs that supply it. Moreover, it occupies the canals from the road margin, or the anthropogenic basins. Although the studied area has a mountainous character, and the species is considered to be specific to the field and hill sectors, the altitude at which it is present here is not a record, in Romania being identified at higher altitudes (Covaciu-Marcov et al., 2003).

*Lacerta viridis* is well represented in the analysed area, being present in most of its parts. Thus, the green lizards are present both in the sectors with open rocks, and at the level of the skirts of the forests, of the bushes and shrubs, or even on the river's shores. We have encountered an individual that had fallen in the same concrete basin as the agile frogs, the respective sample being released as the previous ones.

*Podarcis muralis* is represented in the reservation through large populations. However, the species has a more limited distribution, generally being present at the level of the rocks situated near the river, and more rarely at the skirts of the forests that neighbour the rocky sectors.

*Anguis fragilis* is well represented in the Vălișoara Gorge Natural Reservation, being advantaged by the large surface held by forests. It frequently falls victim to road traffic.

*Natrix natrix* is a common species in the studied region. It was identified especially in the gorge and in the wet areas neighbouring the river. This species also falls

relatively frequently to road traffic, the fact being previously signalled in the case of this species and in the case of snakes in general (Krecsák et al., 2004; Covaciu-Marcov et al., 2009b,c).

*Coronella austriaca* is spread in a more localised manner than the previous species, being observed only in the moister areas neighbouring the river. Moreover, this species also falls victim to road traffic.

## CONCLUSIONS

The herpetofauna of the Vălişoara Gorge Natural Reservation is a typical one for the average altitudes areas from western Romania. Thus, the field and mountainous species lack from here. Most of the herpetofauna species are represented by forms connected to the forested sectors, which still occupy wide surfaces in the reservation. Most of the species from the studied area are important from a conservative point of view, being species that require strict protection. Most of the species identified in the reservation are represented through large populations.

The anthropogenic impact upon the herpetofauna of the studied region is represented by road mortality, which affects both the amphibian and reptile species. Meanwhile, tourists and locals deposit their domestic wastes in many places near the river. The abandoned concrete basins which act as traps for many species also constitute a negative impact upon the herpetofauna. The respective basins should be eliminated, or at least their walls should be partially broken down. Meanwhile, the road that crosses the reservation should be equipped with parapets that stop the access of the herpetofauna on the roads.

#### ACKNOWLEDGEMENTS

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## RESEARCHES CONCERNING THE PROPAGATION THROUGH SEEDS OF SOME LEWISIA SPECIES

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KEY WORDS: Lewisia, propagation, seeds

#### ABSTRACT

Originary from west regions of United States, the species of Lewisia genus are cropped and traded in many other European countries. Because of high adaptability to different environment conditions, the main direction of use represents the establishment of rocks and alpine gardens, but the special decorative aspect thanks to flowers and foliar architecture recommend them as plants in pots. The researches made aimed to establish the efficiency of propagation through seeds but also the behaviour in the conditions of cropping in protected spaces (in conditions of greenhouse) of two species of Lewisia (Lewisia cotyledon and Lewisia pigmaea), uncultivated in our country.

#### **INTRODUCTION**

The name of genus *Lewisia* was given in the honor of captain Meriwether Lewis and he was described for the first time by Frederick Pursh in the "Flora Americae Septentrionalis" in 1814, the first species described being *Lewisia rediviva* (Graham, 2002). Genus *Lewisia* (Pursh) belongs to the family Portulacaceae and encloses over 19 plants semi-succulent perennial species extended in Northern and West regions of USA, the most known being *Lewisia cotyledon, Lewisia tweedyi, Lewisia rediviva, Lewisia columbiana, Lewisia pigmaea, Lewisia longipetala, Lewisia stebbinsii* (Eggli, 2002). Natural hybridization of species from spontaneous flora creates difficulties in species identification.

The species of this genus were used from the beginning as decorative plants, in the establishment of alpine gardens and rocks. But in the past years, the obtaining of new varieties (approximately 155) with special decorative valences made for these species to be more and more used as plants in pots (Houghton, 2000).

Reduced ecological demands and large zoning (areas 4-9) lead to their extension on all continents. In Europe the most popular is *Lewisia cotyledon* (Stuart, 1989).

The researches made in the past years abroad attended especially the methods of generative and vegetative multiplication, especially micropropagation, in order to follow a fast obtaining and of a bigger number of merchantable plants, but especially the creation of

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new hybrid varieties with special decorative values. Less known in our countries are the species of genus *Lewisia* which have not been studied.

Concerning the multiplication through seeds, the researches made aim especially the effect of different physical and chemical treatments on the duration and the germination process. Therefore Aelbrecht, 1989, studying the effect of temperature variations on the seeds germination of *Lewisia cotyledon* observed that the maintaining during 3-4 weeks to temperatures of -5 Celsius degrees, followed by the alternative subjection to temperatures of 5 and respectively 15 Celsius degrees improve the germination process of seeds until 76% to 82%, while for untreated seeds germination was of only 16%.

Deno, 1993 and Pojar J, 1994 recommend as well stratification to cold for propagation through seeds for *Lewisia* species.

The purpose of this study was represented by the observation of behaviour of some species of *Lewisia cotyledon* and *Lewisia pigmaea* in the conditions of cropping in protected spaces and aimed the following of the germination process, as well the monitoring of plants evolution until the stage of adult plant.

## MATERIAL AND METHODS

Biological material (seeds) for the two species, *Lewisia cotyledon* and *Lewisia pigmaea* was obtained from a profile distribution company, this accomplishing the conditions that correspond to quality.

Seeds (in a number of 100 for each species) were put under germination in minigreenhouses, the sublayer used being a mixture of compost, sand and perlite in percentage of 2:1:1 (mentioned by Baulk, 1989). The whole germination process was done in an acclimatization chamber (germinator TG 400). For improving the germination process the seeds were subjected to some thermic treatments (for 2 weeks to a temperature of 4°C, 3 weeks to 18°C, then to 12°C, until the end of the germination period). Observations made on the germination process was made periodically and it was followed the identification of the germination moment, duration and dynamic of the process and the establishment of the germination process. After the conclusion of the germination process the seedlings were moved in pots with the diameter of 8 cm in sublayer of humus, peat and sand in equal parts and it was monitored their evolution, in greenhouse conditions, until the stage of mature plant.

Botanical description upon Hershkovitz, 2003 in "Lewisia. Flora of North America" is the following:

*Lewisia cotyledon* (Watson) - Taproots gradually ramified distally. Stems ascending to suberect, 10-12(-30) cm. Leaves: basal leaves evergreen, sessile or abruptly or gradually narrowed to broad petiole, blade oblanceolate, obovate, spatulat 3-14 cm. Inflorescences dense, paniculate to subumbellate cymes, 10-50-flowered.

Flowers pedicellate usually pink-purple with pale and darker stripes. Capsules 3-5 mm. Seeds 4-15, 1.5 mm, shiny, smooth.

*Lewisia pygmaea* (Gray) - Taproots gradually ramified distally or shortly fusiform, rarely subnapiform. Stems prostrate or suberect, becoming reflexed in fruit, 1-6 cm. Leaves: basal leaves withering at or soon after anthesis, sessile or gradually tapered to long petiole, blade linear to linear-oblanceolate. Inflorescences 2-4(-7)-flowered in racemose cymes or with flowers borne singly. Flowers pedicellate, white, pink, or magenta, sometimes green at base. Capsules 4-5 mm. Seeds 15-24, 1-2 mm, shiny, smooth.

Both species flower on spring until late summer and it is recommended to be used especially for the decoration of exterior spaces.





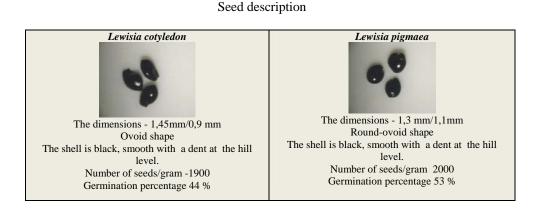
Figure 1. Lewisia cotyledon Figure 2. Lewisia pigmaea

# **RESULTS AND DISCUSSION**

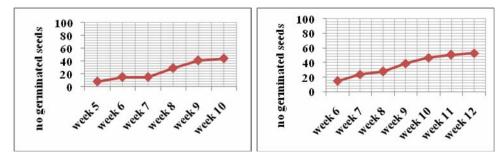
Multiplication through seeds is recommended for *Lewisia* species, but the opinions are shared concerning the period of seeding but also the duration, respectively the germination percentage. Some authors mention as being more favorable the period September - November, when the flower takes place in spring of the following year (Baulk, 1989), the others recommend the months of spring and in this situation the flower will start in the second year (Harcourt, 2000), or during the year (Nicholls, 2002). The germination period can oscillate between one month and three months and in more rare cases it can reach 6 months, according to the species (Baulk, 1989).

The observations and measurements made to seeds for the two species show resemblances concerning the dimensions and their morphology (table 1).

Table 1



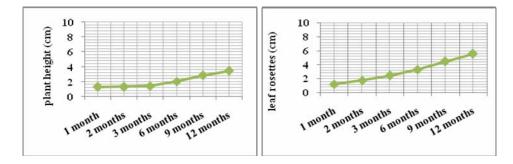
In both species the seeding was done in spring, in April. From data presented in the graphs 1 and 2 we observe that seeds germination was done after 5 weeks for Lewisia *cotyledon* and 6 weeks for *Lewisia pigmaea* and lasted 6 weeks for *Lewisia cotyledon*, respectively 7 weeks for *Lewisia pigmaea*. Even if the seeds were subjected to thermic treatments the germination percentage was of 44% for Lewisia cotyledon and 53% for *Lewisia pigmaea*.



Graph1.The germination at *L. cotyledon* 

Graph 2. The germination at L. pigmaea

After planting into pots, the propagation of development of seedlings was achieved differently for the two species. Analyzing the data from graphs 3 and 4 we can observe that for *Lewisia cotyledon*, the propagation speed of seedlings was slow, therefore, after one year the medium height of the plants was of 3,11 cm and the diameter of resedas formed of leaves did not depass 5,55 cm, these values being closed, but under those mentioned in the specialty literature, that is 6-12 cm, the diameter of foliar resedas (Stuart, 1989) (fig. 1,2).



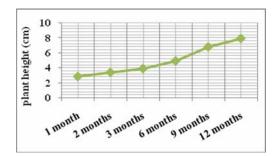
Graph 3. Plant growth dynamics at L. cotyledon Graph 4. Diameter leaf rosettes at L.cotyledon





Figure 1. L. cotyledon at three months Figure 2. L. cotyledon at six months

For the plants *Lewisia pigmaea* the increase speed was bigger, after 1 year, the average value of the plants height was 7,87 cm, the dimensions of the aerial vegetative organs having closed heights with those described in the literature (Hershkovitz, 2003) (graph 5, fig. 3,4).



Graph. 5 Plant growth dynamics at L. pigmaea



Figure 3. L. pigmaea at three months Figure 4. L. pigmaea at six months

In the greenhouse conditions, the flower for *Lewisia cotyledon* was in the month of June, but only to some plants, and the decorative period of approximately 12 days. The blossoms that start from the basis of having 5-7 pink flowers with white margins represented the main decorative element. After the flowers wilt this species remains an ornamental plant through leaves, because of resedas of green dark colour.

*Lewisia pigmaea* can be used as well as a plant in a pot, decorating as well through small pink flowers as well through the architectural aspect, especially given by succulent linear leaves that ramify on a basal way. To this one the blossom took place earlier, but it was still sporadic, the flowers were small, of approximately 0,5 cm diameter in comparison with the value of 1,5-2 cm described in literature (Hershkovitz, 2002) and the decorative period was approximately of 5 days.



Fig. 5 L.cotyledon and L. pigmaea at 1 year Fig.6 L.cotyledon (flower) Fig.7 L.pigmaea (flower)

## CONCLUSIONS

Propagation through seeds can be considered an effective method of multiplication for *Lewisia*. The germination percentage for *Lewisia pigmaea* was of 53% in comparison

with *Lewisia cotyledon* (44%), even if in the case of the second species of germination started a week earlier and the duration of the process was smaller.

For the multiplication through seeds, the seedlings for *Lewisia cotyledon* reached their maturity (to blossom) after a year and two months and those from *Lewisia pigmaea* after a year.

The morphological development of aerial vegetative organs (stem, leaves) for *Lewisia pigmaea* was appropriate, but the flowers had much smaller dimensions while for *Lewisia cotyledon* the development of vegetative parts was short, and the flowers developped normally.

Even if blossom was sporadic and of short period, the behaviour of the two species in conditions of a greenhouse recommends the use of these plants in pots, but also the following in green spaces, taking into account that the main direction of use is the one for alpine gardens and rocks.

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## STUDY CONCERNING THE BEHAVIOUR TO WATER STRESS OF SOME SUCCULENT FLOWER PLANTS

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KEY WORDS: succulent plants, water stress, wilting

#### ABSTRACT

The analysis of the initial water content from leaves to twenty species of succulent flower plants indicated values between 91.53% and 98.24%. After being subjected to total water stress it was stated that theose twenty species standed a period between nine and seventeen weeks, the permanent wilting intervening when the water content from leaves decreased with 4.29% until 19,.9%, according to the species. The establishment of some correlations between the water percentage lost and the biometric values of foliar structures that contain and reduce the water losses (the cells of the mesophyll, the thickness of the cuticle and stomata density) shows the combined effect of foliar structures in the limits of water losses and implicitly to drought resistance.

## INTRODUCTION

The knowledge of the way of behaviour of flower plants in the conditions of application of water stress can represent among the theoretical and practical importance, representing a criteria that must be taken into account in establishing the water that is necessary but also in the association of different species in different flower combinations.

Succulent plants coming from regions with draughty climate bear long periods of draught and those which have their origin in regions with temperate climate from North hemispheres *(Sedum sp.)* have enough water reserves in order to remain alive a longer time, without being watered (Schulte 1989, Van Woert et al. 2005).

The water content from the succulent plants registers high values, between 90-94% (Wickens 1998, Barrera 2009), or even more 95% (Capon 2010), varying according to species, organs, tissue (Willert 1992, Sayed 1998).

The high dimensions of the mesophyll cells and the presence to this level of some substances osmotic active, that increase the capacity of retaining water make that to the level of leaves cumulate the highest water quantity (Esser 2004, Mauseth 2009).

Retaining water is favoured by other structure features of leaves as the cuticle thickness and the low density of stomata (Willert 1992). The analysis of these foliar structures, relieve an interdependence between those that retain water, respectively those who limit the water losses (Cristescu et al. 2009).

The observations made followed the identification of water content from leaves for those twenty species under study with the periodic monitoring of water losses,

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identification of percentage of lost water, which induces the permanent wilting and the period of time upon which this phenomenon is installed. As well there were established correlations between the water losses and the biometric values of different foliar structures (dimension of mesophyll cells, the cuticle thickness and stomata density).

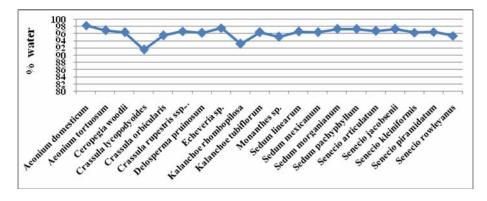
## MATERIAL AND METHOD

In order to analyze the water content there were sampled the leaves from mature plants belonging to the twenty species of succulent plants studied: *Aeonium domesticum*, *Aeonium tortuosum*, *Ceropegia woodii*, *Crassula rupestris ssp marnieriana*, *Crassula orbicularis*, *Crassula lycopodyoides*, *Delosperma pruinosum*, *Echeveria sp.*, *Kalanchoe rhombopilosa*, *Kalanchoe tubiflorum*, *Monanthes sp*, *Sedum linearum*, *Sedum mexicanum*, *Sedum morganianum*, *Sedum pachyphyllum*, *Senecio articulatum*, *Senecio jacobsenii*, *Senecio piramidatum*, *Senecio kleiniformis*, *Senecio rowleyanus*. The analysis of water content from leaves was made with the help of the analytical balance Kem, before and after the application of the water stress, from the apparition of the first signs of fading for some species until the final fading of the last species. After total removal of watering, determinations were made in stages, at determined time intervals. (at 3, 5, 7, 9, 11, 13, 15, and 17 weeks).

Biometric values of different foliar structures, such as the dimensions of the mesophyll cells, the thickness of the cuticle and stomata density were determined before the plants be subjected to water stress, with the help of the optic microscope with the ocular micrometer. The phenomenon of wilting was visually analyzed, for the decorative aspect, and the irreversibility of wilting was analyzed with the help of some plants witness. Correlations were achieved with the help of the statistics Excel programme.

## **RESULTS AND DISCUSSION**

The analysis of the initial content of water from leaves (before the application of water stress) to the twenty species of succulent flower plants indicated high values between 91,53% for *Crassula lycopodyoides* end 98.24% for *Aeonium domesticum* (graph 1).



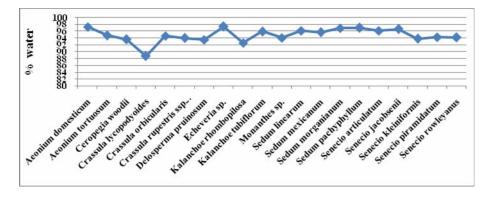
Graph 1. The initial water content (%) in leaves

After three weeks (stage I) from total removal of watering, the percentage of lost water from plants was between 0,19% (*Echeveria sp.*) and 2,99% (*Aeonium tortuosum*)

(graph 2).

Reported to genera, the smallest values registered to the species **Sedum** values between 0,32% for *Sedum pachyphyllum* and 0,74% la *Sedum mexicanum*), followed by *Kalanchoe* (5% for *Kalanchoe tubiflorum* and 0,56% for *Kalanchoe rhombopilosa*), *Senecio* (values between 0,71 % for *Senecio jacobsenii* and 2,54 % for *Senecio kleiniformis*), *Aeonium* (2,1% for *Aeonium domesticum* and 2,61% for *Aeonium tortuosum*) and *Crassula* (with values between 2,82% for *Crassula lycopodyoides* and 2,70% for *Crassula rupestris ssp marnieriana*). Intermediate values presented the species *Monanthes sp.* (1,02%), *Delosperma pruinosum* (2,74%) and *Ceropegia woodii* (2,78%).

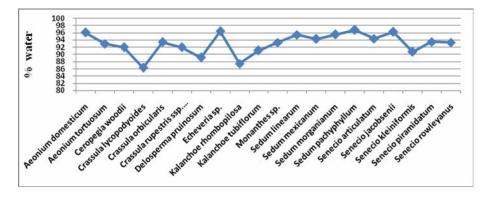
After three weeks from stopping the watering, at the level of the foliar device appeared the first morphological modifications, that at the majority of the species consisted of a change of leaves texture (more soft leaves). At *Senecio articulatum, Senecio kleiniformis, Senecio piramidatum* the modifications are more obvious, displaying through a soft crimping of leaves. They did not present signs of wilting *Echeveria sp., Monanthes sp., Sedum morganianum, Sedum pachyphyllum* and the species of the genus *Aeonium*.



Graph 2. The water content (%) - stage I

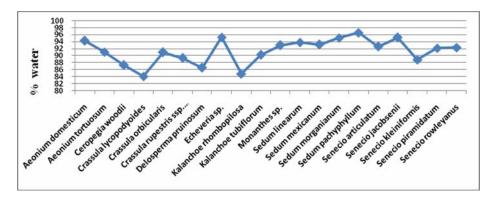
Determinations performed in second stage relieved to some species bigger differences concerning the percentage of lost water. In this stage, the highest percentage of lost water presented the species of the genus *Kalanchoe* (5,10% *Kalanchoe rhombopilosa* respectively 4,80% *Kalanchoe tubiflorum*) and *Delosperma pruinosum* 4,37% (for those species registering the biggest differences in comparison with the previous period), and the smallest *Sedum pachyphyllum* (0,18%), *Senecio jacobsenii* (0,40%), followed by *Monanthes sp.* (0,75%), *Senecio piramidatum* (0,80%) and *Echeveria sp.* (0,92%) (graph 3). For the other species the values were closed to those from the first stage.

The wilting phenomenon could be observed in this moment for the majority of species, more pronounced for *Crassula rupestris ssp. marnieriana, Kalanchoe rhombopilosa, Kalanchoe tubiflorum, Delosperma pruinosum, Senecio articulatum, Senecio kleiniformis Senecio rowleyanus.* The affected species were *Aeonium domesticum, Echeveria sp. Sedum morganianum, Sedum pachyphyllum, Monanthes, Senecio jacobsenii.* For all species, the wilting phenomenon is reversible on this stage.



