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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

MISCELLANEOUS

FRUIT GROWING


EX·TERRA·AVRVM

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STUDIES CONCERNING THE SEROLOGICAL DETECTION ANALYSIS FOR THE PPV (PLUM POX VIRUS) ON SOME PEACH VARIETIES

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Abstract

Plum pox is a serious worldwide problem with a severe impact on the productivity and quality of Prunus fruits (PPV), a virus against which no chemical or biological curative treatment is available. Infections of susceptible plants cause a hormonal disruption in which antagonist hormones are induced simultaneously, while in resistant varieties, the accumulation of antagonist hormones shows a sequential pattern. Identifying key factors underlying the spread of disease is an essential but challenging condition for delivering management strategies. Identification of new genotypes of natural genetic resistance to Sharka; the study of the genetic mechanisms behind the expression of these characters and alternative methods of limiting the propagation of this quarantine virus are objectives of interest in finding long-term solutions to limit propagation of this virus. The Romanian peach varieties 'Alexia', 'Triumf', 'Alex' and 'Flacăra' following the Elisa serological tests proved negative at PPV in conditions of natural infection in the field.

Key words*: peach, varieties, Plum pox virus, Elisa test.*

INTRODUCTION

Plum pox potyvirus (PPV) is a devastating disease of wild and cultivated *Prunus* species. Sharka disease, caused by this *virus* (PPV) is one of the most serious viral diseases of stone-fruit crops, including peach (*Prunus persica* L.), apricot (*P. armeniaca* L.), plums (*P. domestica* L. and *P. salicina* Lindl.) as well as sweet and sour cherries (*P. avium* L. and *P. cerasus* L.) that may be systemically infected by a few unique PPV strains. In areas where plum pox is endemic, such as eastern Europe, the disease can be severe with a possibility of 80-100% yield losses (Kolber et al., 2001). Symptoms vary with virus strain, host species, and cultivar. In susceptible plum cultivars, typical symptoms of leaf chlorosis and necrosis lead to tattered leaves and fruit showing chlorotic rings. Infected apricot cultivars produce misshapen and necrotic fruit. In peach, symptoms are less obvious for many cultivars but chronic infection leads to premature yield reductions and death of trees. Because high concentrations of the virus are not produced and the virus is unevenly distributed in trees, it is difficult to detect and verify in disease surveys. In addition, diseased trees often remain symptom less for several years after infection and function as reservoirs for PPV survival and spread to neighboring trees and orchards.

The virus is transmitted by vegetative propagation and is spread by at least 20 different aphid species in a no persistent manner, allowing epidemics to develop rapidly (Damsteegt, 2001). Among the four identified groups of PPV, the PPV-D and PPV-M strains are the most prevalent and differ in disease severity among *Prunus* species, especially in their ability to infect peach trees via aphid transmission. Fast spreading outbreaks are mostly associated with the PPV-M strain (Zagrai, 2009). The disease manifests as yellow rings and blotches on leaves or fruit. Although not a danger to consumers, PPV can ruin the fruit's marketability by increasing acidity and causing deformities.

In Europe, 100 million trees are infected. PPV eradication efforts hinge on continual surveying of aphid and weed populations. The development and cultivation of new, resistant cultivars could be the definitive solution to this problem (Martinez-Gomez et al., 2000).

In order to plan an efficient breeding program to obtain cultivars resistant to Sharka, it is important to know the genetic control of this resistance.

The objectives for this work are to obtain the serological detection of PPV on local peach varieties ('Flacăra', 'Alexia', 'Alex', 'Triumf'). For study the limitation of PPV infection we are using the Romanian rootstock of interest concerning the resistance to PPV. These genotypes were tested in artificial infected conditions (in greenhouse). After the tests the apricot progenies have been grafted onto a inoculated (PPV-D, provenance of SCDP Voinesti) GF305, susceptible peach. During the vegetation period 2019 was tested by Elisa method.

MATERIALS AND METHODS

Plant material

'Flacăra'. Peach variety 'Flacăra' was created at SCDP Băneasa. The habit of tree is upright to spreading, with a medium anthocyanic pigmentation of flowering shoot, on which floral buds are seated two or more. The type of flower is campanulate with five narrow elliptic petals. The leaf blade is of medium length and width. The length of petiole is medium with reniform shape of nectarines. The size of fruit is large, ovate shaped (in ventral view), the shape of pistil end (without mucrone tip) pointed, the depth of stalk cavity deep and width broad and preeminence of suture medium. The ground color of skin to the fruit is yellow and the hue of over color of skin dark red; the relative area of over color of skin is medium. The color of flesh is orange-yellow with anthocyanin coloration of flesh in central part and around the stone week. The shape of stone is obovate and the intensity of brown color dark. Fruits reach maturity in the first two decades of September, have 240-260 g, the firmness of flesh is medium, without fibers (Figure 1).

Figure 1. 'Flacăra' variety

'Alexia'. Peach variety 'Alexia' was created at SCDP Băneasa. The habit of tree is upright to spreading, with a medium anthocyanic pigmentation of flowering shoot, on which floral buds are seated two or more. The type of flower is rosette with five circular petals. The leaf blade is of medium length and width. The length of petiole is short with reniform shape of nectaries. The size of fruit is medium, circular shape (in ventral view), the shape of pistil end (without mucrone tip) pointed, the depth of stalk cavity deep and width medium and preeminence of suture medium. The ground color of skin to the fruit is cream white and the hue of over color of skin medium red; the relative area of over color of skin is large. The color of flesh is cream without anthocyanin coloration of flesh in central part and around the stone. The shape of stone is obovate and the intensity of brown color medium. Fruits reach maturity in the last decade of June, have 100-110 g, the firmness of flesh is medium, with fibers.