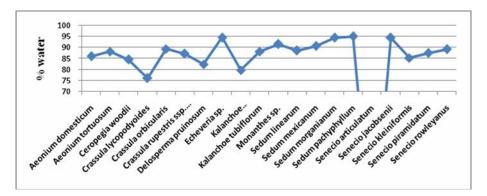
Graph 3. The water content (%) - stage II

In IIIrd stage the differences registered were between 0,23% for *Sedum pachyphyllum* and 2,65% for *Kalanchoe rhombopilosa*, for the majority of the species the values being in the limits closed to those from previous stages (graph 4). The wilting phenomenon is reversible for all species.



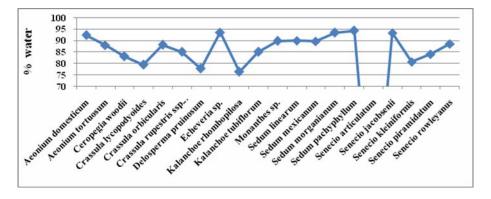
Graph 4. The water content (%) – stage III

After 9 weeks of water stress (stage IV) wilting is permanent for *Senecio* articulatum (graph 5)



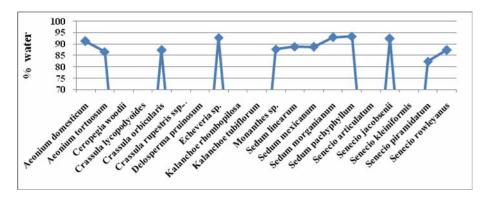
Graph 5. The water content (%) - stage IV

In Vth stage the values of the percentage of dehydrating are between 0,85% for *Senecio jacobsenii* and 4,59% for *Delosperma pruinosum*. In this stage not any other species was affected by permanent wilting (graph 6).



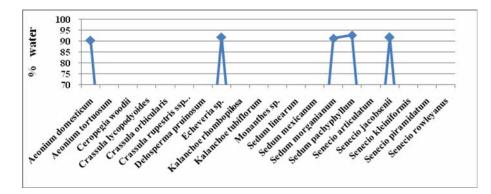
Graph 6. The water content (%) - stage V

Starting with 13<sup>th</sup> week (stage VI), wilting is permanent for other 6 species (*Ceropegia woodii, Crassula lycopodyoides, Crassula rupestris ssp marnieriana, Delosperma pruinosum, Kalanchoe rhombopilosa, Kalanchoe tubiflorum* and *Senecio kleiniformis*). In this stage the percentage of lost water from leaves being between 0,7% for *Sedum morganianum* and 2,22% for *Monanthes sp* (graph 7).

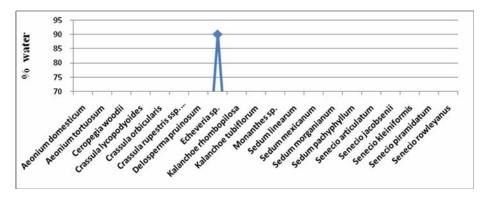


Graph 7. The water content (%) – stage VI

Starting with 15<sup>th</sup> week (stage VII), just five of all species under study has been resisting to water stress, after 17 weeks (stage VIII) the most repellent being *Echeveria sp.* (graph 8,9).



Graph 8. The water content (%) - stage VII



Graph 9. The water content (%) - stage VIII

Data from table 1 indicates for each species what is the water percentage that the species studied may lose in a period of time, then the wilting becomes irreversible.

From the determinations made it was stated that wilting is determined by water losses between 4,29% (*Senecio articulatum*) until 19,9% to (*Delosperma pruinosum*) values under those indicated in literature for heliophile plants (Toma, 1998 states that in the case of heliophile species temporary wilting manifests when plants lose 20-30% from total water)

According to total quantity lost which determines the permanent wilting, those 20 species of succulent plants can be grouped in plants that can suffer losses under 5% (Sedum pachyphyllum, Senecio articulatum), losses between 5% and 10% (Aeonium domesticum, Crassula orbicularis, Echeveria sp, Monanthes sp., Sedum morganianum, Senecio jacobsenii, Senecio rowleyanus), losses between 10% and 15% (Aeonium tortuosum, Ceropegia woodii, Crassula lycopodyoides, Crassula rupestris ssp marnieriana, Kalanchoe tubiflorum, Senecio piramidatum), losses over 15% (Delosperma pruinosum, Kalanchoe rhombopilosa, Senecio kleiniformis).

Table 1.

#### The percentage of water lost to cause permanet wilting

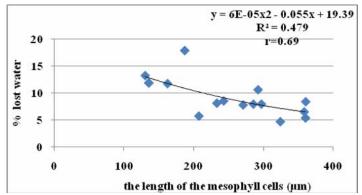
Species	The percentage of lost water to cause permanent wilting	The period (weeks)
Aeonium domesticum	7,99%	15
Aeonium tortuosum	10,65%	13
Ceropegia woodii	13,57%	13
Crassula lycopodyoides	13,27%	13
Crassula orbicularis	8,58%	13
Crassula rupestris ssp. marnieriana	11,92%	13
Delosperma pruinosum	19,09%	13
Echeveria sp.	6,55%	17
Kalanchoe rhombopilosa	17,92%	13
Kalanchoe tubiflorum	11,79%	13
Monanthes sp.	7,82%	15
Sedum linearum	8,16%	13
Sedum mexicanum	7,98%	13
Sedum morganianum	5,41%	15
Sedum pachyphyllum	4,73%	15
Senecio articulatum	4,29%	9
Senecio jacobsenii	5,77%	15
Senecio kleiniformis	16,13%	13
Senecio piramidatum	14,61%	13
Senecio rowleyanus	8,43%	13

The results of the observations concerning the biometrical values of foliar structures for these twenty species are presented in the work *"Study of the structural particularities of leaves in succulent flower plants* "(Cristescu et al. 2009).

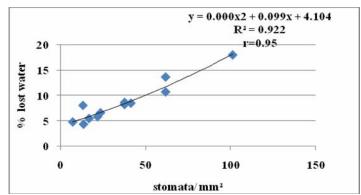
Therefore, for the dimensions of the mesophyll cells are between 135,67 $\mu$ m (L)/108,6  $\mu$ m (w) for *Crassula rupestris ssp. marnieriana* and 495,11  $\mu$ m (L)/433,7  $\mu$ m (w) for *Senecio piramidatum*, the density of stomata (inferior epidermis) is between 7,21 st/mm<sup>2</sup> *Sedum pachyphyllum* and 101,29 st/mm<sup>2</sup> for *Kalanchoe rhombopilosa*, and the cuticle thickness varies from 2,71 $\mu$ m for *Senecio articulatum* and 19,52 $\mu$ m for *Sedum pachyphyllum*. Establishing some correlations between these and the percentage of water lost during the whole period of water stress stated the followings:

- negative significant correlation between the percentage of water lost and the dimension of the mesophyll cells (L) for fifteen species (Aeonium domesticum, Aeonium tortuosum, Crassula lycopodyoides, Crassula orbicularis, Crassula rupestris, ssp marnieriana, Echeveria sp., Kalanchoe rhombopilosa, Kalanchoe tubiflorum, Monanthes sp, Sedum linearum, Sedum mexicanum, Sedum morganianum, Sedum pachyphyllum, Senecio jacobsenii, Senecio rowleyanus) (graph 10).
- positive significant correlation between the density of stomata and the percentage of the water lost for twelve species under study (*Aeonium domesticum, Aeonium tortuosum, Ceropegia woodii, Crassula orbicularis, Echeveria sp., Kalanchoe rhombopilosa, Sedum linearum, Sedum morganianum, Sedum pachyphyllum, Senecio articulatum, Senecio jacobsenii, Senecio rowleyanus*)(graph 11).

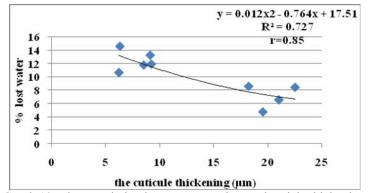
- negative significant correlation between the cuticle thickness and the percentage of lost water for nine of the species (*Aeonium domesticum*, *Crassula lycopodyoides*, *Crassula orbicularis*, *Crassula rupestris ssp. marnieriana*, *Echeveria sp, Kalanchoe tubiflorum*, *Sedum pachyphyllum*, *Senecio piramidatum*, *Senecio rowleyanus*)(graph 12).



Graph 10. The corelation between water lost and the mesophyll cells dimensions



Graph 11. The correlation between water lost and stomata density



Graph 12. The correlation between water lost and cuticle thickening

For the strongest five species (*Aeonium domesticum*, *Echeveria sp*, *Sedum morganianum*, *Sedum pachyphyllum* şi *Senecio jacobsenii*) the establishment of some correlations between the percentage of lost water and the dimensions of the mesophyll cells, the density of stomata and for *Aeonium domesticum*, *Echeveria sp*. and *Sedum pachyphyllum* and for the cuticle thickness, it states the effect combined of these structures on the capacity of containing water.

#### CONCLUSIONS

For the twenty species taken under study, the permanent wilting installed when the total water content from leaves decreased with 4,29% (Senecio articulatum) until 19,9% (*Delosperma pruinosum*). From the analysis made on determined periods of time, we observe that the water losses are constant for some species (*Monanthes sp, Sedum mexicanum, Senecio jacobsenii*) or fluctuate all along different stages (*Delosperma pruinosum, Kalanchoe rhombopilosa, Kalanchoe tubiflorum, Senecio jacobsenii*).

From the twenty species taken under study the least resistant was *Senecio articulatum* where the permanent wilting intalled after nine weeks from stopping waterings.

With the exception of five species that lasted fifteen weeks (and *Echeveria sp.* seventeen weeks), for the rest of the species the permanent wilting installed at thirteen weeks.

For the most resistant five species (*Aeonium domesticum, Echeveria sp, Sedum morganianum, Sedum pachyphyllum* și *Senecio jacobsenii*), the percentage of lost water from one stage to another maintained under the value of 1% (exception *Aeonium domesticum*), and the water content from leaves did not decrease to 90%.

The establishment of some correlations between the percentage of water lost and the structure features of leaves shows the role in the limits of water losses, respectively the resistance to drought.

Taking into consideration the period of resistance to drought, we can state that between the 19 species of succulent plants we can accomplish different types of decorative combinations, taking into account the aesthetic aspect.

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# UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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

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# DATA REGARDING THE FOOD COMPOSITION OF SOME *Pelophylax* kl. esculentus POPULATIONS FROM SATU MARE COUNTY, ROMANIA

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KEY WORDS: Pelophylax kl. esculentus, feeding, period, habitat

# ABSTRACT

The study was carried out in the year 2006 in Satu Mare County. The trofic spectrum varies depending upon the period in which the samples were collected.

The most important preys which were consumed by the analyzed frogs are the Lepidoptera larvae, Araneae and Coleoptera. The feeding diversity is over 2 points in each period of the study.

# **INTRODUCTION**

The completion of a global amphibian assessment (GAA) by the IUCN indicated that amphibians had indeed decreased more rapidly over the previous 30 years than other vertebrates for which data were available (Stuart et al., 2004).

This and other studies highlighted multiple likely causes, including habitat destruction, climate change, agrochemicals, pollution, enhanced UV-irradiation and emerging diseases (Collins & Storfer, 2005, Pounds et al., 2006), thus new data regarding different aspects of amphibian biology are important for their conservation.

*Pelophylax esculentus* is a hybridogenetic frog originating from mating between *P. ridibundus* (RR) and *P. lessonae* (LL). Typically, diploid hybrids (LR) live in sympatry with one of their parental species, upon which they depend for successful reproduction. In parts of their range, however, pure hybrid populations can be found (Arioli et al. 2010).

Studies regarding the feeding of the frogs from the *Pelophylax* kl. *esculentus* complex have been published in the past years at *P. ridibundus* (Covaciu-Marcov et al., 2003, 2005; David et al., 2008), *P. lessonae* (Sas et al., 2005, 2007) and also at *Pelophylax* kl. *esculentus* (Ferenți et al., 2007; Sas et al., 2007, 2009, David et al., 2009).

The trophic spectrum of the green water frogs was also studied without making the difference between the three forms (e.g. Kovacs & Torok, 1995; Low & Torok, 1998; Cogălniceanu et al., 2000).

# MATERIALS AND METHODS

The study was carried out in the year 2006 during April, May and June in three habitats near Gherța Mică. We captured from the habitats a total number of 128 individuals, all females, because we didn't identify any male specimens.

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The habitat from Gherța Mică is represented by a large marsh situated at the edge of a forest at an altitude of 130 m. The marsh is affected by man being used by the villagers as a watering place for cattle.

The other habitat from Gherța Mică (15.04.2006) is represented by a channel of about 500 m, both sides being limited by a pasture. Water depth is of approximately 50 cm, at the bottom of the pond being present a layer of silt of about 20 cm. Vegetation consists of Juncus plants and aquatic cormophytes in some places.

Stomach contents were collected using the stomach-flushing method (Solé et al., 2005). In this way, we can carry out feeding studies without killing the investigated individuals. The samples were preserved in separate airtight test tubes, which contain a 4% solution of formaldehyde. Afterwards, the frogs were released close to the place where they were caught, in an attempt to diminish the impact that our activity could have on them. The processing of the samples was made in the laboratory, with the help of a binocular microscope.

Food composition was evaluated by percentage abundance (% A) and frequency of occurrence (% f). To estimate the feeding intensity we calculated the maximum and average number of preys / individual.

# **RESULTS AND DISCUSSIONS**

We determined a total number of 838 animal preys in the stomach contents of the captured frogs. Beside invertebrates we also identified vegetal fragments, shed skin and minerals.

Table no. 1

Frequency of stomachs with vegetal, shed-skin and mineral. The number of analyzed individuals. The total, maximum and average number of preys per individual. The amount of aquatic and terrestrial preys. The feeding diversity (H)

	15.04.	11.05.	12.05.	9.06.
	2006	2006	2006	2006
%stom. with vegetal parts	70.6	58.5	40	54.5
%stom. with shed-skin	2.94	7.32	20	21.2
%stom. with mineral	-	-	-	3.03
No. of analyzed individuals	34	41	20	33
Total no. of prey	231	341	62	204
Max. no. of prey/individual	15	21	6	14
Average no. of prey /individual	6.79	8.31	3.1	6.18
% terrestrial prey	96.97	98.54	100	97.55
% aquatic prey	3.03	1.46	-	2.45
Feeding diversity (H)	2.7	2.07	2.17	2.51

In all the of the study periods we found individuals which had ingested vegetal fragments, but there were also animal preys together with the remaining plants. In the in the first period, the frequency of the vegetal in the stomach contents was of 70.6%, but in the second period the feeding process was more intense, thus the theory which emphasis the fact that the frequency of the vegetal grows together with the number of ingested preys, can't be applied in our case. The vegetal fragments are ingested accidentally together with the animal preys. High amounts of the vegetal fragments were registered as well at other edible frog populations (Ferenți et al., 2007, Sas et al., 2009, David et al., 2009).

Shed skin consumption is considered by some authors as a form of recycling epidermal proteins (Weldon et al., 1993), being easily ingested from the water mass or can be swallowed because of the confusion which appears between food and other moving individuals. In the case of the analyzed individuals it can be observed that the shed skin fragments were consumed in reduced quantities, except for two periods, 12 May and 9 June, when these were consumed by 20 and 21.2% of the population.

In the habitat from which we captured the individuals analyzed on 12 May, we observed a low trophic offer, thus the shed skin consumption can be considered as a method in order to satisfy their energetic needs. In the other habitat in which we had registered high shed skin consumption, the situation is different, here the medium and maximum number of prey consumed by an individual is higher, the trophic offer is richer, thus the shed skin doesn't constitute an important trophic resource for this population.

The most intense feeding was registered in the habitat from Gherța in 12 April when the medium number of prey was of 13.42 per individual, and the maximum number was of 30 preys. The opposite of this situation was the one already mentioned, registered in 12 May, when the medium number of prey was 3.1. The habitat plays a very important role in the frogs' feeding, thus if the conditions are favourable for the development of a high number of prey taxa, this will reflect in the food composition of the individuals which inhabit the habitat in question.

In the diet of the frogs captured in 15 April, the most important prey is the Oligochaeta, being consumed by 67.6% of the population. In the other periods this prey is missing or is present in reduced quantities, in this habitat the humidity allows the development of this prey, and also other moisture dependent invertebrates like the izopods which were consumed by 29.4% of the population (Table no. 2). The spiders also constitute an important food resource, being identified in the stomach contents of 50% of the frogs.

In 11 May the Lepidoptera larvae represent 44.9% of the total number of preys identified in this period. In this habitat appear in higher quantities the Formicidae, being present in large numbers and having small size, this prey being easy to capture. Also in this habitat the spiders are present in over 40% of the analyzed stomach contents. The larvae are considered rich in lipids and thus more nourishing than the adult forms (Brooks el al., 1996) and having also a more reduced mobility being easily captured by the frogs. This prey category was encountered in the food composition of other edible frog populations like the ones from Ignești and Prunișor in Arad County (Ferenți et al., 2007) but in the case of the population from Hinova (David et al., 2009) the caterpillars were consumed in reduced quantities, and only by the juveniles.

In the habitat analyzed in 12 May we identified only 13 prey categories, this being a habitat in which the feeding conditions are scarce. The prey category that had the higher amount was represented by spiders, which have a value of 25.8% from the total number of the animal preys found in the stomach content, being also ingested by numerous individuals.

The Araneae are present in all of the analyzed habitats, in every period, and in high quantities, these invertebrates being widely spread in nature and having no special environmental requests. These type of preys were also found in the stomach contents of other populations of *Pelophylax* kl. *esculentus* (Ferenți et al., 2007; Sas et al., 2009, David et al., 2009).

# Table no. 2

	15.04	.2006	11.05	.2006	12.05	.2006	9.06	2006
	% a	% f	% a	% f	% a	% f	% a	% f
Annelida - Oligochaeta	24.7	67.6	-	-	3.23	10	0.49	3.03
Gastropoda -snails (a)	-	-	-	-	-	-	2.45	12.1
Gastropoda -snails	5.63	17.6	-	-	3.23	10	-	-
Gastropoda - Limacidae	0.87	5.88	0.29	2.44	-	-	0.49	3.03
Crustacea - Isopoda (t)	6.93	29.4	0.88	7.32	-	-	-	-
Arachnida - Araneae	9.96	50	7.62	43.9	25.8	50	15.7	60.6
Chilopoda	2.16	14.7	0.29	2.44	-	-	0.98	6.06
Diplopoda	0.43	2.94	-	-	-	-	0.49	3.03
Collembola	2.6	5.88	-	-	-	-	0.49	3.03
Odonata	-	-	1.76	14.6	-	-	0.98	6.06
Orthoptera	0.43	2.94	-	-	-	-	2.45	12.1
Heteroptera(t)	2.16	11.8	2.05	17.1	-	-	2.94	12.1
Homoptera- Aphidinea	-	-	0.59	4.88	-	-	-	-
Homoptere-Cicadinea	0.43	2.94	-	-	-	-	2.94	15.2
Lepidoptera (L)	4.76	29.4	44.9	68.3	3.23	10	10.8	42.4
Lepidoptera	5.63	35.3	0.29	2.44	3.23	10	0.98	6.06
Trihoptera (L)	3.03	14.7	0.59	4.88	-	-	-	-
Trihoptera	-	-	0.29	2.44	-	-	-	-
Coleoptera-Dytiscidae(L)	-	-	0.88	7.32	-	-	-	-
Coleoptera-undet.	8.23	29.4	2.93	22	22.6	60	6.37	24.2
Coleoptera-Carabidae	4.76	20.6	2.05	17.1	-	-	2.94	15.2
Coleoptera- Chrysomelidae	1.3	8.82	-	-	3.23	10	-	-
Coleoptera-Coccinelidae	0.87	5.88	0.29	2.44	-	-	-	-
Coleoptera-Curculionidae	2.6	11.8	0.88	7.32	3.23	10	1.47	9.09
Coleoptera-Scarabeidae	0.87	5.88	1.17	7.32	-	-	1.47	9.09
Coleoptera-Elateridae	0	-	4.69	31.7	3.23	10	10.8	45.5
Coleoptera-Stapfilinidae	1.3	8.82	0.88	7.32	-	-	-	-
Coleoptera-Cantharidae	-	-	1.17	4.88	-	-	1.47	9.09
Diptera-Nematocera	2.6	14.7	4.4	26.8	12.9	30	3.43	21.2
Diptera-Brachycera (L) t	0.43	2.94	-	-	-	-	-	-
Diptera-Brachycera	0.43	2.94	2.93	17.1	9.68	20	1.47	6.06
Hymenoptera-undet.	0.87	5.88	1.17	9.76	-	-	0.49	3.03
Hymenoptera-Formicidae	6.06	29.4	16.1	58.5	3.23	10	24.5	57.6
Hymenoptera-Apidae	-	-	-	-	-	-	0.49	3.03
Panorpata	-	-	0.88	7.32	3.23	10	2.94	18.2

# The amount and frequency of the prey taxa (%a – amount; %f - frequency; aq.-aquatic; t. – terrestrial, L.- larvae)

Another prey taxa which had a high amount and frequency of consumption were the Coleoptera, this being consumed in all habitats, representing an important prey category for this populations and also for others previously studied (Sas et al., 2009). The Formicidae were, in the habitat studied in 9 June, the prey category which had the highest amount, but the Araneae were the preys which were consumed by 60.6% of the individuals. The Lepidopera larvae were also important in the trophic spectrum of this population.