'Alex'. Peach variety 'Alex' was created at SCDP Băneasa. The habit of tree is upright to spreading, with a weak anthocyanic pigmentation of flowering shoot, on which floral buds are seated two or more. The type of flower is rosette with five broad elliptic petals. The leaf blade is of medium length and width. The length of petiole is medium with reniform shape of nectarines. The size of fruit is medium, broad oblate shape (in ventral view), the shape of pistil end (without mucrone tip) weakly pointed, the depth of stalk cavity and width medium and prominence of suture medium. The ground color of skin to the fruit is greenish white and the hue of over color of skin light red; the relative area of over color of skin is medium. The color of flesh is white without anthocyanin coloration of flesh in central part and around the stone. The shape of stone is oblate and the intensity of brown color light. Fruits reach maturity in the first decade of August, have 125-150 g, the firmness of flesh is strong, without fibers.

'Triumf'. Peach variety 'Triumf' was created at SCDP Băneasa. The habit of tree is upright to spreading, with a medium anthocyanic pigmentation of flowering shoot, on which floral buds are seated two or more. The type of flower is campanulate with five narrow elliptic

petals. The leaf blade is of medium length and width. The length of petiole is medium with reniform shape of nectarines. The size of fruit is large, circular shape (in ventral view), the shape of pistil end (without mucrone tip) weakly pointed, the depth of stalk cavity deep and width medium and preeminence of suture medium. The ground color of skin to the fruit is yellow and the hue of over color of skin medium red; the relative area of over color of skin is large. The color of flesh is white without anthocyanin coloration of flesh in central part and around the stone week. The shape of stone is obovate and the intensity of brown color light. Fruits reach maturity in the second decade of July, have 245-260 g, the firmness of flesh is medium, without fibers (Figure 2).

Figure 2. 'Triumf' variety

Methods

A. Evaluation of the characteristic symptom of direct viruses on plants

To evaluate the symptoms characteristic of the studied viruses and establish their incidence, in May-July, the following were determined: frequency, intensity and degree of attack on leaves (number of organs analyzed 200) and on fruit (number of organs analyzed 100).

a) The frequency of the attack (F%) is the relative value of the number (n) of plants or organs of the plant attacked by a phytopathogenic agent relative to the total number (N) of observed plants or organs. The obtained results were calculated using the formula: F% = n/N x 100.

b) Intensity of attack (I%) is the value by which the degree of coverage or spread of the attack is given, reporting the surface attacked against the total observed area. The relative expression of attack intensity was calculated using the formula: I% = ∑(i x f)/b, where:

- i = the attacked area (%);
- f = the number of attacked cases per percent;
- n = total number of cases with attack.

c) Degree of attack (DA%) is the expression of the extent of the attack of the crop on the total number of plants we make the observations. The degree of attack was calculated using the formula: DA% = (F% x I%)/ 100.

Interpretation of plum pox genotype behavior in leaf virus attack was performed according to the scale:

* DA% = 1-5% ↔ very weak attack indicating genotypes with high tolerance;
* DA% = 6-10% ↔ weak attack indicating medium-tolerance genotypes;
* DA% = 11-20% ↔ medium attack indicating slightly susceptible genotypes;
* DA% = 21-30% ↔ strong attack indicating genotypes with moderate susceptibility;
* DA% > 30% ↔ very strong attack indicating genotypes with very high susceptibility.

d) Severity of attack and the virus virulence. Interpretation of the response of plum genotypes to Plum pox infection was determined using the interpretation scale of 1-9, the number of samples analyzed being 100. The plum genotypes were framed in:

* Degree of attack 9 ↔ it does not show symptoms on the fruit showing tolerable genotypes;
* Degree of attack 8 ↔ it show slight discoloration of fruit and sporadic premature fall of fruit indicating slightly susceptible genotypes;
* Degree of attack 5-7 ↔ more than 50% of the samples show symptoms and a premature fall of the fruits indicating genotypes with moderate susceptibility;
* Degree of attack 1-4 ↔ more than 75% of the samples show symptoms and a premature fall of fruits indicating very susceptible genotypes.

The influence of the virus on the production of some plum genotypes was determined; the effects of viral infection were expressed in the affected (t/ha) production.

Changes in the physiological characteristics due to the Plum pox virus were determined in 4 peach genotypes: 'Alexia', 'Triumf', 'Alex' and 'Flacăra'.

B. Detection of viral infection

Detection of viral infection was performed by biological methods and serological methods:

- The biological method was performed by bio-testing on wood indicators, respectively on peach GF305, by chip-budding grafting of a variable number of genotypes. Observations on the material were made at 3 months, 6 months and 1 year.
- The serological method comprised 2 variants:

* DAS-ELISA (Double Antibody Sandwitch Enzyme Linked Immunosorbent Assay) for Plum pox viruses (PPV), Prunus necrotic ring spot (PNRSV), Prune dwarf (PDV) and Apple chlorotic leaf spot (ACLSV) (Clark & Adams, 1977);
* TAS-ELISA (Triple Antibody Sandwitch Enzyme Linked Immunosorbent Assay) for identification of strains PPV-M and PPV-D to the virus Plum pox (Cambra et al., 1994).

RESULTS AND DISCUSSIONS

Understanding virus movement in woody plants is complicated by the fact that fruit tree viruses follow the sieve movement. Evidence of infection is based on typical symptoms. The symptoms of infection by a single virus are relatively easy to recognize (Zagrai I. et al., 2005).