The frogs do not seem to select their preys, with some exceptions, capturing all the mobile preys that come in sight and have a proper size to be consumed. This fact can be suggested by the consumption of both larger (Coleoptera) and smaller preys (Isopoda, Formicidae).

The food diversity has the higher value in 15 April (2.7), but also in the other habitats the frogs having at their disposal a wide range of prey categories.

#### CONCLUSIONS

The most important prey categories are represented by the: Araneae, Coleoptera, Lepidoptera larvae, the amount and frequency varying depending on the habitat in question. The fact that the most important preys are the same at different populations indicates the fact that the frogs are opportunistic predators, consuming the preys which are the most abundant in the habitat, this depending on the environmental conditions. The characteristics of the habitat can also produce variations in the trophic spectrum of the frogs, like the presence of the humidity depending taxa (e.g. Isopoda).

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# THE TROPHIC SPECTRUM OF AN *EPIDALEA VIRIDIS* (AMPHIBIA) POPULATION FROM GORJ COUNTY, ROMANIA

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KEY WORDS: feeding, green toad, ants, beetles

### ABSTRACT

We performed a feeding study on 23 green toad individuals from Meri railway station, situated in Jiu Gorge National Park, Romania. The analysis took place in June 2007. The stomach contents revealed the presence of different type of material, animal, vegetal and inorganic. The vegetal, shed-skin and mineral fragments were accidentally ingested by the toads. Over 90% of the preys were insects, the most important ones being ants and beetles. The high quantities of captured ants influenced the high values of the feeding intensity and the fact that all of the analysed samples contained stomach contents at the moment of study. All of the consumed preys had terrestrial origin.

# INTRODUCTION

Despite the fact that amphibians are important components of the ecosystems, the diet habits of some species have yet to be well known. This is the case of the green toad, which has been poorly studied both in the country and abroad in comparison to other amphibian species (Tesio and Teodorescu, 1999; Yiyit et al., 1999; Covaciu-Marcov et al., 2005; Nicoară et al., 2005; David et al., 2008; Nicoară et al., 2008; Ferenți et al., 2009). The feeding information is an important indicator for the quality of the biotope inhabited by the investigated population, thus contributing to its protection. As a result of the fact that the analysed individuals are found in a protected area, Jiu Gorge National Park, it is even more imperative to undertake studies that focus on gathering different data regarding both the studied species and other animal communities from inside the park. Therefore, the present paper aims to bring new data or to complete the existent ones regarding the trophic spectrum of *Epidalea viridis* species.

#### MATERIALS AND METHODS

A feeding analysis was performed in June 2007 on 23 green toads, using the stomach flushing method, a harmless technique recommend by many authors (Solé et al., 2005). The samples were spotted in the artificially lighted areas, around the buildings and

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near the railway tracks from Meri railway station. They were collected by hand during night-time, between 10 p.m. and 1 a.m. This area presents well developed grassy vegetation, with traces of pebbles, rocks, or uncovered soil, under which the toads hide during the day. Despite the railway traffic, the green toads manage to find conditions to undertake their reproduction and feeding activities. Following the analysis of the data, several feeding parameters were studied, respectively the feeding intensity and feeding activity rate, the frequency of vegetal, mineral and shed skin parts, the amount (A%) and frequency (f%)of the animal preys, as well as their origin.

# **RESULTS AND DISCUSSIONS**

The stomach contents of the 23 analyzed individuals revealed the presence of 584 prey specimens, which were grouped in 23 taxonomic categories (Table no. 2). Most of the preys were arthropods (579 samples, representing 99.14%), being constituted of an overwhelming majority of insects (566 individuals, representing 96.91% from the total captured preys) and spiders (13 samples, representing 2.22%). The Oligochaeta class was also present in the stomach contents, 5 worms being identified, which represent 0.85% from the total number of consumed preys. The most well-represented insects were the Hymenoptera (86.4% of the total number of identified insects), followed by the beetles (7.07%) and Trichoptera (2.3%). Other studies that deal with the feeding habits of the green toad indicate that over 90% of the consumed preys also belong to the insect class (Covaciu-Marcov et al., 2005; Nicoară et al., 2008).

The feeding activity rate and the feeding intensity are two important parameters in the analysis of the diet habits of a species (Table 1). The first aspect involves the percentage of individuals that registered stomach contents at the time of study. In our case, the fact that the analysis was performed in the first days of summer, when the climatic conditions were not too harsh and the individuals were found at the middle of their activity, contributed to the fact that all of the individuals presented stomach contents. Almost all of the toads registered animal contents, except one individual that recorded only fragments of vegetal matter. However, this is an isolated case, the rest of the samples from the population presenting prey taxa in their stomachs.

Table 1

No. of studied individuals	23
Total no. of preys	584
% individuals with stomach content	100
Maximum no. of preys/individual	55
Average no. of preys/ individual	25.39
% individuals with vegetal matter	56.52
% individuals with shed-skin debris	4.35
% individuals with mineral parts	30.43
% terrestrial preys	100

The feeding intensity and feeding activity rate. The vegetal, mineral and shed-skin consumption. The prey origin.

The second aspect deals with the total number of preys, the maximum and average number of prey/individual. The high values of both the average number of prey/individual

(25.39) and the maximum number of consumed preys (55) are provided by the high consumption of ants. As a result of their gregarious way of life, these preys were consumed in high quantities, respectively 8 green toads captured over 30 ants each. These high values are a specific characteristic to the species from the *Bufo* genus, being recorded at several species, *Bufo gargarizans* (Yu et al., 2009), *Bufo bufo* (Dimancea et al., 2009). Therefore, the populations of these species, and of others that focus primarily on capturing ants, generally register a higher feeding intensity in comparison to other amphibian species that are not specialised on consuming large quantities of ants, such as *Bombina variegata* (Hodişan et al., 2009), *Rana arvalis* (Covaciu-Marcov et al., 2002).

The stomach contents of the toads also contained, beside prey taxa, different fragments of vegetal matter, shed-skin debris and mineral parts (Table no. 1). These elements are generally accidentally swallowed together with the followed preys that are found on different substrata (Whitaker et al., 1977), in our case on various types of pebbles and grassy vegetation. As a result, over half of the analysed individuals registered vegetal parts in their stomachs and almost a third swallowed inorganic material. However, despite the fact that these two elements registered high values, only one individual recorded shedskin in its stomach content. The scarce presence of skin remains in the trophic spectrum of the *Bufo* genus has been recorded before (Yu et al., 2009), but it is more common for the species that can hunt in the water, such as *Bombina variegata* (Peter et al., 2006) and newts (Covaciu-Marcov et al., 2010; Kovacs et al., 2010). This is due to the fact that it is easier to swallow shed-skin in a puddle, where the surface is smaller, than in a terrestrial habitat, which is much larger.

The animal preys are the elements that represent important trophic resources, revealed by the stomach contents (Table 2). According to their importance for the analysed samples, these can be grouped in two major categories, respectively the preys that were scarcely consumed, generally by very few individuals and in low quantities, and the preys that do not represent an accidental encounter, being consumed in large numbers and by an important part of the analysed individuals. The combination of preys that represent a basis and accidental-consumed ones assures a diversity of the toads' diet and supplements the feeding of the toads, which is mainly composed of ants, beetles and spiders.

Table 2

	A %	F %		A %	F %
Oligochaeta	0.86	17.39	Coleoptera - Curculionidae	1.03	21.74
Araneida	2.23	43.48	Coleoptera (undet.)	1.71	30.43
Collembola	0.51	4.35	Coleoptera (total)	6.85	60.87
Orthoptera	0.34	4.35	Neuroptera	0.17	4.35
Dermaptera	0.17	4.35	Lepidoptera	0.68	17.39
Heteroptera (t.)	0.17	4.35	Lepidoptera (L.)	0.17	4.35
Cicadinea	0.34	8.70	Trichoptera	2.23	30.43
Aphidinea	0.68	17.39	Diptera - Nematocera	0.68	17.39
Carabidae	3.60	39.13	Diptera - Brachycera	0.17	4.35
Scarabeidae	0.17	4.35	Hymenoptera - Formicidae	82.36	82.61
Elateridae	0.17	4.35	Hymenoptera - Vespidae	0.34	8.70

The amount (A%) and frequency (f%) values of the prey taxa
(t. – terrestrial; undet. – undetermined; L. – larvae)

Coccinelidae	0.17	4.35	Hymenoptera (undet.)	1.03	17.39
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On the one hand, almost a half of the prey taxa were consumed by only one individual and in small quantities (generally one sample), thus registering both a low amount and frequency value. These preys represent slightly over 2.2% from the entire trophic spectrum of the analysed toads, most of the preys registering a value of 0.17%. These preys record different types of sizes, both small and large, exclusively belonging to the insect class (Collembola, Orthoptera, Dermaptera, terrestrial Heteroptera, Neuroptera, caterpillar, Brachycera, different types of beetles, respectively Scarabeidae, Elateridae and Coccinelidae). As a result of their low amount value and due to the fact that they were each consumed by 4.35% of the studied individuals, it can be assumed that these preys were accidentally captured by the toads.

On the other hand, there are three prey taxa that represent an important food source for the toads and are therefore consumed by a large proportion of individuals. The most important preys, registering first place regarding both the amount (82.36%) and frequency (82.61%) values, are represented by the ants. It appears that the members from the *Bufonidae* family show a distinct preference towards ants (Toft, 1980), fact sustained by the high values of ant consumption registered by other authors regarding this family (Solé et al., 2005, David et al., 2008). Ant specialists tend to secrete irritating substances that can originate from the formic acid of the ants (Bonansea and Vaira, 2007).

On a whole, the beetles registered second place concerning the amount (6.85%) and frequency (60.87%) values, the toads mostly consuming members from the Carabidae family. Spiders occupied third rank concerning these parameters. The difference between these two indicators is easily understood, being explained by their size. Therefore, due to their larger size, they can be consumed in smaller numbers in order to assure the energetic necessities of the individuals. These preys also represented important food sources for other green toad populations (Nicoară et al., 2005; David et al., 2008; Nicoară et al., 2008).

The combination of several factors, such as the fact that the green toad is a terrestrial species, the type of habitat in which the individuals were captured, the inexistence of temporal puddles around the railway station at the time of capturing, led to the exclusive capturing of terrestrial preys. However, several authors have found aquatic preys in the stomach contents of this species, such as aquatic heteropterans (David et al., 2008; Nicoară et al., 2008), Diptera larvae (Covaciu-Marcov et al., 2005; Nicoară et al., 2005), Gamaridae and Dytiscidae (Covaciu-Marcov et al., 2005), Trichoptera and Coleoptera larvae (Ferenți et al., 2009). Therefore, the individuals are not limited to capturing only terrestrial preys, but will also capture, in smaller amounts, aquatic preys if there are proper conditions (respectively temporary puddles, river valleys).

#### CONCLUSIONS

In conclusion, the analysed individuals recorded high values of the feeding activity rate and feeding intensity, fact which is in relation with the high consumption of ants, the main preys from the stomach contents. Most of the captured preys were insects, which registered different sizes, fact that increases the changes of survival for the green toads. All of the consumed preys were terrestrial, the ones that registered both a high amount and frequency value being the ants, beetles and spiders.

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# CLINICAL-EVOLVING ASPECTS OF THE BESNIER PRURIGO GESTATIONIS

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KEY WORDS: pregnancy induced dermatitis, prurigo, topical therapy.

# ABSTRACT

The Prurigo of pregnancy (the Besnier Prurigo Gestationis) is a pregnancy-induced dermatosis, rare, benign, considered an illness of the entire body. One of its characteristics is that it disappears after the end of the pregnancy, through birth or abortion, and can appear again during a new pregnancy.

Being a border illness, it requires a swift diagnosis and an adequate therapeutic approach. In our study, we have analyzed a number of 14 pregnant women who have been to the Dermatology Clinic of the Craiova County Clinical hospital during 2009. The clinical aspects and the therapeutic behaviour specific to this particular choice are mentions.

# INTRODUCTION

The purpose of this paper has been to conduct a study as detailed as possible of the Prurigo of Pregnancy, from the ethio-pathogenic, clinical, paraclinical, therapeutic and evolutionary points of view. The pregnancy is a physiological state which brings a series of transformations in the entire body. The prurigo of pregnancy is part of the papular-pruriginous dermatosis induced by pregnancy (Tolea, 2002).

On the tegument and phaners these manifestations fall into two categories: physiological changes caused by pregnancy and changes related to the pathological.

All the transformations taking place during the gestation represent proof of functional adaptation, defining the notion of "homeostasis of the pregnancy".

In certain cases the presence of the foetus inside the uterus causes in the mother's body certain reactions outside of the physiological limits and consequently leads to the apparition of pathological manifestations, some of which are minor and do not cause any problem to the normal evolution of the pregnancy (Georgescu Braila and Berceanu, 2001).

Others, quite the contrary, have a major symptomatology and require the fast and appropriate intervention of the clinicians: obstetrician and dermatologist (Kroumpouzos and Cohen, 2001). The pregnant women require special care and attention.

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The prevention and early discovery of the various conditions, as well as their treatment and supervision, ensure a safe passage through pregnancy and birth, both for the mother and for the baby.

We must note that the pregnancy-induced dermatosis are regarded as illnesses of the entire organism. Their rational treatment imposes the mention of the etiology, and if it remains unknown, at least one pathogenic treatment must be given, with a well-known pharmaco-dynamic action. Its role is to interrupt the various links that intervene in the apparition of morbid phenomena. The theoretical and especially practical importance of knowing these "border" affections, which are sometimes ignored or incorrectly diagnosed, has influenced our choice and motivates our endeavours.

# MATERIAL AND METHODS

During the year 2009, 14 pregnant women with cutaneous symptoms have been admitted to the Dermatology Clinic of the Craiova County Clinical Emergency Hospital.

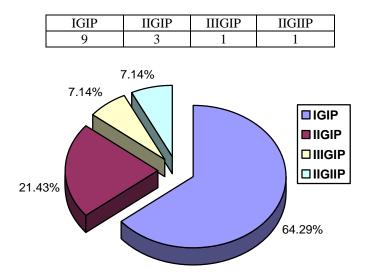
The positive diagnosis has been established by corroborating the data obtained from the anamnesis and from the clinical examination.

Taking into account that the etiology is still uncertain, the anamnesis brought important data concerning the starting point of the condition, as well as the possible existence of an allergic terrain of the pregnant woman.

#### **RESULTS AND DISCUSSIONS**

The examined pregnant women have been divided thus, depending on the gestation (G) and parity (P) (Table 1, Figure 1).

Table 1



The repartition of the cases

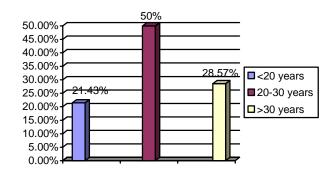
Figure 1. The repartition of the cases

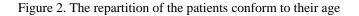
Depending on the age that the pregnant women had when they were admitted, they have been divided into three groups (Table 2, Figure 2).

Table 2

The repartition of the patients conform to their age

Group of age	< 20 years	20 - 30 years	> 30 years
No. of cases	3	7	4





The cutaneous lesions were papular, present on the extension parts of the upper and lower limbs, sometimes on the buttocks. They were accompanied by grating lesions.

A series of risk factors that the patients from our study have shown (various allergies, stress, a heredo-collateral history, parity) have also been shown in the research of other authors (Ambros-Rudolph and Black, 2006; Sherard and Atkinson, 2001).

The reasons for consulting a physician have been the pruritum and the cutaneous eruption. The clinical examination has pointed out the point in the pregnancy: all the pregnant women were in the first half of pregnancy.

The observed cutaneous eruption took the form of papules surrounded by an erythematous halo and grating lesions on the extension areas of the higher and lower limbs and the buttocks region.

The treatment has been complex, obstetrical and dermatologic, local and systemic.

It is a known fact that the treatment of these affections is very difficult, mainly because f the presence of the foetus, who doesn't allow the administering of certain drugs.

The obstetrical behaviour has had as a purpose to maintain the pregnancy through the administering of antispastics, tocolitics (butylscopolamine bromide – scobutil 2f/day, drotaverine chlorum-hydrate – no-spa 2f/day, papaverine -2f/day), inhibitors of the calcium channels, ocytocine antagonists, beta-mimetics, Magnesium sulphate (1-3f/day), nonsteroid anti-inflammatories (indometacine 1-2sup/day), synthesis progestatives (oral or vaginal: micronized progesterone - Utrogestat 2tb/day, dydrogesterone - Duphaston 3tb/day, estroprogestative-Gravibinon 1f/week).

The purpose for administering the antispastics has been to extend the evolution of the pregnancy until an acceptable degree of maturity.

An important part has been played by the prophylaxis of the illness of the hyaline membrane in the case of the imminence of premature birth by using gluco-corticods such as beta-methasone (Celestone 1f every 12 hours) or dexamethasone.

This type of therapy is frequently used by all the obstetricians and the adjuvant medication must be mentioned: central sedatives (diazepam 1tb in the evening before bedtime), vitamins, iron-based mixtures, folic acid, anti-histamines of synthesis (derivates of phenotiazine-romergan 1-2tb/day) (Rennik, 2006).

A special attention has been paid to the psychotherapy of the woman and the couple.

The main purpose of the local medication in this condition was the speeding of the healing of the cutaneous lesions and the elimination or relieving of the extremely distressing subjective symptoms (pruritum) (Sherard and Atkinson, 2001).

Lotions and anti-puriginous mixtures have been administered, containing: menthol 1 %, anesthesine 1-3 %, phenol 1-2 %, ichtiol 0,5-1.5 %, chloral-hydrate 1-5%, resorcin 0,5-1%, camphor 0,5%, lidocaine chlorohydrate 2,5-5%, antihistamines (Doxepin), diphenhydramine, the effect being of diminution of the pruritum.

The antiseptics used have been: oxygenised water, potassium permanganate 0,1 %, zinc sulphate as Dalibour solution and/or Castellani tincture in the case of certain pyodermitis. The best results have been given by the dermato-corticoids (with small potency) applied alone or in various combinations.

The maternal chances of evolution of the pregnancy and foetus are good, the dermatosis being one of the benign conditions (Weisshaar et al., 2005).

# CONCLUSIONS

This affection appears during the pregnancy, disappears after the end of the pregnancy (through abortion or birth) and could appear during a new pregnancy.

The co-existence of a dermatosis and the pregnancy involves a special supervision, the special problems being to give of the right diagnosis.

The clinical poly-morphism very often raises problems for the clinician, of diagnosis but especially of therapeutic behaviour.

It is important to underline the fact that administering drugs to pregnant women requires great care, being necessary to thoughtfully consider if the benefits overcome the risks of adverse reactions. Because these dermatosis have favourable evolution and prognostic, no heroic treatment is justified, most of the times this kind of treatment doing more harm than good.

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# THE ESTABLISHING OF CRITICAL AREAS (VULNERABLE ZONES) TO NITRATES POLLUTION

Dodocioiu Ana Maria<sup>1,\*</sup>, Susinski M.<sup>2</sup>, Tudosie A.<sup>2</sup>

KEY WORDS: vulnerable zones, nitrates, pollution, water protection, fertilizers.

#### ABSTRACT

The Nitrates Directive (91/676/EEC) delivered at 12 december 1991 is dealing with the waters protection against farming originating nitrates, aiming the mitigation of it and prevention of water pollution. It statesthat the member states to identify the waters affected by this type of pollution and to set up the vulnerable zones where agriculture is a major source of pollution in order to establish an action plan. The nitrates vulnerable zones (NVZ) account, after HG 964/13.10.2000, a surface of land that undergo liquid leaches into the polluted waters or susceptible to pollution by nitrates and contribute to the pollution of these waters. Within our country, the vulnerable zones to nitrate pollution have been set up in function of natural conditions of soil, terrain, climate and hydrology as well as in function of farm balance of nitrogen. They account about 13.93% of the agricultural land and 8.64% of the total surface of Romania. There were identified 1,772 villages of which 72 are in Dolj District.

### **INTRODUCTION**

The Nitrates Directive (91/676/EEC) delivered at 12 december 1991 is dealing with the waters protection against farming originating nitrates which will be implemented in Romania, as well and will determine major changes in farming, with social and economical effects and impact on rural development for preserving and increasing the resources, protecting the human and environment health.

The main objectives of 91/676/EEC Directive that are included into the Action Plan are as follows:

- the reduction of pollution produced or induced by farming activity;

- the prevention of waters by nitrates;

The directive assume that the member states to identify the waters affected by this type of pollution and to establish the vulnerable zones where farming is the most important pollution source by nitrates (Jordan and Smith, 2005). For these zones there are established programmes of action that contain compulsory measures on fertilizers control, especially the organic ones (Powlson, 2000).

Some specialists (Grossman, 2000; Soneveld and Bouma, 2003) say the Nitrates directive is one of the most controversial and unsuccesfull directive of EU on environment issues because of slow and difficult process of implementation within the member states and unsatisfactory results of its prerogatives. It is based on legaly established tresholds of

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nitrates concentrations in drinkable water (50 mg per liter) and stiff requirements for farmers on manure applying that do not has to overpass 170 kg nitrogen per hectare (the equivalent of dung produced by two milk cattle during an year).

In some states of EU, the Directive became operational short after its adoption by the European Counceil. As a consequence, in Netherlands began the extensive monitoring of nitrates concentration in water table and there were evaluated the trophycity of the surface waters and there was established a Code of good practices.

The romanian document that include the Nitrate Directive 91/676/EEC is the Commitment of Romanian Gorvernment 964/2000 on complying with the Action Plan for water protection against nitrates pollution originating from farming.

The development and concentration of the husbandry activity in specific zones has conducted to the deterioration of the water quality from the following causes:

- high animals density as compared with the farming land;
- concentration and large scale of husbandry farms nearby surface waters;
- wrong management of slurry;
- excessive application of animal wastes on soil (Panoiu, 2009).

# MATERIAL AND METHOD

The nitrates vulnerable zones (NVZ) is defined as "farming surface on country teritory that drains animal slurry into the polluted waters or susceptiple to pollution by nitrates" (AG 964/13.10.2000).

The delimitation of the zones that are vulnerable to nitrates pollution from farm activity has been one of the obligations assumed by our country in order to join the European Union. The working metodology and high volume of informations have allowed the delimitation of those areas where this type of pollution unfolds.

The establishing of these areas have consisted in marking the reloading areas which means those zones where the water from surface hydrographic basin that can content pollutants leaches into the water table. The aproach was made on the basis of maps with water table and terrain digital maps (Simota, 2009). There was considered that with a slope higher than 8% (so called mountain zones) the predominant process is surface runoff and considered vulnerable. With the other zones (plain) the predominant process is infiltration into the soil of nitrates till the water table, leaching, which is related by the ET value (the difference between the rainfall and the potential evapotranspiration) expressed as mm/month. Both with mountain and plain the runoffs and leaching depend of the soil type (Dumitru, 2010).

# **RESULTS AND DISCUTIONS**

The National Institute for Pedology and Agrochemistry and the National Administration of Waters has made a delimitation of NVZ from Romania, mainly, based on the soil maps, soil characteristics related to surface runoff and the leaching capacity which can offer informations on nitrates pollution of a certain region. In this manner, every soil type has different values of vulnerability in function of many criteria (hydraulic conductivity, total water capacity, soil type, the existence of an impermeable horizon, etc.). By combining all these criteria for every soil type there were obtained values of general vulnerability.

The vulnerable zones to nitrates pollution have been designed on the basis of natural conditions of soil, terrain, climate and hydrology as related to nitrates transfer toward subterraneous and surface waters as well as on the basis of nitrogen balance (from animal manure or from pulses) in every village. The vulnerable zones to nitrates pollution originating from farming designed then are the perimeter of 255 villages in Romania that represents 8.64 % of the country surface, respectively, 13.93% of the total agricultural surface of the country.

There were identified the following three types of vulnerable zones:

- potential vulnerable zones: nitrates transfer conditions toward water reservoires are favorable yet there is no positive balance of the nitrogen at village level and the nitrates concentration from water table measured in AMAR net is under 50 mg/l;
- Vulnerable zones with present sources: nitrates transfer conditions toward water reservoires are favorable and the balance of the nitrogen at village level is positive;
- Vulnerable zones from old sources: the transfer conditions of nitrates toward water reservoires are favorable yet there is no positive balance of nitrogen at the village level but formerly there were husbandries within the zone and the nitrates concentration from water table measured by AMAR net is higher than 50 mg/l.

The statistics of designed surfaces as vulnerable to nitrate pollution is presented in the first table.

The statistics of the surfaces considered vulnerable to mitrates pollution									
Hydrological	Surface	Affected surface	Agricultural	Bridges					
institution	GIS	from statistics	surface	Km <sup>2</sup>					
	$\mathrm{Km}^2$	Km <sup>2</sup>	Km <sup>2</sup>						
Arges Vedea	2188	2191	1339	605					
Banat	1701	1697	1521	55					
Buzau Ialomita	2026	2030	1629	190					
Crisuri	916	780	674	114					
Dobrogea Litoral									
Danube									
Jiu	305	272	212	51					
Mures	976	965	687	183					
Olt	1593	1592	981	458					
Prut Barlad	2052	2051	1381	407					
Siret	3092	3051	1513	1134					
Somes Tisa	1961	1925	1112	661					
Total	16810	16554	11049	3858					
Romania	238391	238391	147412	67428					
% NVZ from	7%	7%	7%	6%					
country surface									

The statistics of the surfaces considered vulnerable to nitrates pollution

Table 1

According with the Nitrates Directive, the reviewing of the vulnerable zones must be performed every 4 years accounting the following:

- the high nitrates concentration measured at wells from vulnerable zones and potentially vulnerable;
- economic difficulties as a result of including some remote zones as vulnerable zones to nitrates pollution;
- avoiding introduction/elimination of NVZ during reporting process in function of increasing/decreasing the number of animals in the village;
- set up a complex and coherent monitoring system at regional level for gathering informations required by the reporting process to Nitrate Comitee of EU;

- the presence of eutrophicated surface water reservoires;
- the expertise of EU countries which reviewed the nitrate vulnerable zones.

According with the Nitrates Directive requirements there was reviewed the vulnerable zones by including within this category the communes considered in 2003 as potentially vulnerable. In this manner, the number of the communes reach 1,772 of which, Dolj District has 79.

The integrated monitoring system of waters and soils from vulnerable zones to nitrates pollution is still on progress it still functioning after data gathered by the wells system AMAR and the Monitoring system elaborated by ICPA.

There is no a system of monitoring wells yet in communes that belong to vulnerable zones of which most of rural population drink water. In 2008 there was set up by ICPA an action of evaluation of water quality from wells that belongs to the vulnerable zones. There were made about 800 wells in 140 villages from 31 Districts. Of a 779 measurements, 42% have higher nitrates concentrations than 50 mg/l which is the limit imposed by Nitrates Directive.

#### CONCLUSIONS

The Nitrate Directive elaborated by EU deals with the protection of surface waters by nitrate pollution of farming origin and it states that the member states to identify the vulnerable zones to this kind of pollution and to establish the action programs for reclaiming them. The nitrate vulnerable zone (NVZ) is the surface of land that undergo liquid leaches into the polluted waters or susceptible to pollution by nitrates and contribute to the pollution of these waters. These zones have been set up on the basis of natural conditions of soil, climate and hydrology as refered to nitrates transport to water reservoires and the farm balance of nitrogen. The vulnerable zones to pollution by nitrates originating from farm are encountered in our country in 1,772 villages and within Dolj District, in 79 villages.

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# NEW BRUGNONE CULTIVARS WHICH IMPROVE THE ROUMANIAN FRUIT ASSORTMENT

Dumitru Liana-Melania<sup>1</sup>

KEY WORDS: peach, cultivars, nectarine, brugnone, productivity

#### ABSTRACT

Near peach, pavie and nectarine the brugnone enriched the fruit assortment who give us a considerable contribution of vitamins, mineral salts, fibres and caress us by their sweet and pleasant flavour.

Two brugnone cultivars "Valerica" and 'Anemona' were obtained at Research Station of Fruit Growing Constanta. Both of then have excellent quality of fruit, high and constant yield and more over cover the shortage of fruit from the second part of July and August.

# INTRODUCTION

Brugnone, like nectarine, derive from peach, not from plum and they are natural variation of *Prunus persica*-lanuginosa. (Dumitru et al., 2002, Zhivondov, 2007).

Brugnone result in France and they were named first "bignon" (1600) and then "brugnon" (1680) (Encyclopedia Britannica-Nectarine, 2004; Peach, 2004). They are similar to pavie (specially peach for processing), who were mentioned also in France (1560). (L'encyclopedie libre, 2009-Brugnon). Brugnone and pavie have a ferm flesh, big adherence of stone; they are very sweet and flavoured. (Encyclopedia des aliments, 2008). Brugnone and nectarine have many terapeutical propreties (Dumitru, 2003, Ivan and Radu, 2009, Neyrat, 2008).

# MATERIALS AND METHODS

The studied genotypes was observed from phenological point of view; there were made physico-chemical analyses; mesurements on fruits and trees; appreciations on productivity and ripening time; behaviour to the attack of main diseases and parasites, etc.

The new genetic material obtained was observed in the competition-trials, organized in different Romanian areas: Valu lui Traian, Bucuresti, Dabuleni, etc.

The density was 833 trees/ha (4/3 m) for standard trees and 2222 trees/ha (3/1.5 m) for dwarf trees.

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#### **RESULTS AND DISCUSSIONS**

The nectarine assortment from R.S.F.G. Constanta comprises some varieties like: 'Cora', 'Delta' (extremely early cv.); 'Romamer 2', 'Costin' and 'Crimsongold' (early cultivars); 'Flavortop' 'Fantasia' and 'Fairlane' (tardiv varieties). Between these, Fairlane is a brugnone cv. (Dumitru et al., 2006; Dumitru, 2007; Neyrat, 2008).

The new brugnone cultivars: 'Valerica' (omologated in 2003) and 'Anemona' (2010) have a middle ripening time (July-August). Both of then have a middle flowering time. Their flowering intensity are maximum.

Their yield is to much that control one 'Crimsongold' (Table 1) and are constant year by year (21.5 kg/tree for 'Valerica' and 28.0 kg/tree for 'Anemona').

The dry matter is high 13.7% to 'Valerica' and 15.5% to 'Anemona' (Table 2).

The fruit are very attractive, bright coloured and very good as for processing as for fresh consumtion too.

#### CONCLUSSION

The results show that the new brugnone cultivars 'Valerica' and 'Anemona' have a high productivity, good proprieties of fruit, big percentage of dry matter, pleasant flavour and are very good for fresh consumption and canneries.

#### ACKNOWLEDGEMENTS

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			of			le	Yie	ld
No.	Genotype	Start of flowering buds	Beginning o flowering	End of flowering	Flowering intensity	Ripening time	kg/tree	t/ha*
1.	Crimsongold	19.03-	24.03-	07.04-	4	06.07-	14.7	12.2
	(control) standard tree	29.03	04.04	16.04		17.07		
2.	Anemona	18.03-	26.03-	18.04-	5	24.07-	28.0	23.3
	standard tree	13.04	18.04	27.04		08.08		
3.	Valerica	14.03-	21.03-	15.04-	5	04.08-	21.5	47.7
	semidwarf	28.04	09.04	23.04		23.08		
	tree							

# Phenological stages and average yield (multiannual date), R.S.F.G. Constanta, Valu lui Traian

Table 2.

No.	Genotype	Average fruit weight (g)	Stone / weight (g)	Stone / flesh (%)	Dry matter (%)	Acidity* (mg%)	Fruit appearence	Flesh quality	Destination
1.	Crimsongold (control)	80.0	7.6	9.5	9.3	0.72	Spherical; orange-with 80-90% purple	Yellow ferm; semi free stone	Fresh consumption Processing (nectar)
2.	Anemona	113.0	9.7	8.6	15.5	0.37	Spherical- ovoidal; orange-with 80% redcrimson	Yellow- orange; very ferm; clingstone	Processing (jam and compote- slices) Fresh consumption
3.	Valerica	105.0	8.1	7.7	13.7	0.60	Ovoidal; orange with 90% red	Yellow- orange; very ferm; clingstone	Can (compote and jam jam by pieces of fruit) Fresh consumption

Quality test of the fruit, R.S.F.G. Constanta, Valu lui Traian

Acidity: mg malic acid / 100 g flesh fruit

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# THE MINERAL COMPOSITION OF SOME WILD GROWING EDIBLE SPECIES OF MUSHROOMS

Elekes Carmen Cristina<sup>1\*</sup>, Busuioc Gabriela<sup>1</sup>, Dumitriu Irina<sup>2</sup>

KEY WORDS: mushrooms, minerals, potassium, calcium, phosphorus

#### ABSTRACT

Many mushrooms species are known to accumulate metals to a higher level than the plants and are considered as a source of proteins, vitamins – riboflavin, biotin and thiamine, fats, carbohydrates, amino acids and minerals. The trace metals concentrations were established by Inductively Coupled Plasma - Atomic Emission Spectrometry method. The results are varying with the analyzed species of mushrooms between 11869.85 and 32088.68 mg/kg for potassium, 240.81 to 716.98 mg/kg for calcium and between 0 to 5350 mg/kg for phosphorus.

#### **INTRODUCTION**

In various cultures, the mushrooms have been a food supplement, some specific group of people traditionally eating wild mushrooms. They are eaten because of them delicacy and their chemical and nutritional proprieties, as for their therapeutic and preventing disease characteristics due to the chemical composition (Agrahar-Murugkar and Subbuakshmi, 2005; Manzi et al., 2001). Mushrooms are considered as a source of proteins, vitamins – riboflavin, biotin and thiamine (Buigut, 2002) -, fats, carbohydrates, amino acids and minerals (Jiskani, 2001). Ogundana and Fagade (1981) indicated the mushrooms are 16.5% dry matter, which contain 7.4% crude fiber, 14.6% crude protein and 4.48% fat and oil. The protein content of mushrooms is very high, is twice the content in asparagus and potatoes, four time the content in tomatoes and carrots and six times the content in oranges (Jiskani, 2001). Their energy value varies according the species and is about equal with that of an apple (Adejumo and Awosanya, 2005).

Many mushrooms species are known to accumulate metals to a higher level than the plants (Kalač et al., 2004). Within the fruiting body, the metals are distributed unevenly. The highest concentrations have been observed in the spore-forming part, but not in the spore, a lower content in the rest of the cap and the lowest level in the stipe (Thomet et al., 1999). Also, the level of metal concentrations varies with the metal content in the soil. High level of metals concentration was observed in the vicinity of metals polluted area and metals smelter (Collin-Hansen and Andersen, 2003; Kalač et al., 1996; Svoboda et al., 2000).

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The aim of this paper is to determinate the minerals content of some wild growing mushrooms, which may be useful in the phytopharmaceutical biotechnologies in order to obtain important quantities of biominerals accessible for the human body. In addition, by analyzing the bioaccumulation factor of these species we can conclude which species are accumulators and hyperaccumulators for these minerals.

# MATERIALS AND METHODS

#### Species and ecology

Five species of mushrooms were harvested from a wooded area, near Sinaia city, from Bucegi Massif of Carpathian Mountains. All these macrofungi were founded in deciduous forest, at 800 m altitude, relatively close to the road Targovisite - Sinaia. They growth in a cold period, in November, on the soil, but the mycelium was founded also in the mixture of litter leaves and shoots from the ground. The analyzed species are edible (*Marasmius oreades* and *Boletus griseus*), edible with low nutritive value (*Collybia butyracea* and *Hygrphorus virgineus*) or with conditioned edibility (*Calvatia excipuliformis* – can be used only when is very young).

The harvested mushrooms were mature, with spore forming part, and were collected the whole fruiting bodies, cap and stipe. The species of mushrooms were identified using guides book (Bielli, 1999).

#### **Analytical methods**

For each mushroom we sample 6-9 exemplars from different places and the substratum near the mycelium, down to the depth of 5 cm. Samples, mushrooms and soil, and them processing were done with plastic, glass and pottery instruments to avoid any metal contacts which can influence the final results.

After harvesting, the mushrooms were clean up by the soil particles, dried at 60 °C and then grinding to fine powder. The substratum samples were dried at 40 °C until the complete process, then grinding to a fine powder and sieved at 250  $\mu$ m (conform SR ISO 11464).

The estimation of metallic content in the analyzed mushroom and them soil was done by the Inductively Coupled Plasma - Atomic Emission Spectrometry method (ICP-AES). For the analyzes with ICP-AES method, the biological samples (mushrooms) were mineralized, in Berghof microwave digestor, by mixture with 10 ml of nitric acid concentrated 65% and 2 ml of hydrogen peroxide, and for the soil samples were done hot extractions with nitric acid 1:1.

In present paper, the metals contents of mushrooms were established with a 110 Liberty Spectrometer type of Varian brand. To disintegrate the sample in constituents atoms or ions is used a plasma source, which will stir up them on superior energetic layer. They will revert to the initial form by the emission of characteristic energy photon, emission recorded by an optical spectrometer. The radiation intensity is proportional with each element concentration in the sample and is intern calculated by a couple of calibration curves to obtain directly the measured concentration.

The concentrations represent the mean of many exemplars and are expressed in mg of metal related with kg of dry soil or plants.

#### **RESULTS AND DISCUSSION**

The contents of trace metals are related to species of mushrooms, collecting area of the sample, age of fruiting bodies and mycelium, and distance from any source of pollution (Kalač et al., 1991). Accurate and adequate mushrooms composition data are important to evaluate the intakes of essential nutrients and assessing exposure risk from intake of toxic elements. The average intakes are 1000 mg per day for potassium and calcium and 4000 mg per day for phosphorus. This daily nutrient intake is likely to pose no risk of adverse effects (Food, 2001). The results of mineral composition for the analyzed species of mushrooms indicate the potential for their use as a source of good quality food.

Potassium is an essential mineral for the body's growth and maintenance and plays an important role in proper hearth function. In the analyzed species of mushrooms the concentration of potassium is different from one species to another, ranging between 11869.85 mg/kg in *C. excipuliformis* and 32088.68 mg/kg in *Boletus griseus* species. In the left hand side of figure 1 we can see that, except *C. excipuliformis*, the concentration of potassium in the substrate, under the fruiting body of mushrooms, is few times lower than the K concentration in the mushroom. This ratio can be expressed as the bioaccumulation factor (the metal concentration in mushrooms reported to the metal content of the soil). For potassium, the bioaccumulation level has high values, over the unit threshold which means that the analyzed species of mushrooms are accumulators and hyperaccumulators for this metal.

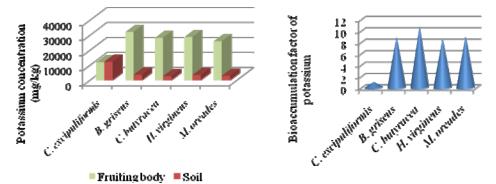


Figure 1. Potassium concentrations in mushrooms and soil, and the bioaccumulation factor

Calcium is needed for so many different functions in the body, for the formation and maintenance of bones, the development of teeth and healthy gums, for blood clotting, stabilizes many body functions and is thought to assist in bowel cancer. It has a natural calming and tranquilizing effect and is necessary for maintaining a regular heartbeat and the transmission of nerve impulses. It helps with lowering cholesterol, muscular growth and the prevention of muscle cramps. Furthermore it also helps with protein structuring in DNA and RNA. It provides energy, breaks down fats, maintains proper cell membrane permeability, aids in neuromuscular activity and helps to keep the skin healthy. Calcium also stops lead from being absorbed into bone.

Mushrooms are not an excellent food concerning the contribution with calcium for the human body, the concentrations in the fruiting body ranging from 240.81 mg/kg in *C. butyracea* to 716.98 mg/kg in *Hygrophorus virgineus* species (fig. 2). These concentrations are few times lower than the calcium content of the soil where the mean concentration is

about 40 g/kg of dry soil. Analyzing this difference, the bioaccumulation factor shows values lower than neither 0.1, which means that mushrooms are nor accumulators for this metal.