The incidence of symptom development and the use of serological and molecular tests revealed the pathogenic effects of this virus. Local Romanian varieties of peach were tested concerning the resistance to the PPV.

In Table 1 the peach varieties were tested by serological tools the Romanian varieties and rootstocks, and among them, the variety 'Alexia' and 'Alex' are resistant to artificial infection with PPV-D.

To highlight the influence of rootstock resistant to PPV, we are grafted the peach resultants progenies on to the peach GF305 used like susceptible to PPV (Figures 3 and 4). Grafted plants and also peach varieties were monitored by visual inspection and ELISA, completed by RT-PCR for the PPV symptoms (Table 1). Results showed that infection not spread after inoculation on the different resistant rootstocks. Results confirm that, PPV infection was translocated from the inoculums buds to varieties but the virus remained close to the inoculation site for the varieties 'Alexa' and 'Alex', indicating a possible inhibition of virus replication. Some Romanian local varieties could be an important promise to limit the infection of the virus on peach orchards.

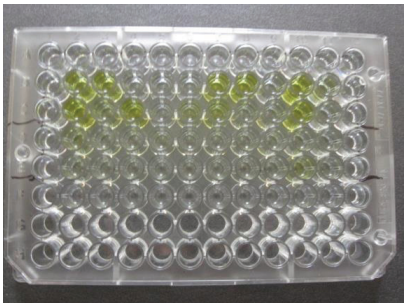

Figure 3. DAS-ELISA for Plum pox viruses (PPV)

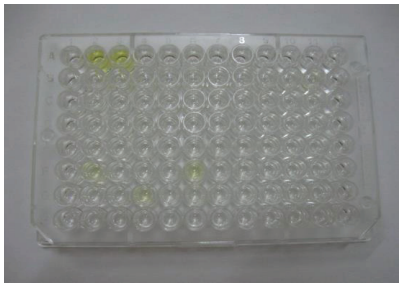

Figure 4. DAS-ELISA for Plum pox viruses (PPV)

Figure 5. Symptoms of Plum pox viruses (PPV)

Figure 6. Symptoms of Plum pox viruses (PPV)

Table 1 Observation of peach response to PPV-D strains infection and PPV detection in different parts of the graft-inoculated four peach genotypes, under artificial conditions

Nr. crt	Genotype	Analyzed part of plant	2011 (1rst scoring)			2011 (2nd scoring)	
			DASI-ELISA (DO = 405nm)	IC-RT-PCR	PPV symptoms intensity	DASI-ELISA	PPV symptoms intensity
1	'Falcăra'	bottom half	++	+	+	++	+
		top half	+	+	+	+	+
2	'Alexia'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
3	'Alex'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
4	'Triumf'	bottom half	+++	+	++	+	+
		top half	+	+	+	+	+
C-	Negative Control	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
C+	'Tuleu dulce' (control)	bottom half	++++	+	+++++	+++++	+++++
		top half	++++	+	+++	++++	+++

CONCLUSIONS

The peach GF305 rootstocks are a good indicator for susceptibility to PPV. The subsequent grafting protocol was optimized, and a Romanian PPV-D isolate was identified and used as inoculum's source. Analyzing the Alexa's and Alex's response to PPV infection remained close to the inoculation site, and revealed an interaction between the host genotype and the virus.

The PPV infection was translocated from the inoculums buds to varieties but the virus remained close to the inoculation site indicating a possible inhibition of virus replication.

Therefore, this genotype can be characterized at most as resistant to PPV.

Considering the results of peach 'Alexa' and 'Alex' were manifested a resistance to the artificial infection with PPV, the detection of the virus was performed by serological and molecular tools.

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TWENTY YEARS OF JUJUBE (*ZIZIPHUS JUJUBA* MILL.) RESEARCH IN ROMANIA

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Abstract

Chinese jujube is a new fruit specie for Europe with a high potential to be planted on arid and semiarid areas on marginal, poor and even salty soils. Being a multimillennial fruit crop in China, jujube has a high importance in the Chinese diet due to its complex nutraceutical properties. Nearly 1000 varieties and local genotypes are cultivated in China on over 2 million hectares on low input production systems. Even if it was introduced in Dobrogea region, some 2,000 years ago by the Greek and Roman colonists, jujube plants and fruits are nearly unknown, as it happens in other countries from the Mediterranean basin. The first cultivated varieties were introduced at the Faculty of Horticulture in Bucharest from Shanxi Province, China within a common research project in 1997. During more than twenty years, a complex research program has been carried out. The first task was to determine the adaptation of the most important jujube genotypes at the local growing conditions. A second topic was to establish the best propagation method by using seedling production for rootstocks, grafting techniques and in vitro propagation. Other researches on jujube phenology, fruit biochemical composition, fruit storage and consumer preferences have been realized. The local jujube genotypes from Ostrov and Jurilovca have been studied and described. The paper presents a synthesis of the most important results achieved during two decades of jujube research and the opportunity to extend the cultivation of jujube in Romania as a new "super fruit".

Key words: *genotypes, seeds, seedlings, in vitro propagation, fruits, storage, sensorial analysis.*

Foreword:

I dedicate this work to the memory of my Chinese friend, Mr. Bi Ping from Shanxi Province that had an outstanding contribution to the first studies of *Ziziphus jujuba* Mill. in Romania.

INTRODUCTION

Gaius Plinius Secundus (AD 23 - 79), better known as Pliny the Elder, mentioned in his Historia Naturalis that a Counsellor of the Roman Emperor Octavian Augustus, introduced the Chinese jujube from Syria to Italy and from there it was distributed to other Mediterranean countries.

Most of ancient Chinese date populations are located in spontaneous status in Dobrogea region, between the Danube and the Black Sea (Ciocârlan, 2000) close to the antique sites (Greek, Roman and Byzantine ruins). It is possible that they have been introduced in those times by the colonists (Stănică, 1997).

Locals named jujube, "Dobrogea olive", for its bushy canopy and small olives live fruits. The most known jujube population can be found in Jurilovca, near Doloşman Cape on the Razelm lake shore, close to the Ancient Greek Colony Argamum. Recently, another jujube local genotype was identified close to Sasovia Fortress at Mahmudia, Tulcea, on Sfântu Gheorghe Danube Arm (Stănică, 2019).

In Ostrov, Constanța county, there exists another genotype with tree habitus and bearing bigger fruits near the Byzantine Fortress Vicina - Păcuiu lui Soare and the Roman City of Durostorum on the right shore of Danube.

Beginning with 1996, following a cooperation project between the University of Agronomic Sciences and Veterinary Medicine of Bucharest and Shanxi Academy of Agricultural and Forestry Sciences and Taigu Fruit Research Institute, few genotypes of jujube have been introduced in Romania, at the Faculty of Horticulture.

The collection was completed in May-June 1998, during the visit of Mr. Bi Ping from Taigu Fruit Research Institute, when few other varieties were grafted on a Romanian rootstock

(Figure 1). The Korean variety Hongan was introduced by grafting from Italy, soon after.
Other new varieties were introduced after 2016, within the scientific cooperation between Hebei Agricultural University from Baoding and USAMV of Bucharest. In January 2018, it was inaugurated the China-Romania Joint Jujube Key Research Laboratory with the same partner.

Figure 1. Mr. Bi Ping, doing Jujube grafting (May 1998)

MATERIALS AND METHODS

After the introduction of the first jujube genotypes from China, researches were conducted at the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Stănică, 2016).
The trees were planted in the jujube collection, at 4.0 m between rows and 2.5 m between plants on the row. The soil was maintained covered with grass by regular mowing. No irrigation, chemical fertilizers or phytosanitary treatments were applied.
Regular studies regarding the behaviour of jujube genotypes at Romanian conditions, by registering the plant phenology, tree growth, characteristics of leaves, flowers, fruits and seeds, resistance to pest and pathogens, etc. were applied. Several studies on generative and vegetative propagation, including *in vitro* micro-propagation of jujube genotypes were also finalized. A study regarding the microbial charge on the fruit in the preharvest phase, prior to picking was done, underlining the high density of different pathogens existing on an untreated orchard (Mardare et al., 2016). In the same time, the presence of several emerging pest was monitored in the jujube orchard (Ciceoi et al., 2017).
Lately, for several genotypes, studies on fruit behaviour in controlled atmosphere storage conditions (temperature: -2-0°C, humidity: over 95%, O_2 between 3% and 5% and CO_2 lower than 2%) were conducted.
Recently, the consumer preferences were analysed by using a test group of 116 persons with an age range from under 14 to over 66 years
Some fruits physical characteristics related to fruit size as weight (g), length and diameter (mm) and fruit shape index were analysed prior to the sensorial test in order to notice any correlation between them and the fruit sensorial appreciation. A constant research work along the years was done in Dobrogea region for the identification and characterization of the local genotypes of jujube (Ciocârlan, 2000; Stănică F., 2009)

RESULTS AND DISCUSSIONS

Phenology studies

The earliest bud breaking was recorded in the beginning of April, having even 7 days difference between genotypes, according to the temperature in the spring. When the bud breaking occurred later, the difference between genotypes was of only 2-3 days.
It was noticed that the flowering starts at the beginning of June, with no major differences between the genotypes and continues till end of July-beginning of August.
Instead, the fruit ripening varied with the genotype, from the third decade of September till the third decade of October. From the genotypes analysed, three had a compact ripening period of only 10 days (selection R2P4, Da Bai Ling and Feng Mi Guan), while selections R1P8 and R1P9 maturated their fruits during 30 days. Selections R2P2, R2P3, R1P8 and R1P9 were early ripening, while Xuan Cheng Jian and Cheng Tuo, were late ripening genotypes (Stănică & Vasile, 2008).

Propagation by seeds

For rootstock propagation, the use of sour jujube (*Ziziphus acido-jujuba* Mill.) seeds is most common practice. One of the most difficult phase of seed propagation was the extraction of the seeds from the hard-woody kernels. The raw kernels have a long duration and a very low germination rate. The mechanical seed extraction is difficult without the use of an appropriate equipment an having a small quantity of kernels. Instead, the kernel endocarp digestion using concentrated acids can be a simple method. Studies regarding the influence of different time treatments from 9 to 24 hours with concentrated H_2SO_4 on seed germination were done on Ostrov and sour jujube genotypes. The optimum time for the concentrated acid treatment was 12 hours, with 77% germination rate for Chinese sour jujube (Stănică et al., 1997).

***In vitro* propagation**

In order to find a rapid clonal propagation method, some studies regarding the *in vitro* multiplication were done. Two culture media: Murashige and Skoog (1962) and Quoirin and Lepoivre (1977) modified by Standardi and Catalano (Standardi & Catalano, 1985) and four hormonal balances with 0, 1.0, 2.0 and 3.0 mg/l BAP were tested. Best results were obtained on MS medium with the macro elements reduced in half (Stănică, 1997). The highest shoots multiplication rate was recorded at the concentration of 1.0 mg/l BAP. The highest shoots rooting percentage was obtained on MS culture media without cytokinin and with low auxins concentration (IBA 0.1 mg/l) (Stănică, 2002).