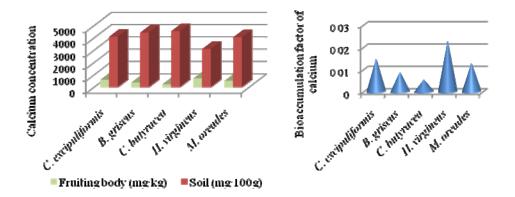


Figure 2. Calcium concentrations in mushrooms and soil, and the bioaccumulation factor

Phosphorus is important to keep in balance with calcium and magnesium. It plays a role in every metabolic reaction in the body and is important for the metabolism of fats, carbohydrates, and protein for proper growth and production of energy. Phosphorus is absorbed through the intestines, transported in the bloodstream, and stored in the bones and teeth. 70% of ingested phosphorus is absorbed. The content of phosphorus varies in a wide range in the analyzed species of mushrooms. In *C. excipuliformis* the phosphorus concentration are under the detection limit of method, but the highest concentration founded was for *Marasmius oreades* species, which show a value of calcium of 5350.19 mg/kg. The concentration of phosphorus in substratum varies also in wide range, and we can observe in the left hand side of fig. 3 that the concentration of metal in the fruiting body increases with the decreasing of the phosphorus content in the soil. The bioaccumulation factor has different values for the analyzed species of mushrooms, ranging between 0 and 2.72. Only two of five species are accumulators for this element: *Hygrophorus virgineus* and *Marasmius oreades*.

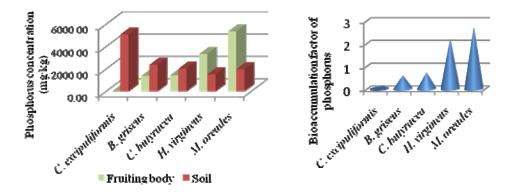


Figure 3. Phosphorus concentrations in mushrooms and soil, and the bioaccumulation factor

#### CONCLUSIONS

For potassium, the concentrations in the fruiting body excel the potassium content in the soil which means that these species of mushrooms are accumulators and hyperaccumulators for K. The highest concentration if potassium was founded in *B. griseus* species, 32088.68 mg/kg.

The concentrations of calcium are not indicating the accumulating capacity of the analyzed species of mushrooms, the highest concentration showing *Hygrophorus virgineus* species, 716.98 mg/kg.

Only two species, *Hygrophorus virgineus* and *Marasmius oreades* show a phosphorus concentration in the fruiting body higher than in soil, indicating the accumulation capacity of these species. The level of phosphorus in the fruiting body was up to 5350 mg/kg.

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# DATA UPON THE FEEDING OF A SALAMANDRA SALAMANDRA (AMPHIBIA) POPULATION FROM VODIȚA VALLEY, MEHEDINȚI COUNTY, ROMANIA

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KEY WORDS: Salamandra salamandra, food composition, altitude, season

# ABSTRACT

The most important preys for the Salamandra salamandra population from Vodiţa are Diplopoda, followed by Araneida. Together with the animal preys belonging to 22 prey taxa, the salamanders also presented vegetal remains, shed-skin fragments and inorganic elements in their stomach contents. Over 99 % of the consumed preys were terrestrial invertebrates. The feeding of the studied population is influenced by the study period and the characteristics and geographical position of the populated habitat.

# INTRODUCTION

The presence of adult salamanders is conditioned by the existence of forests and valleys with streams in the case of their larvae (Manenti et al., 2009). In Romania, it is considered that salamanders are present from 200 m upwards (Cogălniceanu et al., 2000). However, in some areas of the country, due to certain particularities of the relief or of the climate, the species descends under 200 m (Paşcovschi, 1956, Covaciu-Marcov et al., 2007, 2008). The lowest point from the country at which it was encountered lies on Vodiţa Valley, at only 100 m altitude (Covaciu-Marcov et al., 2009). Although well represented in Romania (Cogălniceanu et al., 2000), *S. salamandra* it is considered to be a vulnerable species (Iftime, 2005), the fact being mainly put upon the destruction of its habitat, respectively forests.

Therefore, it is very important to know its ecology in order to protect the species and its habitat. In this respect, food is considered to be the main connection between an animal and its environment (Kenett and Tory, 1996). Moreover, feeding could indicate the quality of the medium in which amphibians live (Kovács et al., 2007). Studies regarding the trophic spectrum of the salamanders from the country were realised after the year 2000 (Covaciu-Marcov et al., 2002a, Cicort-Lucaciu et al., 2007, Ferenți et al., 2008, Cicort-Lucaciu, 2009). Thus, our study aims to complete the data regarding the feeding of the species in Romania with data concerning one of the most southern populations from the country, situated at the lowest altitude and at the beginning of spring.

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#### MATERIALS AND METHODS

The samples were taken in 12.04. 2007. On a whole, we captured 43 salamanders, surrounding Vodiţa Monastery, near Orşova. The animals were captured by hand, between 8 and 9 in the morning, after a rainy night. All of the salamanders were released in their habitats, after the collecting of the stomach contents. The salamanders were captured from approximately 200-300 m from the valley, at altitudes of 110-130 m. Vodiţa stream is tributary to the Danube, normally having a low flow, rocky substratum, with a typical mountainous character despite the low altitude. The sectors with salamanders is situated upstream of Vodiţa monastery, where the stream flows through the forested slopes, and different oak species and beech are present. The stomach contents were drawn using the stomach flushing method (Solé et al., 2005), being afterwards preserved in sealed test tubes, using formalin. The preys were determined in the laboratory using the scientific literature.

# **RESULTS AND DISCUSSIONS**

The salamander population from Vodiţa Valley consumed a larger number of prey taxa in comparison with other populations from Romania (Ferenți et al., 2008, Cicort-Lucaciu, 2009). This fact is determined by the geographical position of the studied region in the extreme southern part of the country, which is found under sub-mediterranean climatic influences (Drugesu and Geacu, 2004). Thus, certain prey taxa, such as scorpions, are present here, lacking from the trophic spectrum of the salamanders from other regions of the country (Covaciu-Marcov et al., 2002a, Cicort-Lucaciu, 2009). These results prove once more that the low altitude is not a limitative factor for the feeding of this species (Cicort-Lucaciu, 2009).

Despite the fact that the population from Vodița is found at the lowest altitude from the country (Covaciu-Marcov et al., 2009), the food diversity is quite high. The positioning of the salamanders' habitat at low altitudes allows the species' presence at its level, meanwhile also assuring the fulfilment of the requirements connected to the available trophic offer.

The feeding intensity is lower at Vodiţa than in the case of other similar studies, despite the number of consumed prey taxa (Cicort-Lucaciu et al., 2007, Cicort-Lucaciu, 2009). Thus, the maximum number of preys/individual was only 9, while the average number of preys/individual was 3.27. The positioning of the sample collection period, at the beginning of April, is responsible for this situation. The low feeding intensity during this interval confirms the previous data generally obtained at different amphibian species, according to which their feeding is precarious at the beginning of spring, due to the low temperatures that affect both prey and predator (Covaciu-Marcov et al., 2002b). In addition, during the interval, the salamanders were found in the areas neighbouring the stream, which restricted their hunting territory.

The salamanders from other areas were found at a larger distance from the valleys later in the year (Cicort-Lucaciu, 2009). Moreover, it is highly possible that this is more difficult at Vodiţa, due to the steep slopes from some areas.

From the 43 analysed salamanders, only one individual did not present animal prey in its stomach content. However, this individual did not have an empty stomach, presenting shed-skin remains. Thus, although all of the salamanders presented stomach contents, the case of the individual without animal preys, therefore without a proper food, is also a result of performing the study at the beginning of spring, as is the low feeding intensity. The cases in which all of the amphibian individuals present stomach contents generally indicate optimal feeding conditions (Sas et al., 2009).

It is normally considered that amphibians accidentally consume vegetal fragments, which are swallowed together with the followed prey (Whitaker et al., 1977). Thus, basically it has been established that once with the increase of the feeding intensity there is an increase in the frequency of the animals that consumed vegetal parts (Kovács et al., 2010a). Therefore, in our case, once with the lower feeding intensity, the frequency of the salamanders that consumed vegetal remains should have also been lower. This fact is partially valid, the frequency of vegetal consumption being lower than in the case of other populations from Arad and Bihor County (Covaciu-Marcov et al., 2002a, Cicort-Lucaciu et al., 2007). However the situation is not a general one, due to the cases in which the salamander populations presented an intense feeding, at which the frequency of vegetal consumption was lower or similar to the one registered at Vodița (Ferenți et al., 2008, Cicort-Lucaciu, 2009). This fact does not mean that the theory regarding the relation between the feeding intensity and the frequency of vegetal consumption is annulled, but it rather indicates its adaptation to a specific situation of the salamanders. Thus, the previously studied populations inhabited dense and shadowy beech forests, where the chance of accidentally swallowing grassy vegetation was not too high, even the beech leaves are too large. However, in the present case, the valley also has opened, sunny, grassy sectors, the southern oaks from the forest being rare, therefore the grassy substratum is highly developed. In this situation, the salamanders had the opportunity to swallow more vegetal parts than the other populations that had a higher feeding intensity, but were situated in denser forests.

A similar explanation can be provided in the case of the mineral consumption. Cases in which the salamanders did not present this type of stomach contents were described in the scientific literature (Cicort-Lucaciu, 2009). The respective populations occupied wet beech forests that did not present rocks in the substratum. In the case of the salamanders from Vodița, these hunted near the valley, where there are wide surfaces with sand and gravel brought by the water. These could have been easily swallowed together with the prey.

Shed-skin was also consumed by other *S. salamandra* populations (Cicort-Lucaciu, 2009). Amphibians generally consume more abundant fragments of shed-skin in the periods in which the trophic offer is lower (Kovács et al., 2010b). This seems to be the case of the population from Vodiţa, although shed-skin was frequently consumed in other periods. In the case of this species, it seems that shed-skin represents a trophic resource easy to consume, being swallowed with any opportunity. The fact can be connected to the species' preference for slow preys with continuous movements (Luthardt & Roth, 1979), thus for feeding with a low energetic consumption. It is obvious that shed-skin consumption, considered a way of epidermal recycling (Weldon et al., 1993), does not imply great effort, indicating the trophic opportunism of this species. All of the shed-skin belonged to its own species, being either of the respective individual or of one that attracted another salamander. We consider that the consumption of shed-skin in the case of this species cannot be the result of an unsuccessful hunting directed towards another individual from the population, as a result of its movement during shedding (Sas et al., 2005).

The most important prey taxa for the *S. salamandra* population from Vodița, regarding both the amount and frequency of consumption, are the Diplopoda and Araneida. These are also important prey taxa for other salamander populations from Romania (Covaciu-Marcov et al., 2002a, Cicort-Lucaciu, 2009). Chilopoda and crustacean Isopoda

also register high amount and frequency values. In comparison to other studies, there are some differences regarding the consumption of some preys.

For example, the caterpillars had high values in May (Cicort-Lucaciu et al., 2007, Cicort-Lucaciu, 2009), but are less important at Vodiţa. This fact indicates the importance of the feeding period, of both this species and of the amphibians in general (Covaciu-Marcov et al., 2010).

Table no. 1.

Prey taxa	A %	F %	Prey taxa	A %	F %
Vegetal remains	-	65.11	Blatoidea	0.7	2.32
Shed-skin	-	13.95	Orthoptera	0.7	2.32
Inorganic elements	-	11.62	Coleoptera undet.	2.12	6.97
Lumbricidae	0.7	2.32	Coleoptera Carabidae	2.12	6.97
Gastropoda (with shell)	2.83	9.3	Coleoptera Lampiridae	4.25	11.62
Gastropoda (limax)	7.8	18.6	Trichoptera (L.)	0.7	2.32
Araneida	14.89	41.86	Lepidoptera	1.41	4.65
Opilionida	6.38	20.93	Lepidoptera (L.)	1.41	4.65
Scorpionida	0.7	2.32	Nematocera undet.	0.7	2.32
Crustacean Isopoda	9.21	23.25	Hymenoptera undet.	0.7	2.32
Diplopoda	34.74	55.81	Hymenoptera Formicidae	0.7	2.32
Chilopoda	5.67	23.95	Hymenoptera Apide	0.7	2.32
Plecoptera	0.7	2.32			

The amount (A%) and frequency of consumption (F%) of the different categories of stomach contents (undet. – undetermined; L. – larvae)

Most of the preys consumed by the salamanders, which are strictly terrestrial (Fuhn 1960), had terrestrial forms (99.29 %). However, a small part of the preys were aquatic (0.7 %), being represented by the Trichoptera larvae. The aquatic preys were also extremely few in the case of other populations (Covaciu-Marcov et al., 2002a, Cicort-Lucaciu, 2009). The Trichoptera larvae were consumed from the water shore, in the areas with low water-depth, where the larvae remained partially on land, thus being observed by the salamanders. A similar explanation was also provided in the case of the consumption of Plecoptera larvae (Covaciu-Marcov et al., 2002a).

#### CONCLUSIONS

The *S. salamandra* population from Vodița consumed 141 preys belonging to 22 prey taxa. The maximum number of preys/individual was 9, while the average one was 3.27. Most of the consumed preys were terrestrial ones. The aquatic preys, represented by the Trichoptera larvae, were consumed from the water shore, from the rocks that remained partially uncovered by water. The most important preys are the Diplopoda and Araneida, followed by the Chilopoda and Isopoda.

The particularities of the feeding of this population are determined by the geographic region and the conditions of the habitat. Due to the southern position, submediterranean prey taxa were consumed here, such as scorpions. The lower feeding intensity is a consequence of the study period, which was realised at the beginning of April, when the low temperatures limited the trophic offer, both in a quantitative and qualitative manner. The appearance of an individual that consumed only shed-skin was also determined by the period. The habitat particularities explain the high consumption of vegetal and mineral parts.

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#### DEVELOPMENT OF *IN VITRO* PROPAGATION PROTOCOL FOR THE NEW VARIETY OF PLUM *ALUTUS*

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KEY WORD: Plum, in vitro propagation, acclimatization, protocol

#### ABSTRACT

The study aimed to establish a working method in the laboratory and the behavior of the new plum variety Alutus, in various stages of micro propagation from the initialization phase to rooting phase. Basic media used, with the best results was Murashige&Skoog (1962), added with different specific phytohormones, for each phase of in vitro propagation. For the starting phase, the best results were obtained with basic Murashige&Skoog media and concentrations of BAP 1 mg / 1 and for multiplication and rooting phases IBA 0,1 mg / 1 and GA<sub>3</sub> 0,1 mg / 1. The growth chamber ensured a photoperiodism 16 / 8 hours light (3500 lux) and a temperature of 22-25 ° C.

## INTRODUCTION

The variety studied was created in 2009 at University of Craiova - SCDP Valcea and there is in National Collection of the genus *Prunus*, existing at University of Craiova - SCDP Valcea.

Most species of the genus *Prunus* have vegetative propagation by grafting and less by in "in vitro" culture.

By in vitro culture the plum cultivars show a lot of problems in different stages op micro propagation. Tissue culture is a healthy way to ensure a good final material having a high quality of protection (Hanzer et al, 1995).

Alutus plum variety (H 74-17-83) is recommended as table fruit variety by having a very large fruit, with semi-clinging stone to flesh, dark blue color of the fruit without pruine and yellow flesh color. Age of fruit ripening is middle August.

Good results obtained by micropropagation of this new variety of plum, encourage researches to optimize the method for being used to industrial scale.

# MATERIAL AND METHOD

The experiments were done in "in vitro" culture laboratory, of University of Craiova - SCDP Valcea. For producing a good quality and good phytosanitary plants we have proposed some objectives to achieve:

Establishing the specific culture media (optimal) for each phase of in vitro propagation;

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Establishing appropriate time for harvesting the biological material;

The biological material was formed of new shoots growth during one year, 30-50 cm in length cut in one bud. The buds were in dormancy from November to March.

Studies were made using two culture media *Murashige&Skoog and Lepoivre*, both supplemented with 30 g / 1 sucrose.

As gelling agent was used Phytagel 1,5 g / l.

Disinfection of material was accomplished by immersion in 70% alcohol, for 10 minutes, followed by immersion in Ca hypochlorite for 15 minutes, and three washes with distilled and sterile water.

The growth room temperature was achieved of 20 to 25  $^{\circ}$  C and a photoperiodism of 16 / 8 hours.

Acclimatization was done in insect proof depositaries during summer and in greenhouse during winter.

#### **RESULTS AND DISCUSSIONS**

## 1. Starting phase

Biological material collected as one year shoots has been fragmented into pieces with one bud and sterilized according to the work protocol.

The four types of nutrient media for the starting phase were V1= *Murashige&Skoog*, V2 = *Lepoivre*, both supplemented with 1 mg / 1 BAP and 0,1 mg / 1 IBA, V3= *Murashige&Skoog* and V4 = *Lepoivre* without phytohormones. (Table 1).

Gelling agent used was Phyagel 1,5 g / l. The media were autoclaved at 121  $^\circ$  C for 25 minutes.

Inoculation of biological material (one year buds) on media was carried out under laminar flow hood.

Cultures thus obtained were transferred to growth chamber at a temperature of 25  $^{\circ}$  C and light intensity of 3500 lux, under artificial light conditions and a photoperiodism of 16 / 8 hours.

Initiation phase is always one of the most difficult for "in vitro" micro propagation, because it depends on several factors: an appropriate material disinfection, harvesting period of the material, working mode.

Recorded data showed that the *Murashige&Skoog* culture medium, initiation rate was 76%, *Lepoivre* (Quoirin and Lepoivre, 1977), culture medium initiation rate was 70% compared to variants V3 and V4, which had no growth hormones in their composition (Table 2).

Table 2

Variant	No. of buds at starting phase	No. of young plants	Initiating rate
V1	50	38	76
V2	65	46	70
V3	50	6	12
V4	65	10	15

Results obtained from initiating rate of plants at *Alutus* variety

The best results were obtained for inoculation using *Murashige&Skoog* culture media with a hormonal balance BAP = 1 mg / 1 and IBA = 0.1 mg / 1, achieving a 76% initiating rate.

Table 1

Composition of the growth basic media						
Substance	Murashige&Skoog (1962) V1	Lepoivre (1977) V2	Murashige&Skoog (1962) V3	Lepoivre (1977) V4		
	Μ	acroelements				
NH <sub>4</sub> NO <sub>3</sub>	1650 mg/l	400 mg/l	1650 mg/l	400 mg/l		
KNO3	1900 mg/l	1800 mg/l	1900 mg/l	1800 mg/l		
CaCl <sub>2</sub> 2H <sub>2</sub> O	440 mg/l	-	440 mg/l	-		
MgSO <sub>4</sub> 7H <sub>2</sub> O	370 mg/l	360 mg/l	370 mg/l	360 mg/l		
KH <sub>2</sub> PO <sub>4</sub>	170 mg/l	270 mg/l	170 mg/l	270 mg/l		
K <sub>2</sub> SO <sub>4</sub>	-	-	-	-		
$Ca(NO_3)_2 4H_2O$	-	1200 mg/l	-	1200 mg/l		
NaH <sub>2</sub> PO <sub>4</sub>	-	-				
	Μ	icroelements				
FeSO <sub>4</sub> 7H <sub>2</sub> O	27,9 mg/l	-	27,9 mg/l	-		
MnSO <sub>4</sub> 4H <sub>2</sub> O	22,3 mg/l	0,75 mg/l	22,3 mg/l	0,75 mg/l		
ZnSO <sub>4</sub> 7H <sub>2</sub> O	8,6 mg/l	8,6 mg/l	8,6 mg/l	8,6 mg/l		
H <sub>3</sub> BO <sub>3</sub>	6,2 mg/l	12,0 mg/l	6,2 mg/l	12,0 mg/l		
CuSO <sub>4</sub> 5H <sub>2</sub> O	0,025 mg/l	0,025 mg/l	0,025 mg/l	0,025 mg/l		
Na <sub>2</sub> MoO <sub>4</sub> 2H <sub>2</sub> O	0,25 mg/l	0,25 mg/l	0,25 mg/l	0,25 mg/l		
CoCl <sub>2</sub> 6H <sub>2</sub> O	0,025 mg/l	0,025 mg/l	0,025 mg/l	0,025 mg/l		
KI	0,83 mg/l	0,08 mg/l	0,83 mg/l	0,08 mg/l		
Na <sub>2</sub> EDTA	-	-				
Na <sub>2</sub> SO <sub>4</sub>	-	-				
		Vitamins				
Inozitol	100 mg/l	100 mg/l	100 mg/l	100 mg/l		
Tiamină HCl	1,1 mg/l	1,1 mg/l	1,1 mg/l	1,1 mg/l		
Ac. Nicotinic	1 mg/l	-	1 mg/l	-		
Piridoxină HCl	0,5 mg/l	-	0,5 mg/l	-		
Glicină	2,0 mg/l	-	2,0 mg/l	-		
Colină	-	-	-	-		
Biotină	-	-	-	-		
Pantotenat de	0,5 mg/l	0,5 mg/l	0,5 mg/l	0,5 mg/l		
Са						
Riboflavină	0,5 mg/l	0,5 mg/	0,5 mg/l	0,5 mg/		
Ac. ascorbic	-	-	-	-		
IBA	0,1mg/l	0,1mg/l	-	-		
GA <sub>3</sub>	1mg/l	1mg/l	-	-		
6-BAP	1.5 mg/l	1.5 mg/l	-	-		

**2. Multiplication phase** Once plum crop was established, there were no notable changes, taking into account that were used the same culture media Murashige&Skoog and Lepoivre, but with a different hormonal balance.