Propagation by cuttings

Several tests for the jujube clonal propagation by cuttings were made during the vegetation period and during dormancy, using rooting hormones: NAA, IBA, Radistim, Rhizopon, mist atmosphere and basal heating. Until now, no satisfactory results obtained.

Propagation by grafting

Grafting is the most used and the most efficient jujube propagation method that we applied. Since 1998, the new introduced varieties were over grafted on mature rootstocks using the top under bark method. Scions were taken from mother plants during dormancy and preserved at 3-4°C, wrapped in plastic film till the grafting moment (May). The individual one node scion waxing with white paraffin at 80-100°C is another possibility for long term preservation of scions (Stănică, 2002). Top grafting was use also for the propagation of the new varieties on Romanian rootstock as Ostrov and Jurilovca selections. The varieties 'Da Gua Zao', 'Shengli', 'Ji Dan Zao', 'Da Bai Zao', 'Da Bai Ling Zao', 'Da Ma Ya', 'Feng Mi Guan Zao', 'Long Zao', 'Tai Li Hong Zao', 'Li Zao' and 'Ban Zao' were grafted with a success rate between 92.5 and 100%. After grafting, scions grew fast and 7 from 9 varieties produced fruits in the grafting year (Figure 2). Fruit average weight varied between 4.63 and 20.89 g.

Figure 2. Jujube fruit formation in the grafting year

Jujube fruit characteristics

A special attention was applied on fruit analyses, both morphological and biochemical. There were conducted comparative researches between Chinese, Korean genotypes and the local biotypes.

Sour jujube including the Jurilovca population was compared with Chinese sour jujube and

Ostrov biotype (Stănică, 2009). The weight of fruits varied from 0.96 g (Chinese sour jujube) to 1.35 g (Jurilovca 2 population). The Ostrov biotype weight was 6.29 g.
There were not registered significant differences between kernel shape and size at the sour jujube biotypes from China and Jurilovca (Table 1).

Table 1. Characteristics of Romanian jujube local populations fruit and kernel

Selection	Fruit weight (g)	Kernel weight (g)	Kernel length (mm)	Kernel diam. (mm)
Sour jujube	0.96	0.21	8.2	6.5
Jurilovca 1	1.30	0.22	7.8	6.7
Jurilovca 2	1.35	0.25	9.7	6.8
Ostrov	6.29	0.35	13.9	7.6

In the Faculty of Horticulture collection, the genotypes recorded a fruit weight between 5.89 g (R3P10 selection) to 28.57 g (Cheng Tuo Zao). The fruit length varied between 27.0 mm (Feng Mi Guan Zao) to 53.0 mm (R3P4 selection) and the fruit diameters varied between 10/20 mm (R3P10 selection) to 39/40 mm (R3P7 selection).
Chinese date is very well known for the highest content in soluble solids and ascorbic acid.
Most of the analysed genotypes had the soluble solid content higher than 30% Brix. R2P4 selection recorded 39.60 % Brix. Fruit content in minerals varied between 0.16% and 3.38% with an average of 1.78%. The ascorbic acid content varied between 110.0 mg/100 g fw and 1020.0 mg/100 g fw (R1P11 selection) with an average of 306.1 mg/100 g fw. Fruit acidity, expressed in content of malic acid, varied from 0.16% to 0.82% with an average of 0.36%.

Phytosanitary studies on jujube fruits
Several analyses on jujube fruits immediately after harvest and during storage period were conducted in the last years, comparing the differences between genotypes from Bucharest collection. It is important to mention that, no chemical treatments were applied after the establishment of the jujube orchard.
The Chinese jujube is highly tolerant to pests and diseases and only few phytopathological agents have been recorded (Ciceoi et al., 2017).
On the fruit samples, the following pathogens, *Alternaria* spp., *Rhizopus* spp., *Fusarium* spp. and *Monilinia* spp., were identified after harvest. The infection rate ranged from 2% to 30% on *Alternaria* spp., 20% to 75% on *Rhizopus* spp., 0 to 68% on *Fusarium* spp. and 0 to 70% on *Monilinia* spp. (Mardare et al., 2016).
The stored fruits are susceptible also to fungal diseases and quality losses. Several studies showed that in the ripening period several pathogens as *Alternaria* spp., *Stemphyllium* spp., *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp. can be present on the fruits and can affect the fruits quality during storage (Dicianu et al., 2017). Some basic antifungal treatments has to be applied during the preharvest phase in order to ensure a "clean" fruit production at the ripening moment and during postharvest period.

Jujube pests
During the last 20 years, since we first introduced the jujube trees in the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, no pests were observe with the exception of the Mediterranean fruit fly (*Ceratitis capitata* Wied.), identified in 2013 (Chireceanu et al., 2013). Since only few early matured fruit have been affected, no chemical applications were needed. Instead, the year 2016 was totally exceptional from the point of view of climatic conditions and pests evolution, especially for the new invasive species. Four species were damaging the jujube crop, namely *Metcalfa pruinosa* Say, *Ceratitis capitata* Wiedemann, *Nezara viridula* (L.) and *Halyomorpha halys* Stal, while another 2 species producing damages were not yet identified (Ciceoi et al., 2017). Using online databases, we estimate the possible risk raised by other recorded pests of jujube crop in Romania.

Jujube fruit storage
After one month of storage, the jujube fruit weight losses varied between 1.05% at Taigu Ban Zao and Xuan Cheng Jian Zao and 4.65% at Hongan. After 60 days of storage the highest weight losses was registered at Xuan Cheng Jian Zao (5.70), while the lowest at Taigu Ban Zao (0.07%).

The qualitative losses were determined by several fungi infections as: *Alternaria* spp., *Rhizopus* spp., *Fusarium* spp. and *Monilinia* spp. (Figure 3). Even the fruits are carrying the pathogen agents from the field, the infection occurring in the preharvest phase, the incidence of qualitative losses was influenced by genotype. After one month of storage, few genotypes lost most of the fruits: R1P4 (81.64%), R1P7 (83.11%) and Taigu Ban Zao (89.65%). No qualitative losses were registered at: Sour jujube, Xuan Cheng Jian Zao, Shanxi Li Zao and Hongan (Dicianu et al., 2017).

Figure 3. Qualitative losses of Hu Ping Zao fruit after two months of storage, determined by preharvest fungi infections

The highest percentage of healthy fruits left after two months of CA storage were registered at Da Bai Ling Zao (75.00%), Sour jujube (73.45%) and Hongan (63.46%) (Stănică et al., 2018a).

Fruit Sensorial Analysis

Sensorial analysis was made beginning with the first fruits obtained in the Bucharest collection. Several external parameters like fruit size, typical shape, skin colour and skin status were studied. In the same time, some internal parameters like flesh colour, firmness and juiciness, taste and aroma were analysed.

The target public were very diverse, from experts in fruit growing, students to general public in different open day's events.

The results showed no significant correlations between the fruit weight and the consumer appreciation of the fruit size, the correlation coefficient being 0.45. In the same time, there were no significant correlations between the shape index and the consumer appreciation (correlation coefficient - 0.30).

By analysing the rates of different aspects that the panellists had to appreciate, one can see that the fruit size was noted with only an average of 3.22 while the colour with 3.11 out of 5 points possible. We think, both characters were subjective judged in comparison with similar know fruits and without having a clear base reference. Instead, the flavour and the general taste received both higher scores 4.14 and 4.15 respectively, showing that jujube fruits are appreciated by the consumers, even the analysed genotypes are mainly used for dried products and not for fresh consumption (Stănică et al., 2018b).

Identification of local Romanian jujube genotypes

After 1997, several expeditions have been organized in Dobrogea Region in order to identify Romanian jujube genotypes (Stănică, 1997; Ciocârlan, 2000). The Ostrov population located in the Southern part of Dobrogea is represented by highly productive trees (Figure 4) producing small fruits of over 6 g, sweet and sour, resistant to cracking (Figure 5).

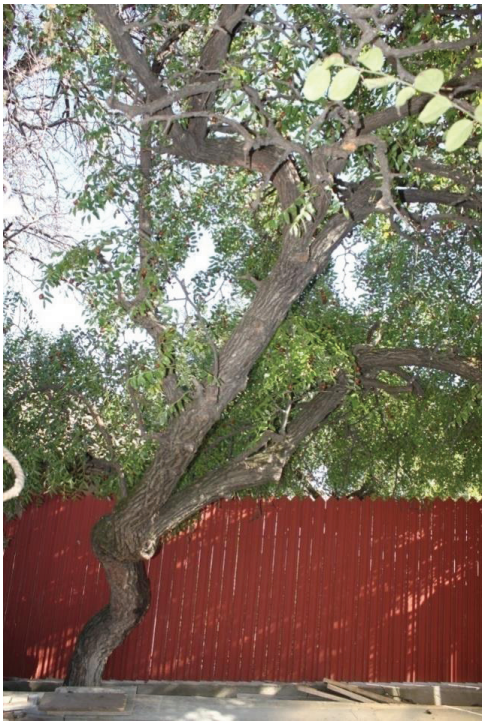

Figure 4. Tree of Ostrov Jujube population

Figure 5. Fruits of Ostrov Jujube population

In Jurilovca area at Doloşman Cape, jujube plants grow as high vigour bushes (Figure 6), while the fruits are similar with the sour jujube ones with 1.3 g, average size.

Figure 6. Jujube high bushes at Doloşman Cape, Jurilovca

Three Jujube genotypes were identified at Jurilovca, maybe being natural hybrids produced by free pollination followed by seed propagation (Figure 7).

Figure 7. Fruits of Jurilovca 1, 2 and 3 Jujube genotypes (from left)

In October 2009, a new local Jujube genotype was identified at Mahmudia, on the Sf. Gheorghe Danube Arm (Figure 8), close to the Sasovia Fortress (Stănică, 2019). The plants are similar with the Jurilovca genotypes, even they are located some 60 km farer. Two fruit types were found and described (Figure 9).

Figure 8. Jujube plants at Mahmudia, close to Sasovia Fortress on Sf. Gheorghe, Danube Arm

Figure 9. Fruits of Mahmudia 1 Jujube genotype

Rumours that some other Jujube genotypes are growing in Dobrogea has to be verified.
In the same time, genetic finger prints of the Romanian Jujube genotypes have to be made in order to see their parentage with some Chinese genotypes, and to establish from which Province of China they are originated.

CONCLUSIONS

Chinese Jujube is a new fruit specie for Romania being studied during over 20 years at the Faculty of Horticulture in Bucharest. The plant can be successfully propagated both by seeds or by vegetative ways. Even the grafting can be applied with good results, for the fast propagation of the new varieties, *in vitro* multiplication is recommended. The planting material starts to produce fruits in the first year after planting due to the Jujube high precocity.

Considering the complex composition of sugars, vitamins, amino acids, etc, Jujube fruits have an important nutraceutical effect, being extremely useful for the human healthy diet. Jujube dried fruits can be used as a source of raw material for other products. Having in mind that, in Romania, Jujube can be cultivated with organic technologies gives a highly value to this crop. The jujube trees are resistant to drought, tolerant to salt and can be cultivated on sandy soils. Over 700,000 ha are affected by these two problems especially in the Southern and North-Western part of Romania. With a high resilience to extreme temperatures: very low and very high, Jujube is the fruit tree recommended to cope with the climate change effects and to valorise the marginal soils. The interesting ornamental look of some varieties, recommends Jujube trees for landscaping too (Stănică, 2000).