In vitro multiplication phase is considered the most important link in biotechnology, because its performance, is basic for production and multiplication of fruitgrowing seedlings.

Thus, it is estimated that a well controlled experimental system, starting from a single explant can be obtained in a period of 6-12 months to one million plants (Gregory et al., 1995).

Table 3

media					
Variant	Time	Shoots/ explant			
V1	Nov.	10			
V I	Feb.	12			
V2	Nov.	6			
V 2	Feb.	8			
V3	Nov.	7			
, s	Feb.	2			
V4	Nov.	6			
v4	Feb.	3			

Results obtained in multiplication phase of Alutus variety, on different culture

Hormonal balance used for V1 and V2 with the best results was BAP = 1 to 1.5 mg / 1 GA<sub>3</sub> = 1mg / 1 and vitamin-*Lepoivre and Murashige&Skoog* normal.

# 3. Rooting phase

The "in vitro" rooting is the final step in the multiplication process in controlled laboratory conditions requiring a perfect technological discipline, as by failure rules work with sterile equipment and aseptic environment can be undermine all the work, without recovery of biological material

Following experiments revealed that *Murashige&Skoog* culture media is indicated for all stages of in vitro propagation, *Lepoivre* media giving satisfactory results only in the rooting phase. Addition of IBA (0,5-1 mg / 1) occurs in the 10-12 week of plant life.

In terms of growth room with a temperature of 21-25 ° C and a photoperiodism of 16 / 8 light, it was made a good proliferation of plants with an elongation produced after 5-8 weeks, followed by rooting after 10 - 12 weeks.

Regular maintenance was performed transferring cultures explants in fresh culture media. The two culture media used in the four variants, have worked well achieving a rate of over 60% vitro plants rooted plants, that have been aclimatized (Table 4). Roots were well developed, having a length between 1-2 cm.

Tal	ole	4
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The *Alutus* variety root formation on "in vitro" conditions

Variant	No of inoculated plants on rooting media /vessel	<b>Rooted plants</b>	Aclimatized plants
V1	9	7	6
V2	9	5	5
V3	9	2	-
V4	9	1	1

Well acclimatized plants were transferred to insect proof depositaries, first in the substrate of perlite, then in vessels with a mix of earth, sand and peat, the ratio of 1:1:1.

At normal summer temperatures, a good acclimatization of new plants was obtained, accomplished at low temperatures is more difficult and with poor results.

## CONCLUSIONS

Previous research showed that plum tree propagation by in vitro culture (meristem culture) is a process rather difficult, starting with the time of sampling of biological material and establishing a work protocol for this species.

- Behaviour of *Alutus* variety micropropagation process was very good, biggest problems amounting to acclimatization;
- Best variant as culture media for the *Alutus* plum variety, proved to be V1 (*Murashige&Skoog*, basic medium + hormonal balance BAP, GA<sub>3</sub> and IBA specific for each phase of micropropagation);
- V2 variant (*Lepoivre* media) gave the best results in rooting phase, achieving a 55% acclimatized plants.
- The results obtained both in laboratory conditions and in conditions and natural life, proving feasibility of organogenesis and the propagation through tissue culture of *Alutus* variety.

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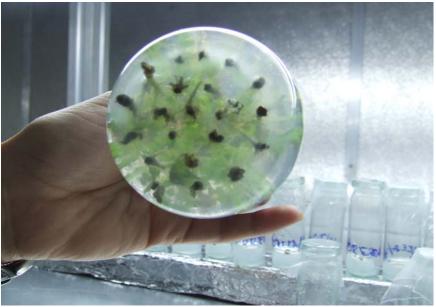
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*Alutus – in multiplication phase* 



Alutus – rooting phase

## UNIVERSITATEA DIN CRAIOVA UNIVERSITY OF CRAIOVA

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## THE INSTRUMENTAL METHODS OF ANALYSIS USED IN THE CONTROL OF OENOLOGICAL PRODUCTS

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**KEY WORDS:** the instrumental methods, wines, spectrophotometry, quality

#### ABSTRACT

In this paper presents two instrumental methods of analysis used in the study of the quality of wines, namely: the optical method - refractometry and the spectrophotometric method. Are shown the benefits of using these methods; are presented the results concerning the colour, the microbiological stability, the content of methanol, citric acid and antocyans for three wines of the Valea Călugărească area.

## INTRODUCTION

The technology and quality control of oenological products are currently made using the instrumental methods of analysis, the methods based primarily on the relationships between the physical properties of substances and their chemical structure.

The advantages of these methods are: the rapid implementation, the analytical sensitivity and the accuracy (the perfect reproducibility of the results), the wide applicability (allow to determine of a large number of molecules: alcohols, acids, sugars, antocyans, flavours, etc.), the low consumption of reagents. In addition, the most methods are suitable for the automation. (Târdea, 2007)

Depending on the nature of investigations on the oenological products, the methods of the instrumental analysis can be:

- *the optical methods* (refractometry, polarimetry, colorimetry) - which determining, indirectly, the sugar content and their chemical nature (at the mash), the antocyans content (at the red wines);

- the spectrophotometric methods (in the fields of VIS, UV and IR) - from the absorption and transmission spectra can be determined: the colour of wine, the methanol, the citric acid, the antocyans, etc.;

- *the electrochemical methods* (potentiometry, voltammetry) - which determine the pH and the oxidation-reduction potential of the wine, the content of amino acids and protein, the content of mineral substances;

- *the chromatographic methods* - which separated and determined: the antocyans, the flavourings, the volatile phenols, the alcohols, acids, etc.;

- *the isotopic methods* - to detect the addition of water and the origin of alcohol from wine by the establishment of the isotopic ratios D/H and  ${}^{18}O/{}^{16}O$ .

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The paper will present the results obtained by two instrumental methods described above, namely: refractometry and UV-Vis absorption spectrophotometry, used to control the quality of wines produced in the Valea Călugărească area by a local private producer.

#### MATERIAL AND METHODS

The studied wines are: Pinot Gris (2007), Cabernet-Sauvignon (2007) and Fetească Neagră (2007), from the Valea Călugărească area.

In the preliminary analysis of wine samples was appreciated the colour (the intensity and the hue) and the microbiological stability.

**The wine colour** is due to phenolic compounds from the wine. The colour of white wines is due flavones (the yellow pigments) and phenolic hydroxyacids, which accumulate in the grapes. At the forming the colour of red wines participate polyphenols (antocyans and tannins), plus some flavones and phenolic acids, found in the white wines. The most important are antocyans that determine the blue-red colour of the wine.

The wine colour is determined by the spectrophotometric methods as: is measured the absorbances of the wine samples at the wavelengths of 420, 520 and 620 nm and is calculated (Sudraud, 1990):

- the colour hue or the colour tint (N<sub>C</sub>):

$$N_{\rm C} = \frac{A_{420\rm nm}}{A_{520\rm nm}}$$

- the colour intensity  $(I_C)$ , obtained by the amount of absorbances:

$$\mathbf{I}_{\rm C} = \mathbf{A}_{420} + \mathbf{A}_{520} + \mathbf{A}_{620}$$

The yeasts are the microorganisms commonly used in the classical or modern biotechnological processes (Florea-Dumitru et al., 2002). During spontaneous fermentation, the yeast species are not consistently found; the fermentation process is initiated by the nonspore forming species and spore forming species continue it. Different types of bacteria contribute to improve the quality of wine or lead to unwanted changes of it (Popa et al., 1990).

The indirect technique for the determination of the number of viable cells was used for assessing the microbiota of investigated wines. Each 0.2 ml of  $10^{-1}$  and  $10^{-2}$  wine dilutions was inoculated in three repetitions on the culture media: solid YPG medium (yeast extract - peptone - glucose) for the yeasts isolation, Carr medium for the acetic acid bacteria isolation and Lafon-Lafourcade medium for the lactic acid bacteria isolation. After 24 hours incubation at  $27^{\circ}$ C, the colonies were counted using the Funke Gerber colony counter and the number of colony - forming units per ml (CFU/ml) was calculated, taking into account the factor of dilution.

With **the refractometric methods** can be detect some illegal practices at the wine by comparing the refractive index with the density of wine. The addition of the salts and the fruit juices into wine, to increase the extract of wine, helps to increase the density of wine, without the changing the value of refractive index. Using a hand Zeiss refractometer, with a scale from 0-30% dry, we evaluated the amount of total sugar from the samples studied. Previously, were removed from wine the alcohol and the volatile compounds, which changed the value of the refractive index. The refractometric readings were corrected according to temperature, to 20°C.

#### The spectrophotometric methods

The spectral analysis has a high sensitivity and is very accurate. The absorption spectrum can give us the clear information about wine and its colour - one of the main parameters of wine quality (Niskanen et al. 2009). The studies were made using a spectrophotometer UV/VIS Perkin Elmer Lambda 25, with double-beam.

The analysis of wine seeks also establish the maximum limits for the chemical compounds that can the harm consumers.

Par example, **the methanol** is a component that affects the quality of wine and a value over 500 mg methanol/L, the wine becomes unfit for drinking. The methanol from the wine is obtained in during the alcoholic fermentation by the enzymatic pathway, by the hydrolysis of the soluble pectins from the grapes, under the action of pectin-methyl-esterase enzyme. Has occurs a demethylation of galacturanopiranozic esters from the pectin composition, with the pectic acid insolubilisation and the methanol formation. The determination of methanol from the wine is made after its separation from wine by the distillation, by the spectrophotometric dosage. Methanol from the wine distillate is oxidized to formaldehyde with potassium permanganate, acidified with phosphoric acid. The amount of formaldehyde resulting is proportional to the methanol content from the wine. Formaldehyde formed is determined by the colour reaction with chromotropic acid, measuring the colour intensity with spectrophotometer at 575 nm. To determine the methanol content of wine is necessary to draw the calibration curve.

Usually, another element from wine, which may be determined by spectrophotometrically analysis, is **citric acid**. The addition of citric acid in wine is certified by the International Organization of Vine and Wine and, also, the European Union to correct the deficient acidity of wines, the condition is that, ultimately, the wine does not contain more than 1g/L citric acid. The determination of citric acid is based on the manganic oxidation reaction, which the hidroxycarboxylic group from the middle molecule is oxidized, with formation of acetone-dicarboxylic acid, carbon dioxide and water. The method is based on the reaction of citric acid from wine with acetic anhydride in the basic medium, when is formed a compound whose maximum absorbance is measured with spectrophotometer at a wavelength of 363 nm.

Antocyans represent the visible phenolic compounds (pigments), which accumulates in grapes and gives the red wine colour. They represent 38% of total phenolic compounds present in the wine. The quantitative determination of anthocyans from the red wines aims to track the evolution of the phenolic ripening process of the grapes and the establishing of the anthocyans content, for their quantitative evaluation. The quantitative determination of antocyans is made by the spectrophotometry in visible and is based on the colour change of antocyans function of pH. The absorbance variation for the antocyans colour is measured, at two pH values, namely 0.6 and 3.5, compared with the distilled water. The measurements are taken at the wavelength of 520 nm, the absorbance of the samples being proportional to the antocyans content.

#### **RESULTS AND DISCUSSION**

The primary analysis of the samples showed that the wines do not have uniformity in terms of specific microbiota. Thus, the sample of white wine, Pinot Gris, had presented only lactic acid and acetic acid bacteria (over 3500 CFU/ml); this sample had stability in terms of alcoholic fermentation, but also the possibility of undesirable organoleptic changes, caused by malolactic fermentation in a white wine, not too acid and with the sugar level of 10 g/l. Can be possible a contamination of the wine during his maturation with the bacteria from the storage vessels.

The red wine sample, Cabernet Sauvignon, has presented the yeast (4700 CFU/ml), lactic acid bacteria (1445 CFU/ml) and acetic acid bacteria (1425 CFU/ml), showing a lower microbiological stability and a possible disease of the wine. Since the citric acid concentration of this wine was quite high, it appears that there was no efficient malolactic fermentation, beneficial for the organoleptic characteristics of wine.

The wine sample Fetească Neagră has contained only yeasts (Figure 1), the absence of lactic acid bacteria justifying the high concentration of citric acid determined.

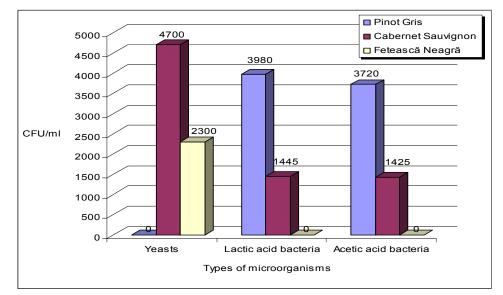
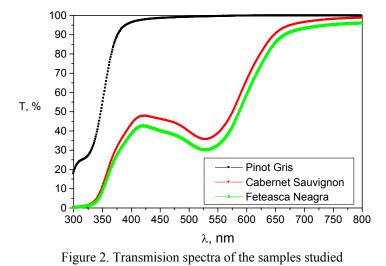


Figure 1. Types of microorganisms contained in the studied samples

The transmission spectra of the samples studied are shown in Figure 2.





From the values of absorbances, we can establish the colour intensity and the colour hue.

#### Table 1

Table 2

values of the color parameters						
Wine type	Rec	White wine				
Analysis	Fetească Neagră	Cabernet Sauvignon	Pinot Gris			
Colour hue (N <sub>C</sub> )	0.72	0.73	0.51			
Colour intensity $(I_C)$	1.0239	0.8771	0.151			

Values of the color parameters

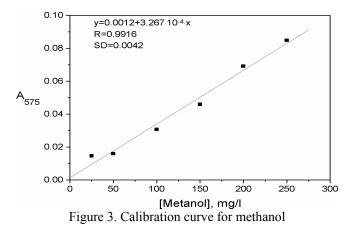
The N<sub>C</sub> values are situated in the range 0.5 to 0.6. Only for the old wines, the value is supra-unit (1-1.5) because the copolymerization of antocyans with tannin from wine decreases the absorbance corresponding the red colour of wine, the wine becoming the redbrick. Thus, one can observed the high values of colour hue for the red wine samples, probably due to its easy oxidation. The I<sub>C</sub> values are subunit at the white and rose wines and supra-unit at the red wines. The sample Cabernet-Sauvignon shows a subunit value of the colour intensity.

The total sugar content from the wine samples studied, determined by refractometric method is shown in Table 2.

Total sugar content						
Wine type Red wines White wine						
Analysis Fetească Neagră Cabernet Sauvignon Pinot Gris						
Dry, %		3.2	3.0	3.3		
Total sugar, g/l 9.0 6.8 10.0						

The content in methanol, citric acid and antocyans from the wine samples studied was determined by spectrophotometric dosage.

The calibration curve obtained for methanol is shown in Figure 3.



The content in methanol, citric acid and antocyans obtained for three wines from Valea Călugărească area is presented in Table 3.

Table 3

	Fetească	Cabernet-Sauvignon	Pinot Gris
	Neagră		
[CH <sub>3</sub> OH], mg/l	212	284	140
[citric acid], g/l	1.86	1.46	0.32
Antocyans, mg/l	124.5	234	-

Content in methanol, citric acid and anthocyans from samples

Since the maximum limits permitted by the OIV for methanol are: 150 mg/l for white and rose wines and 300 mg/l for red wines, it is clear that the samples studied fall into this area.

For citric acid, it is noted the overcoming of the limit allowed (1 g/l), in the case of samples of Cabernet-Sauvignon and Fetească Neagră. In addition, usually, the red wines containing less citric acid than the white wines, because by malolactic fermenting that affecting normally the red wines, the lactic bacteria decompose some of citric acid with the formation of volatile acids. The high quantity of citric acid measured at Fetească Neagră was correlated with the lack of lactic bacteria in this sample of wine.

The Cabernet-Sauvignon wine has a fingerprint of antocyans stronger than Fetească Neagră wine; for old wines, the antocyanic spectrum is modified because the wine Cabernet-Sauvignon tends to form polymerized antocyans (Rădoi et al., 1999).

# CONCLUSIONS

In terms of the microbiological stability, the studied wines tally with the wines produced by the local producers. They do not have a industrialized technological line, the alcoholic fermentation is spontaneous, under the action of natural microbiota, the fermentation vessels and the storage vessels are not fully sterilized and the sulfitation for the wines stabilization is not very emphasized.

The samples of Cabernet Sauvignon and Feteasca Neagra, with high values from citric acid, they have lost from naturalness and they are suspected of supracitraj.

To a better define the wine colour, will be used the spectrophotometric method in the tristimulus coordinates (CIE-Lab-76), which enables to determine the chromatic parameters.

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## STUDIES OF TOTAL PHENOL CONTENTS, ANTHOCYANS AND ANTIOXIDANT ACTIVITY OF SOME GREEK RED WINES

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*KEY WORDS*: wine, grapes, total phenols, antiradical activity, antioxidant activity

#### ABSTRACT

The study involved 16 red-wine brands produced from more than one grape cultivars originated from different regions of Greece. The total phenol contents ranged from 1360 to 3970 ppm gallic acid equivalents, and their anthocyans varied from 10 to 428  $\mu$ M malvidin-3-glucoside equivalents. The antiradical activity, assessed through inhibiting the free stable radical DPPH varied from 2.96 to 14.80  $\mu$ mol DPPH/ml and the antioxidant capacity determined by the FRAP assay ranged from 9.95 to 26.75  $\mu$ mols FRAP/ml wine. The antioxidant and antiradical activity of the wines tested depended strongly on the total phenolic content. This work showed that Greek red wine is important antioxidant source, a valuable tool for local nutraceutical industries which will make them great competitors in the global market.

## **INTRODUCTION**

The favourable climate and soil conditions of Greece, as part of Mediterranean basin, meet the optimum requirements for growth of a wide spectrum of grapes varieties and production of good quality wines. Grape and wines are an important source of natural antioxidants (Kanner et al., 1994). In particular, red wine has a phenolic content considerably higher than white wine due to winemaking procedure (Frankel et al., 1993; Leighton et al., 1998). Phenol rich foods and beverages contribute to the prevention of several diseases associated with oxidative stress (Muselík et al., 2007). Furthermore, grapeseed polyphenolic compounds combined with antioxidant vitamins are currently used with success in nutraceutical industry (Nutall et al., 1999).

Due to industrial importance of wine antioxidant activities, there is a need for more in-depth-analysis of traditional wines around the globe. The aim of this work was to determine the levels of total phenols, anthocyans, antioxidants in Greek red wines and measure their antiradical attributes.

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## MATERIAL AND METHODS

Sixteen Greek red wines produced from one or more grape varieties were used in our study (Table 1). Samples from wines had been produced from 2004 to 2006 in different wine regions of Greece were used. The total phenol content was determined using the Folin-Ciocalteu assay (Singleton and Rossi, 1965; Badenschneider et al., 1999) and the results expressed as gallic acid equivalents (GAE) or catechin.

The anthocyans were measured using the method of Ribereau-Gayon and Stonestreet (1965) and expressed in malvidin-3-gllucoside equivalents (molar extinction coefficient of 28000). The ferric reducing antioxidant activity was assessed *in vitro* by the FRAP (Ferric reducing/antioxidant power assay) method of Benzie and Strain (1999), using Fe<sup>3</sup>-tripyridyl triazine complex (Fe<sup>3+</sup> -TPTZ). The antioxidant activity was expressed as  $\mu$ mol/ml wine. The antiradical activity was assessed by bleaching the free stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) using the method of Brand-Williams et. al. (1995). The initial DPPH concentration was calculated by the equation:

$$A_{515nm} = 12509 \text{ C}_{\text{DPPH}} - 2.58 \times 10^{-3}$$

The antioxidant activity of the L-ascorbic acid standard was 11304  $\mu$ mol/g (Simonetti et.al., 1997; Fogliano et.al., 1999).