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NEW TENDENCIES IN FRUIT TREES TRAINING AND ORCHARD PLANTING SYSTEMS

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Abstract

In the last 40 years there was a continuous struggle to increase the orchard productivity specially by increasing the fruit trees planting density. The availability of low or medium vigour rootstocks in most fruit species created the possibility of raising the planting densities in apple and pear. The general tendency is to create canopies with vertical axes garnished with non-permanent, renewable fruit branches. The number of axes varies from one to three per canopy: more vigorous is the tree, more axes are formed and the total vigour is split on more growing directions. The main concern is to ensure a sufficient productive canopy volume per hectare and a high light interception in order to obtain high quality fruits and high and constant yield. The paper presents some new ideas on rootstocks use and alternative methods to control the tree vigour, new planting systems and discussions on actual canopies as Vertical axe, Bi-Baum®, Parallel V and Trident. New pruning techniques to balance the tree growth and fruiting capacity are explained.

Key words*: rootstocks, canopy, trellis systems, light efficiency, tree growth, productivity.*

INTRODUCTION

In the last 40 years there was a continuous struggle to increase the orchard productivity specially by increasing the fruit trees planting density especially in apple and pear (Wertheim et al., 2001) and recently, in sweet cherry and peach and other stone fruits.

The availability of low or medium vigour rootstocks in most fruit species created the possibility of raising the planting densities in apple and pear, to more than 4,000 trees per hectare and in stone fruits, to over 2,000 trees per hectare. This new approach was possible by the introduction of low vigour rootstocks like M9, P16, M27 for apple (Stehr, 2011), quince for pear (Wertheim, 2002) and Gisela 5 for sweet cherry (Franken-Bembenek, 2005).

The used of feathered planting material, launched by Fleuren nursery in Netherlands at the beginning of ’90, with side fruit branches, took the advantage of early fruiting.

In parallel, a constant reduction of the tree permanent structure and simplification of the canopy occurred with a correspondent reduction of the man work costs for tree training and pruning.

A large number of publications presented results on testing high and very high planting densities, the new proposed planting systems, reaching densities from 2,500 trees/ha to 6,000, or even more than 8,000 trees/ha.

Many of the high-density planting systems were promoted by different nurserymen, being characterized by high initial investment costs, rapid fruit bearing, short orchard life, early replacement (Hoying et al., 2012).

All these means an obvious increase of the planting material quantity per hectare and an early return to the nursery for buying new trees. Not all the farmers were happy with those ideas and there was a counter tendency on finding alternative solutions.

As a reaction to the initial tendency of the increase in tree density to very high numbers, researchers and farmers tried to keep the orchard density below 2,500-3,300 trees/ha in order reduce the orchard investment costs.

New canopies were designed, by increasing the number of tree axes to two in Bibaum® (Musacchi, 2008), Bi-Axis (Dorigoni et al., 2011), Tatura trellis, Parallel Y, etc. or to three axes in Drilling (Widmer & Krebs, 1997; 2001; Stănică & Platon, 2011), Candlestick (Chandelier) (Vercammen, 2011), 3 - Leader system (Elkins & DeJong, 2011), Parallel trident (Stănică & Eremia, 2012; Stănică et al., 2014a; 2014b), or even four axes in Mikado

(Widmer & Krebs, 1997; 2001; Stănică & Platon, 2011).
Besides the reduction of the number of planted trees, the multiple axes canopies, were also suitable for more vigorous species like peach (Caruso et al., 1997), apricot (Stănică & Eremia, 2012), plum (Meland, 2001) and sweet cherry (Stănică et al., 2014) or, for apple cultivar grafted on medium vigour rootstocks.
Nowadays, the general tendency for all fruit species is to create canopies with vertical axes garnished with non-permanent, renewable fruit branches.
Starting from simplified canopies like Vertical axis, Tall spindle (Robinson et al., 2011; Robison & Dominguez, 2015), Super spindle, Solaxe (Lauri & Lespinasse, 2000) etc., the tree management, pruning techniques and the orchard "philosophy" and technology changed radically.
The number of axes varies from one to three per canopy: more vigorous is the tree, more axes are formed and the total vigour is split on more growing directions.
The main concern is to ensure a sufficient productive canopy volume per hectare and a high light interception in order to obtain high quality fruits and high and constant yield.
Recently, at the Faculty of Horticulture in Bucharest, several planting systems and canopies designs are evaluated in different species in order to find efficient solutions for the new fruit orchards.

MATERIALS AND METHODS

The paper presents some new ideas on rootstocks use and alternative methods to control the tree vigour, new planting systems and discussions on actual canopies as Vertical axe, Bibaum®, Parallel V and Trident.
New pruning techniques to balance the tree growth and fruiting capacity are explained.