Data were analyzed using the Minitab statistical package. Standard deviation was calculated for all measurements. The linear regression between total phenolic content and antioxidant activity as well between total phenolic content and antiradical activity was examined.

## **RESULTS AND DISCUSSION**

The total phenols content varied between wines. 'Ktima averof' red wine had the highest concentration of total phenols (3820 ppm GAE) while 'Daphnes' the lowest (1360 ppm GAE). The mean concentration of total phenols was 2605 ppm GAE (Table 1). Several researchers have also been observed differences in the phenolic content among grape varieties (Frankel et al., 1995; Simonetti et al., 1997; Burns et al., 2000). The difference in the content of total phenols depends on several factors such as variety, climatic and ecological factors, cultural practises, harvesting method and winemaking procedure.

The content of anthocyans on red wines fluctuated from 10 to 428  $\mu$ M malvidin-3glucoside equivalents. Their quantity at red wine depends on the wine production method and aging. Old wines have lower content from new ones (Mazza, 1995). In our study wines produced on 2006 had greater anthocyans content than wines produced on 2004 with 'Rapsani' wine to have the greatest concentration.

The antioxidant activity (FRAP) differed between 16 red wines brands (table 2). All wines had high antioxidant activity which reduces Fe. The wine 'methistanes' had the greatest FRAP activity (26.75  $\mu$ mol FRAP/ml wine) and 'Grand Rose' the lowest (9.95  $\mu$ mol FRAP/ml wine). Antioxidant capacity of wines is in close relation with their concentration in total and individual phenolic acid (Pulido et al., 2000). In our study there was linear correlation between the amount of total phenols and antioxidant capacity of red wines (r<sup>2</sup>=7822), (Figure 1).

The antiradical activity of wines was ranged from 2.96 to  $14.80 \ \mu mol DPPH/ml$  wine with mean  $5.96 \ \mu mol DPPH/ml$  (table 2). 'Chevalier de Rhodos' had the highest antiradical activity and 'methistanes' the lowest. Burnes et. al. (2000) reported negative

relation between phenolic content and antiradical activity of several red wines. From the 16 red wine brands evaluated here 'Chevalier de Rhodos' had low total phenolic content (1630 ppm GAE) and high antiradical activity (14.80  $\mu$ M DPPH/ml). 'Grand Rose' had greater total phenolic content (1430 ppm GAE) but five times lower antiradical activity (2.96  $\mu$ M DPPH/ml) than 'Chevalier de Rhodos'. 'Rapsani', 'Peza' and 'Archodiko' had intermediate content of total phenols and high antioxidant activity with 22.40, 20.02 and 22.45  $\mu$ mol FRAP/ml respectively. The relation between total phenolic content and antiradical activity are shown in Figure 1.

Table 1.

Wine Number	Wine Trade and Region	Variety	G.A.E. (ppm±sdev)	Catechin (µM)	Anthocyans (µm malv.)	Year, Grape category
n.1	Naoussa (Naussa)	Ksinomavro	3805±75	13121	193±3	2005 V.Q.P.R.D.
.n.2	Gumenissa (Gumenissa)	Ksinomavro + Negoska	3710±30	12793	193±3	2005 V.Q.P.R.D.
n.3	Ktima Averof (Ioannina)	Cabernet Sauvignon+ Cabernet Franc+ Merlot	3820±56	13172	209±7	2006 Regional
n.4	Rapsani (Larissa)	Ksinomavro + Krasato + Stavroto	2320±12	8000	428±8	2006 V.Q.P.R.D.
n.5	Ktima Katsaros (Larissa)	Cabernet Sauvignon + Merlot	2840±20	9793	208±9	2006 Regional
n.6	Messenikola (Karditsa)	Mesenikola + Syrah + Karinian	1420±45	4897	46±3	2004 V.Q.P.R.D.
n.7	Methistanes (Larissa)	Ksinomavro + Krasato + Stavroto	3970±68	13690	24±1	2004 Table grape
n.8	Sitia (Sitia)	Liatiko	3050±60	10517	10±1	2004 V.Q.P.R.D.
n.9	Peza-(Peza)	Kotsifali + Mandilari	2320±79	8000	29±3	2004 V.Q.P.R.D.
n.10	Daphnes (Daphne)	Liatiko	1360±22	4690	17±1	2004 V.Q.P.R.D.
n.11	Critikos (Crete)	Kotsifali + Mandilari	2740±11	9448	40±3	2005 Regional
n.12	Archodiko (Rodos)	Cabernet Sauvignon	2220±90	7655	65±2	2005 Regional

Total Phenolic and anthocyans content of Greek Red Wines.

		+ Grenache Rouge				
n.13	Chevalier de Rhodos (Rodos)	Mandilaria	1630±31	5621	30±1	2005 V.Q.P.R.D.
n.14	Egeopelagitikos (Paros)	Monemvasia + Mandilaria	2880±31	9931	89±3	2006 Regional
n.15	Kalabaki (Limnos)	Limnio	2160±29	7448	76±2	2006 Table grape
n.16	Grand Rose (Rodos)	Amorgiano	1430±39	4931	20±1	2005 Table grape

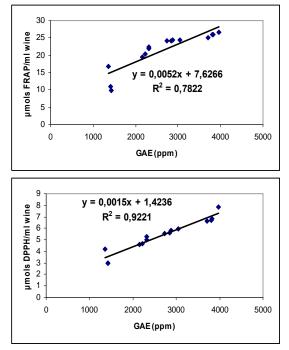


Figure 1. Relation between red wines antiradical and antioxidant activity and total phenolic contend (GAE).

Table 2.	
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Antiradical activity (DPPH) and Antioxidant activity (FRAP) of Greek Red Wines.

Wine			150 m	l wine	
Number	Trade	µM DPPH/ml	µM FRAP/ml	µmol FRAP	eq. mg vit. C
n.1	Naoussa	6.70±0.10	26.00±0.10	3900	345
n.2	Goumenissa	6.64±0.20	25.11±0.22	3767	333
n.3	Ktima Averof	6.82±0.11	26.06±0.33	3909	346
n.4	Rapsani	5.28±0.25	22.40±0.10	3360	297

-				1	
n.5	Ktima Katsaros	5.60±0.26	24.10±0.80	3615	320
n.6	Messenikola	2.96±0.12	10.90±0.32	1635	145
n.7	Methistanes	7.83±0.12	26.75±0.35	4013	355
n.8	Sitia	5.95±0.15	24.50±0.34	3675	325
n.9	Peza	4.98±0.15	22.02±0.50	3303	292
n.10	Daphnes	4.22±0.17	16.84±0.30	2526	223
n.11	Critikos	5.52±0.22	24.10±0.42	3615	320
n.12	Archodiko	4.70±0.17	20.45±0.40	3068	271
n.13	Chevalier de	14.80±0.20	22.12±0.28	3318	294
	Rhodos				
n.14	Egeopelagitikos	5.85±0.28	24.40±0.88	3360	297
n.15	Kalabaki	4.58±0.11	19.48±0.35	2922	259
n.16	Grand Rose	2.96±0.04	9.95±0.10	1493	132

#### CONCLUSION

Greek red wines exhibit remarkable antioxidant and antiradical activity. Flavonoid and total phenol concentrations in Greek red wines provide a valuable tool for local nutraceutical industries, a key-role which will make them great competitors in the global market. High interest have the red wine brands 'Chevalier de Rhodos', 'Grand Rose' 'Rapsani', 'Peza' and 'Archodiko' as shown high relationship between total phenolic content and antioxidant or antiradical activity.

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# FERTILIZATION MANAGEMENT OF GREENHOUSE CROPS BASED ON SOIL SALINITY LEVEL

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KEY WORDS: Electrical conductivity, salinity, fertilizer, greenhouse crop

## ABSTRACT

In greenhouse cucumber plants which were growing in soil with increased salinity (EC 0.66 dS/m), the omission of basic fertilization was tasted. During cultivation period the fertigation programme was organized according to soil salinity level, plant's growth and leaf tissue analysis. Soil salinity declined through cultivation period while plant's yield did not affected by fertilization process. Plant's need for nutrients was partially covered by soil salinity was observed after soil solarisation.

## INTRODUCTION

Soil salinity assessment is based on measurement of soil electrical conductivity, a quick, reliable and easy method which could be used during cultivation period for indication of soil fertility in a greenhouse crop (Rhoades et al., 1999).

The application of high fertilizer rates in intensive cultures, like greenhouse crops, affects electrical conductivity. In soils from several greenhouses in Thessaly, which have irrigated with low salinity water, the high electrical conductivity (0.4 dS/m) in soil extracts (soil: $H_2O$  ratio 1:5) is related with the increase concentration of soluble N and K. In these soils the application of N and K fertilizers in the following culture is not recommended (Chouliaras et al., 1991). In addition, the concentration of phosphorus in soil is not affect salinity (Chouliaras et al., 1991).

The mobility of P in soil is very limited and therefore could remains in soil for many years, in contrast with N and K. Thus, the knowledge of P fertilizer applications in formers cultures consists a valuable guide to efficient plant nutrition management.

The aim of this work was to develop a fertilization method for a greenhouse cucumber culture based on soil salinity and fertilizer inputs of the former crops.

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#### MATERIALS AND METHODS

Soil samples (to a depth of 15 cm) were taken from a greenhouse located at the Technological Education Institute (TEI) of Larissa, Greece, for physical and chemical soil properties estimation. Soil organic matter, ammonium and nitrate nitrogen, available P and exchangeable K were measured following the Page *et al.* (1982) method. Organic matter content was calculated by chemical oxidation of soil with 1 mol/l  $K_2Cr_2O_7$  and titration of the remaining reagent with 0.5 mol/l FeSO<sub>4</sub>.

Soil organic matter was estimated by multiplying soil organic carbon content by the factor 1.724 as reported by Hesse (1972). Both ammonium and nitrate nitrogen were extracted with 0.5 mol/l CaCl<sub>2</sub> and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P (P-Olsen) was extracted with 0.5 mol/l NaHCO<sub>3</sub> and measured by spectroscopy. Finally, exchangeable K was extracted with 1 mol/l CH<sub>3</sub>COONH<sub>4</sub> and measured by Flame Photometry (Essex, UK).

According to analysis, the greenhouse soil was loamy sand, slightly calcareous, with alkaline pH, low organic matter content and high Cation exchange capacity (Table 1). The electrical conductivity of soil extracts (water soil ratio1:5) was 0.66 dS/m, indicating marginally increased soil salinity (Chouliaras et al., 1996).

Table 1.

Chemical soil properties	
Soil properties	Values
pH	8.13
CaCO <sub>3</sub> (%)	5.8
Organic matter (%)	0.74
Cation exchange capacity (cmol/kg)	23
Electrical conductivity in soil extract (1 soil : 5 H <sub>2</sub> O, dS/m)	0.66

Cucumber plants (var. Gador) was transplanting at early April 2008 in greenhouse. During cultivation period (April-July 2008) plants were watering with good quality water (EC = 0.5 dS/m) while the fertigation programme organized according to plant growth, blooming and fruit load, and soil salinity changes. Forty days after transplanting leaf samples were taken for plant inorganic elements assessment.

As the concentration of N and K in plants was found to be low, fertigation programme modified according to CTIFL guide (1989). Totally, during the whole growing period 110 Kg N, 80 Kg P ( $P_2O_5$ ) and 130 Kg K ( $K_2O$ ) per hectare were used for plant fertigation. At the end of growing season, soil covered by transparent polyethylene plastic for soil solarisation and the soil electrical conductivity was measured three months later.

The experimental design was completely randomized with four replications. Data analysis was made using the MINITAB statistical package (Ryan et al., 2005). Analysis of variance was used to assess treatment effects. Mean separation was made using Tukey's test when significant differences between treatments were found.

#### **RESULTS AND DISCUSSION**

Soil salinity was reduced during growing period (Figure 1). Plant fertilizer application based on soil salinity did not only reduce salt concentration in soil but led to electrical conductivity decline from 0.66 dS/m to 0.24 dS/m at the end the growing season. Table 2 shows the balance of available inorganic elements in the soil during growing period. The availability of minerals is due to fertilizer residues from previous crops and nutrients applied via irrigation in the current crop. These data confirms the use of soil salts from plants.

In addition, it is remarkable the increase of salinity (1.23 dS/m) after soil solarization due to soluble salts mobility to soil surface caused by intensive evaporation. In this case, the salinity level has to be considered for the following crops. According to Chouliaras (1990), is recommended the improvement of soil properties by organic matter application for these greenhouses.

Finally, the fertigation programme did not affect plants yield. The total production of greenhouse cucumber was  $6.4 \text{ Kg/m}^2$ .

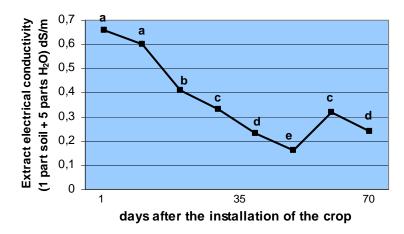


Figure 1. Changes in soil salinity during cultivation period.

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	Inorganic elements availability										
Specification	N (Kg/ha)	$P_2O_5$ (Kg/ha)	K <sub>2</sub> O (Kg/ha)	Electrical							
-	Inorganic	P-Olsen	Exchangeable	conductivity							
Start of growing	490	70	1040	0.64 dS/m							
season											
Surface fertilizer	110	80	130								
application											
After soil solarization	730	80	1180	1.23 dS/m							

#### CONCLUSION

This study shows that greenhouse soil salinity due to accumulation of fertilizers could be taken into account for basic fertilizer omission. In this way, plant needs for nutrients might partially be covered by soil salts leading to limited surface fertilizer application and soil salinity reduction by the end of the growing season.

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## INFLUENCE OF SOME ENVIRONMENTA3L FACTORS ON MAIN PHYSIOLOGICAL PROCESSES ON APPLE TREES

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KEY WORDS: environmental, factors, physiological, processes, apple

#### ABSTRACT

In order to obtain good quality crops it is necessary to know the interaction between plants and environmental factors (factors (light, temperature, the  $CO_2$  concentration in the air, soil humidity, soil fertility, etc.). This paper's aim is to study the physiologic reaction (photosynthesis rate, transpiration rate, stomatal conductance of  $CO_2$ ) of apple cultivars to environmental factors (temperature, light). Determination of intensity in physiologic processes in accordance with climate factors has been carried out by using LCpro + portable photosynthesis system.

#### **INTRODUCTION**

Plants can regulate the movements of water vapour,  $O_2$  and  $CO_2$  through the leaf surface. This is accomplished by opening and closing pores, called stomata (sing., stomate), usually found on the bottom side of the leaf. Opening and closing of stomata is controlled by specialized cells called guard cells (Martin et al., 2010).

The photosynthetic activity is conducted by seasonal changes and diurnal changes (light intensity fluctuations, leaf temperature, air temperature and humidity) (Kositsup et al., 2010).

Under low light levels, the available light is insufficient to support the maximal potential rate of the light-dependent reactions, and thus limits the overall rate of photosynthesis. As light levels are increased the rate of increases photosynthesis (Hogewoning et al., 2010).

At a particular light intensity, the so-called "light saturation point", the rate of  $O_2$  evolution levels off. Any further increase in the amount of light striking the leaf does not cause an increase in the rate of photosynthesis the amount of light is said to be 'saturating' for the photosynthetic process (Zeiger, 1990). At the light saturation point, increasing the light no longer causes an increase in photosynthesis. (Marenco et al., 2009).

In same fruit tree species, there is a diurnal variation in photosynthesis activity. Maximum value was recorded in the morning, followed by its reduction. (Chen and Cheng, 2009).

Research has been made on environmental factors' effect on physiological processes and on growth and development in fruit tree species, in Romania as well (Burzo et al., 1999; Cosmulescu, 2004, 2007, 2008).

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#### MATERIALS AND METHODS

This study was made at Valcea Research and Development Station for Fruit Tree. Five apple cultivars were taken under study: Royal Gala, Rubinette, Fuji, Akane and Braeburn.

Determinations of photosynthesis, transpiration intensity and stomatal conductance were made by using the portable Lcpro system that enables automatic recording of other parameters as well (stomatal conductance, leaf temperature, incident photosynthetic radiation etc). LCpro is designed to carry out precise measurements of photosynthesis and transpiration, by automatically controlling the leaf chamber environment.

Lcpro leaf chamber contains a system for analyzing and measuring the  $CO_2$  and  $H_2O$ . Measuring of  $CO_2$  is carried out through a miniature infrared gas analyzer.

Measuring of  $H_2O$  is done by using high quality water vapors sensors. Beside gas exchanges, other relevant parameters are being measured as well; various calculations are also automatically carried out, based on recognized formulae.

All measurements, calculations and experimental programs were stored in files on memory cards. The results were graphically represented and statistically interpreted.

## **RESULTS AND DISCUSSION**

Fruit growth is influenced by environmental factors and internal factors. Most representatives environmental factors are: light, temperature, the  $CO_2$  concentration in the air, soil humidity, soil fertility. Sun radiation reaches soil and trees canopy in two forms: direct light and diffuse light. As a result of sun radiation leaf is worming and the process of photosynthesis is beginning.

This process increased, reaching maximum value at 37-39  $^{\circ}$ C (Figure 1). Any further increase in the amount of temperature striking the leaf does not cause an increase in the rate of photosynthesis. The relationship between temperature and photosynthesis is showed in table 1 (Tch/A).

Under experimental conditions given, at cultivar Akane, 25% of photosynthetic rate variation was determined by leaf temperature. (the lowest value). At the either side the biggest influence of temperature on photosynthetic rate was obtained at Braeburn. (60.69%)

Higher temperatures cause the assimilation stop, respiration intensification and fast decrease of reserve substances; in case of fruit-trees, temperature of 39°C, is considered to be maximum limit for normal development of photosynthetic and growth activity. Photosynthetic rate is lower at Braeburn and higher at Rubinette cultivars (Figure 1).

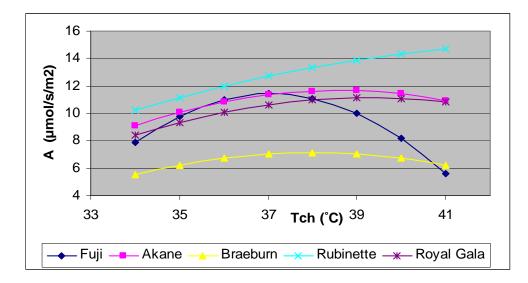


Figure 1. Influence of temperature on photosynthesis rate

Transpiration in fruit trees appears as a result of high temperature. Transpiration is made through all aerial organs, with priority in leaves. Analysing correlation between leaf temperature and transpiration rate, (Table 1, Tch/E) it was found positive correlation - as temperatures increase, so the transpiration rate increases too. Temperatures of parts exposed to sun have reached values of more than  $40^{\circ}$ C.

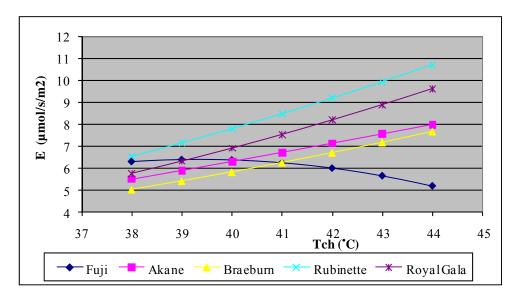


Figure 2. Relationship between temperature and transpiration rate

Percentage of the temperature affects transpiration is in the range 76.04% (Fuji) - 97.26% (Royal Gala). Effect of temperature response of all varieties is showed in Figure 2.

Another factor that was taken under study was stomatal conductance for  $CO_2$ . Stomatal conductance for  $CO_2$ , expressed in mol/m<sub>t</sub>/s, represents the value expression of stomatal permittivity for carbon dioxide passing through.

The study of this parameter offer information on the way how the plant reacts under different conditions of water supply or temperature, to adjust the intensity of transpiration process, so that to reduce water loss.

Relationships between stomatal conductance and photosynthesis (gs/A) for all cultivars are showed in Table 1. Under experimental conditions given, approximately 17% (Braeburn) – 61% (Rubinette and Akane) of photosynthetic rate variation was determined by stomatal conductance.

If water availability is reduced, stomata close and evaporation decreases, leading to improved water use efficiency. Total  $CO_2$  assimilation (the photosynthesis) also decreases, but the plant conserves water and increases its chances of survival.

 $CO_2$  used during photosynthesis first must pass through stomata into internal spaces within the leaf. It then diffuses into mesophyll cells where it becomes available for photosynthesis. When the stomata close,  $CO_2$  levels drop rapidly within the leaf, inhibiting the light-independent reactions. This then causes photosynthesis to stop (Figure 3)

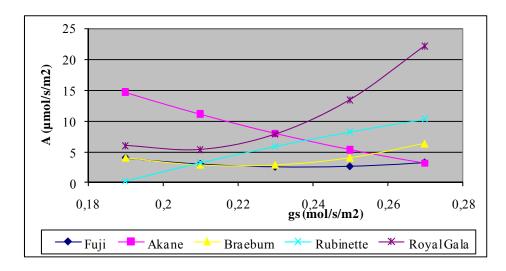


Figure 3. Relation between stomatal conductance and photosynthesis

arameters
between p
Relationship

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			Parameters	eters	
	CULUVAIS	Tch\E	Tch∖A	gs\A	A\E
	Euji	$y = -0.0552x^2 + 4.3405x-78.921$ $R^2 = 0.7604$	$y = -0.0552x^2 + 4.3405x-78.921 \qquad y = -0.3749x^2 + 27.796x - 503.8  R^2 = 0.7604  R^2 = 0.4993$	y= 684,43x <sup>2</sup> - 324,32x + 40,893 R <sup>2</sup> = 0,3434	y= 1,0289x <sup>2</sup> - 7,5932x + 16,823 R <sup>2</sup> = 0,7105
	Akane	$y = 0,0028x^{2} + 0,187x - 5,6577$ $R^{2} = 0,953$	$y = -0,1239x^2 + 9,5564x - 172,61 \qquad y = 558,68x^2 - 401,33x + 70,761  R^2 = 0,2541  R^2 = 0,6116 $		y= 0,2512x <sup>2</sup> - 1,5932x + 10,503 R <sup>2</sup> =0,5066
	Braeburn		$y = -0,104x^2 + 7,8949x - 146,66$ $R^2 = 0,6091$	y= 1405,5x <sup>2</sup> - 618,23x + 70,761 R <sup>2</sup> = 0,1773	$y = -1,1562x^2 + 11,675x - 21,734$ $R^2 = 0,6403$
	Rubinette	$y=0,0159x^2-0,607x+6,6495$ R <sup>2</sup> = 0,9492	y= -0,0477x <sup>2</sup> + 4,2243x -78,288 R <sup>2</sup> = 0,4886	$y = -380, 21x^2 + 301, 04x - 43, 56$ $R^2 = 0, 6107$	$y = -0,4812x^2 + 7,4689x - 12,584$ $R^2 = 0,7152$
	Royal Gala	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	y= -0,1018x <sup>2</sup> + 7,9805x- 145,27 R <sup>2</sup> = 0,6026	y= 3914, $1x^2 - 1598, 3x + 168, 37$ y= -0, 6329 $x^2 + 7, 9778x - 13, 01$ R <sup>2</sup> = 0, 2773 R <sup>2</sup> = 0, 5299	y= -0,6329x <sup>2</sup> + 7,9778x - 13,01 R <sup>2</sup> = 0,5299

Table 1

283

#### CONCLUSIONS

Photosynthesis era influenced both by environmental factors and internal factors. Transpiration rate and photosynthetic rate are influenced by closure or aperture of stomata.

Unfavorable environmental conditions (temperature, light, too much or too little) cause the change in the development of physiologic processes.

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# Vol. XV ( XLXI ) - 2010

# CONTRIBUTIONS TO THE KNOWLEDGE OF FAUNA OF BEETLES (ORDER COLEOPTERA) IN SOME ECOSYSTEMS OF SWEET CHERRY AND SOUR CHERRY IN EASTERN ROMANIA

Herea Monica<sup>1</sup>\*, Tălmaciu M<sup>2</sup>., Tălmaciu Nela<sup>2</sup>

KEY WORDS: Coleoptera, cherry, Barber traps, beating

#### SUMMARY

Contributions to the knowledge of fauna of beetles (order Coleoptera) in some ecosystems of sweet cherry and sour cherry from Iasi and Vaslui counties

The beetles were sampled using traps soil type Barber, and beating in some sweet cherry and sour cherry where, have been applied various control methods.

From the collected material were selected Coloptera species following the determination of each specie.

Among the most common Coleoptera we identified: Stethourus punctilum Weisse, Carabus violaceus L.; Silpha obscura L., Cymindis humeralis Fourc., Apion atomarium Kirby, Otiorrynchus ovatus L., Dermestes laniarius Illig., Harpalus calceatus Duft., Silpha carinata Herbst.

# MATERIALS AND METHODS

Research on entomofauna of beetles were made in plantations of sweet cherry and sour cherry farm Loturi Service Deleşti, and Farm Vasile Adamachi in 2009.

Gathering material was made using 6 traps soil type Barber wih phormol solution 3-4% and beating (Talmaciu et al., 2001). Installation traps type Barber was in mid May and they worked until late August.

Beating method samples were taken were the sudden shaking of two branches with a length of 30-50 cm from 10 trees. Each time sampling was done in five trees remained stable and marked distinctive signs the beginning of the season and five random trees chosen (Talmaciu et al, 2003)

## **RESULTS AND DISCUSSION**

Investigations are made for the first time this year in 2009, and will continue to establish a structure of wild beetles in this area by the number of treatments applied by default and depending on the degree of pollution.Of material collected species of beetles were selected, which were then determined (Chatened du Gaetan, 1990).

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No	Name of species		Number	of stampl	es colecte	d / harves	ting
	_	Ι	II	III	IV	V	VI
1	Cymindis humeralis Fourc.	22	0	0	0	0	0
2	Otiorrynchus ovatus L.	6	0	0	0	0	0
3	Omias rotundus F.	5	0	6	3	3	0
4	Harpalus distinguendus Duft.	2	0	2	0	0	2
5	Harpalus calceatus Duft.	6	0	10	0	0	0
6	Cryptophilus obliteratus Rtt.	4	0	0	0	0	0
7	Phyllodrepa melanocephala F.	1	0	0	0	0	0
8	Silpha obscura L.	1	0	0	0	0	0
9	Opatrum sabulosum L.	1	0	0	0	0	0
10	Apion apricans Herbst.	4	0	0	0	0	0
11	Psylliodes chrysocephala L.	4	0	1	0	0	0
12	Orchestia minor Walk.	1	0	0	0	0	0
13	Harpalus aeneus L.	2	0	2	0	0	0
14	Calathus fuscipes Goeze.	2	2	0	0	0	4
15	Orchesia micans Panz.	2	0	0	0	0	0
16	Carabus coriaceus L.	3	0	0	0	0	0
17	Dermestes laniarius Illig.	1	0	11	0	0	0
18	Sitona inops Gyll.	1	0	0	0	0	0
19	Bembidion lampros Herbst.	1	0	0	0	0	0
20	Harpalus tardus Panz.	2	0	2	0	0	0
21	Brachysomus hirtus Boh.	1	0	0	0	0	0
22	Carabus violaceus L.	0	2	0	0	0	0
23	Otiorrynchus raucus F.	0	0	3	0	0	0
24	Halyzia 14guttata L.	0	0	3	0	0	0
25	Amara ovata F.	0	0	2	0	0	0
26	Pterostichus niger Schall.	0	0	4	0	0	0
27	Pseudophonus rufipes Mull.	0	0	1	0	0	0
28	Amara aenea DeGeer.	0	0	2	0	0	0
29	Licinus casideus L.	0	0	1	0	0	0
30	Polystichus connexus Fourc.	0	0	1	0	0	0
31	Combocerus glaber Schall.	0	0	1	0	0	0
32	Leptinotarsa decemlineata Say.	0	0	0	5	0	0
33	Mordella fasciata f.	0	0	0	1	8	1
34	Longitarsus tabidus F.	0	0	0	0	1	3
35	Ennearthron cornutum Gyll.	0	0	0	0	1	0
36	Phyllotreta vittula Redt.	0	0	0	0	2	0
37	Baryplithes araneiformis Schrank.	0	0	0	0	2	0
38	Lagria hirta L.	0	0	0	0	0	1
Total		72	4	52	9	17	11

Structure and dynamics of species of ground beetles collected in traps in sweet cherry and sour cherry groves belonging Farm Vasile Adamachi Iasi

Overall in 2009, sweet cherry and sour cherry plantations in Iasi county were identified 165 specimens, belonging to a total of 40 species (table 1). The harvest situation is as follows:

Take the collection dated 18.05, collected a total of 72 samples belonging to 21 species. Species collected were: *Cymindis humeralis* Fourc. (22 samples); *Otiorrynchus ovatus* L. (6 samples), *Harpalus calceatus* Duft .(6 samples), *Omias rotundus* F. (5

Table1

samples), *Apion apricans* Herbst. (4 samples); *Psylliodes chrysocephala* L. (4 samples); *Cryptophilus obliteratus* Rtt. (4 samples); *Brachynus crepitans* L. (1 samples) *Carabus coriaceus* L.(3 samples) *Harpalus distinguendus* Duft. (2 samples), *Harpalus aeneus* L.(2 samples), *Calathus fuscipes*. Goeze. (2 samples), *Orchesia micans* Panzer. (2 samples) and other species are represented by only onesamples. Harvesting II dated 05.06, were collected four specimens of beetles belonging to the following two species: *Carabus violaceus* L. (2 samples), and *Calathus fuscipes* Goeze. (2 samples). Harvesting III dated 22.06, were collected 52 samples beetles belonging to 16 species, we noted: *Pterostichus niger* Schall. (4 samples), *Omias rotundus* F. (6 samples) *Harpalus calceatus* Duft. (10 samples) *Dermestes laniarius* L. (11 samples). Harvesting IV from 15.07.2009, were collected 9 samples, belonging to three species: *Leptinotarsa decemlineata* Say. (5 samples), *Omias rotundus* F. (3 samples), and *Mordella fasciata* F. (one sample).

The collection V dated 28.07, were collected 17 samples of beetles belonging to 6 species: *Mordella fasciata* F.(8 samples); *Omias rotundus* F (3 samples), with 2 samples are represented and *Barypeithes araneiformis* Schrank., *Phyllotreta vittula* Red. and species *Longitatsus tabidus* F.and *Ennearthron cornutum* Gyll. were represented by one single sample. The collection of VI dated 18.08, were collected 11 samples beetles belonging to five species: *Mordella fasciatta* F.(1 sample)., *Calathus fuscipes* Goeze. (4 samples); *Longitatsus tabidus* F. (3 samples), *Harpalus distinguendus* Duft.(2 samples) with 2 samples each species are represented Phyllotreta vittula Redt. and *Baryplithes araneiformis* F., *Mordella fasciata* F.and *Lagria hirta* L. species were represented by one single sample.

Follow the structure and dynamics of species of soil beetles collected in traps follows (Chatened du Gaetan, 1990; Panin I., 1951) in cherry and sour cherry groves belonging Farm SC. Loturi Service SRL Deleşti-Vaslui situation is as follows (table 2):

Take the collection, dated 18.05, were collected 27 samples from a number of beetles belonging to 11 species. The largest number of samples was the *Dermestes linarius* Illig. (6 samples) and also the lowest number of samples was the major species *Cymindis humeralis* Fourc., and *Panageus crux-masjor* L.

Table 2.

No.	Name of species	Nu	mber of s	stamples	colected /	/ harves	ting
		Ι	II	III	IV	V	VI
1.	Dermestes laniarius Illig.	6	6	21	2	1	0
2.	Cantharis fusca L.	3	0	0	0	0	0
3.	Panageus crux major L.	1	0	0	0	0	0
4.	Harpalus tardus Panz.	2	2	0	0	0	0
5.	Harpalus calceatus Duft.	3	3	0	0	0	0
6.	Otiorhynchus ovatus L.	2	0	0	0	0	0
7.	Adalia bipunctata L.	2	0	0	0	0	0
8.	Cymindis humeralis Fourc.	1	0	0	0	0	0
9.	Omias rotundus F.	2	0	0	0	0	0
10.	Opatrum sabulosum L.	3	0	0	0	0	0
11.	Phyllotreta nemorum L.	2	0	0	0	0	0
12.	Amara fasciata F.	0	1	0	0	0	0
13.	Coccinella 7punctata L.	0	5	0	0	0	0
14.	Otiorhynchus raucus F.	0	3	2	0	0	0
15.	Brachinus crepitans L.	0	0	2	0	0	0
16.	Omias rotundatus F.	0	3	0	0	0	0

Structure and dynamics of species of beetles collected in soil traps sweet cherry and sour cherry plantations belonging farm SC. Loturi Service SRL Deleşti Vaslui

17.	Amara aenea DeGeer.	0	3	4	0	0	0
18.	Harpalus distinguendus Duft.	0	0	2	0	0	0
19.	Harpalus calceatus Duft.	0	3	7	0	0	0
20.	Harpalus pubescens Mull.	0	0	2	0	0	0
21.	Harpalus azureus F.	0	0	3	0	0	0
22.	Curculio nucum L.	0	0	1	0	0	0
23.	Harpalus griseus Panz.	0	0	0	0	0	1
24.	Psylliodes chrysocephala L.	0	2	2	0	0	0
25.	Harpalus aeneus F.	0	0	3	0	0	0
26.	Carabus coriaceus L.	0	0	3	0	0	0
27.	Dermestes lardarius L	0	0	0	2	0	0
28.	Meligetes aeneus F.	0	0	0	2	0	0
29.	Harpalus punctifolis L.	0	0	0	1	0	0
30.	Harpalus tardus Panz.	0	0	0	0	1	0
31.	Carabus violaceus L.	0	0	4	2	4	17
32.	Cymindis vaporariorum L.	0	0	0	1	0	0
33.	Propylaea quatuordecimpunctata L.	0	0	0	0	0	2
34.	Longitarsus tabidus F.	0	0	0	0	2	0
	Total	27	25	30	10	6	20

Harvesting II, dated 29.05, have been collected in all 28 samples of beetles belonging to a total of 9 species. The largest number of samples (3) and species (6) were collected in the trap no. 6. other traps were between one and three samples belonging to 1, 2 or 3 samples. Harvesting III, dated 22.06, were collected 59 samples from a number of beetles belonging to 15 species. The largest number of samples was the *Dermestes lanirius* Illig. (21 samples) and also the lowest number of samples was the *Curculio nucum* L. and *Psylliodes chrysocephala* L. Harvesting the IV, dated 06.07, have been collected in all 10 samples of beetles belonging to a total of 6 samples, as follows: *Dermestes lardarius*L., *Carabus violaceus* L., *Dermestes linarius* Illig., *Melighetes aeneus* F. were represented by 2 samples each, and *Harpalus punctifolis* L., *Cymindis vaporariorum* L. were represented by 0 one single sample. Will the collection of 15.07, have been collected in all 8 samples of beetles belonging to a number of 4 species.

At the VI harvest, dated 28.07, have been collected in all 20 samples of beetles belonging to a number of 3 species and *Carabus violaceus* L. was seconded by a number of 17 samples. Research of entomofauna on tree crown was made of sweet cherry and sour cherry groves of the farm Vasile Adamachi Iasi. The situation of samples(tablel 3) were we use beating is as follows (Chatened du Gaetan, 1990; Panin, 1951; Rogojanu and Perju, 1979).

Table 3

No.	Name of species		Numb	per of s	tample	es col	ected /	harvest	ing
		Ι	II	III	IV	V	VI	VII	VIII
1	Catodere elongata Thomson	1	3	0	6	2	0	3	0
2	Stethourus punctilum Weisse.	1	0	0	0	0	0	0	5
3	Otiorrynchus ovatus L.	20	0	0	1	0	0	0	0
4	Longitarsus jacobae Waterhouse	0	0	0	1	0	0	2	1
5	Phyllotreta vittula Red.	0	0	0	0	0	0	2	0
6	Cymindis humeralis Fourc.	0	0	0	0	0	0	1	0

Situation on the species and number of samples use beating of sweet cherry and sour cherry orchards belonging Farm Vasile Adamachi Iasi

7	Coccinella 7punctata L.	2	0	0	0	0	0	0	0
8	Apion longirostre Olivier	1	0	0	0	0	0	0	0
9	Apion varipes Germ.	1	0	0	0	0	0	0	0
10	Psylliodes chrysocephala L.	1	0	0	0	0	0	0	0
11	Psylliodes chalconerus Koch.	0	0	0	2	0	0	0	0
	Total	27	3	0	10	2	0	8	6

The first harvest, dated 06.05, were collected 27 samples belonging to 7 species. The largest number of samples we recorded the species *Otiorrynchus ovatus* L.(20). Harvesting II, dated 05.06, have been a single species *Cartodere elongata* Thomson with 3 samples. Harvesting III, dated 18.06, did not have a sample of the beetle. Harvesting IV dated 02.06, I caught a total of 10 species of beetles, the species with the highest number of samples (6) is *Cartodere elongata* Thomson. Harvesting V dated 14.07, I caught only two samples of the *Cartodere elongata* Thomson. At the VI harvest, dated 28.07, did not have a sample of the beetle. Harvesting VII dated 18.08, I caught four species of beetles total number of eight samples. Harvesting VIII dated 07.09 has been a total of 6samples belonging to two species of beetles.

Following nine harvesting we collected a total of 108 samples of beetles (Table 4).

Table 4.

Situation on the species and number of samples collected use beating in sweet and sour cherry orchards belonging SC. Loturi Service Delesti Vaslui

No.	Name of species		Num	ber of	stampl	es co	lected ,	/ harvest	ing
INO.		Ι	II	III	IV	V	VI	VII	VIII
1	Catodere elongata Thomson	1	3	0	0	1	0	0	0
2	Stethorus punctillum Weise.	2	0	1	0	0	7	20	25
3	Otiorrhynchus ovatus L.	2	0	0	0	0	0	0	0
4	Apion nigritarse Kirby.	1	0	0	0	0	0	0	0
5	Apion viciae Kirby.	1	0	0	0	0	0	0	0
6	Apion atomarium Kirby.	0	9	0	0	1	0	0	0
7	Ceuthorrhynchus sulcicollis Payk.	0	0	1	0	0	0	0	0
8	Halyzia 14 gutata L.	0	0	1	0	0	0	0	0
9	Coccinella bipunctata L.	0	0	1	0	0	0	0	0
10	Athorus rufus Gyll.	0	0	0	0	2	0	0	0
11	Phyllotreta armoracie Koch.	0	0	0	0	2	0	0	0
12	Longitarsus apicalis Beck.	0	0	0	0	2	0	6	0
13	Longitarsus atricillus Gyll.	0	0	0	0	0	0	6	0
14	Longitarsus jacobeae Waterhouse	0	0	0	0	0	0	0	2
15	Longitarsus anchusae Payk.	0	0	0	0	0	0	0	1
16	Coccinella 7punctata L	0	1	0	0	1	1	3	4
17	Anthonomus varians Payk.	0	0	0	0	0	0	1	0
18	Psylliodes chrysocephala L.	0	0	0	0	0	0	0	4
	Total	7	13	4	0	9	8	36	36

The largest number of samples (36) occurred in the VIII harvesting on 09.09 and harvesting IV on 19.06 I had no sample of the other 6 coleoptera. Harvest IV, the number of individuals collected ranged from 4-31, the total number of species of beetles was 17.

#### CONCLUSIONS

In 2009, in sweet and sour cherry orchards were collected using soil traps type Barber was collected 317 samples of beetles, belonging to 40 species, of which the largest number of samples were: *Carabus violaceus* L., *Harpalus calceatus* Duft., *Amara aenea* Geer, *Calathus fuscipes* Goeze., *Dermestes linarius* Illig., *Cymindis humeralis* Fourc., *Otiorrynchus ovatus* L., *Mordella fasciatta F*.

The largest number of samples (165) was collected using soil traps in sweet cherry and sour cherry groves belonging farm Vasile Adamachi, Iasi

The sweet cherry and sour cherry orchards from SC Loturi Service Deleşti-Vaslui was collected using soil traps a number of 152 samples of beetles belonging to 32 species.

In 2009, the groves of sweet and sour cherry were made bimonthly by beating in the period from May to September.

Following collection of the entomological material used beating method in sweet cherry and sour cherry groves belonging Farm Vasile Adamachi Iasi we totaled number of 56 samples of beetles of which: *Otiorrynchus ovatus* L., *Stethourus punctilum* Weisse., *Cartodere elongata* Thomson, *Longitarsus jacobae*Waterhouse, *Coccinella 7 punctata* L., and plantations belonging to SC Loturi Service Deleşti-Vaslui have made a total of 108 samples of beetles of which: *Stethourus punctilum* Weisse., *Longitarsus apicalis* Beck., *Coccinella 7 punctata* L., *Otiorrynchus ovatus* L., *Apion atomarium Kirby*.

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