RESULTS AND DISCUSSIONS

Rootstock use

In the last years the breeding programs in different countries created medium and low vigour rootstock for the most important fruit species. Generally, the low vigour rootstocks transfer to the grafted tree: reduced vegetative growth, precocity, high productivity, high quality fruits (Ghena et al., 2004). Reducing vigour opened the way to increase the planting densities and to imagine simplified canopies and planting systems. But because of reduced root volume, most of the low rootstock need trellis systems.
In apple, M 9 rootstock was generalized in most of the modern orchards. Even M 9 has the above-mentioned advantages, it showed to have also some important problems as: high susceptibility to woolly apple aphid, to fire blight (*Erwinia amylovora*), to *Phytophtora cactorum*, to replant diseases, etc. There are no results yet regarding the resistance to deep frost in the Romanian conditions, especially when the soil is not covered with snow.
Taking in consideration the necessity of new plantings within the Romanian Rural Development Sub Program for the Fruit Industry, the new orchards will be planted in areas with multiple risks regarding the fire blight, drought, replanting diseases, deep winter frost, high summer temperatures etc.
In this situation, a new range of rootstocks has to be tested in order to avoid the mentioned challenges. Promising new apple rootstocks have been recently released by American, Polish and Russian breeding programs.
The Geneva Apple Rootstock Breeding and Development Program started at Cornell University in 1968 by Dr. James Cummins and Herb Aldwinckle. From 1998 to present the program continues in cooperation with USDA having as leaders Dr. Gennaro Fazio, Herb Aldwinckle and Terence Robinson. Several Geneva® released rootstocks appear to have tolerance/resistance to apple replant disease, fire blight and *Phytophtora*: G.41, G.214, G.935, G.202 and G.210. The resistance may be due to the initial screening for *Phytophtora* disease which may also have selected for tolerance to other soil microorganisms (Robinson et al., 2013).
Resistant rootstocks offer better performance in organic orchards too. A few selections of Geneva® rootstocks are available in Europe since 2013 for large scale testing.
In Poland at Skiernievice the apple rootstock breeding program. Lewandowski et al., 2012 tested the susceptibility of selected apple rootstocks to fire blight caused by *Erwinia amylovora*.

From the Russian breeding program initiated by Budagowski, B 9 seems to be extremely promising and interesting for Romanian conditions, for its low vigour, productivity, high resistance to fire blight and frost. For M9 rootstock vigour, the planting distances for apple have been generalized at 3.2-3.5 x 0.7-1.0 m.
For pear, besides the quince rootstocks that have a vigour reducing effect, some pear rootstocks as Farold 40 or 60 are used, giving a much higher tree vigour and three axis canopies as Trident, are recommended (Cean & Stănică, 2013). The *in vitro* propagation of some pear varieties and their cultivation without grafting can be used with good results (Stănică et al., 2002).
For sweet cherry, Gisela 5 is already spread in most of the high-density orchards (Asănică et al., 2013) and recently, while lower vigour rootstock, Gisela 3 started to be tested. However, in more difficult conditions and soils, Gisela 6 gives better results even the tree vigour is superior to the previous ones.
For peach, plum and apricot, besides some specific rootstocks as GF 677, plum and apricot seedlings, there at least three clonal rootstocks that are quite commonly used: Myrobalan 29C (high vigour), Saint Julien A and Ishtara (medium vigour).

Planting systems and canopies
Vertical axe is a popular canopy nowadays for most of the temperate fruit trees species. Being introduced initially at apple, it was then used for pear (Figure 1) and lately it was introduced also to more vigorous species as sweet cherry, peach, apricot and plum. It has few import features that makes it so popular:
- it is close to the natural growing tendency of the fruit trees, besides the axis vertical training;
- it doesn't need complicated and labour-intensive trellising;
- it allows a good illumination of the canopy and a periodical renewal of the fruiting branches.
The main principles for Vertical axe training and pruning are:
- the axe is the leader and it has to be kept vertical and stimulated to grow annually;
- all the lateral fruiting branches are disposed in spiral on the main axe, their vigour decreasing from base to top;
- each fruiting branch has its own growing space and a single growing direction;
- a diameter ratio of 1: 3 has to be maintained between the axe and the lateral branches and between the main and the secondary branches.

a b
Fig. 1. Vertical axe at apple (a) and pear (b)

The Vertical axe can be used equally for low vigour trees at stone fruits. The planting distances are slightly increased taking in consideration the general higher vigour of the tree (Table 1).

Table 1. Planting distances and trees density for the Vertical axe canopy

Species	Planting distances (m)	Planting density (trees/ha)
Apple, pear	3.2-3.5 x 0.6-1.0	2,857 - 5,208
Sweet cherry, peach, plum, apricot	4.0-5.0 x 1.5	1,333 - 1,666

In some special conditions (low fertility soils, slopes,) sweet cherry can be planted at 3.5 x 0.5 m, on very high density (5,714 trees/ha).
It is a typical approach on Trento region in Italy (Figure 2).
Generally, sweet cheery need to be planted for Vertical axe at 4.0-4.5 x 1.5 m (Figure 3).
Vertical axe trees at different stone fruit species are presented in the Figure 4.
For peach, 3-4 short skeleton branches can be formed at the canopy base, while on the axe, only mixed fruiting branches and 2-3 buds short pruned replacing branches are kept.

Fig. 2. Very high-density planting at sweet cherry in Trento region (3.5 x 0.5 m)

Fig. 3. Vertical axe in high density planting at sweet cherry in Însurăței, Brăila (4.5 x 1.5 m)

a b

Fig. 4. Vertical axe at peach (a) and plum (b) in Însurăței, Brăila (4.5 x 1.5 m)

Parallel U and Bi Baum® are two different canopies that in principle aim to create two parallel axes oriented on the row in order to divide/reduce the tree growing vigour. Designed by the Italian nursery Mazzoni, Bi Baum® is created in the nursery by budding two opposite scion buds on the rootstock. By the competition for resources between the "twin shoots", a reduction of the vigour and a balanced growth of the axes is obtained. The technique was firstly proposed for vigorous scion/rootstock combination in apples and pears (ex. Fuji/M9, Florina/M9, other varieties/M26) (Figure 5). Even the tree cost is higher, there are some other advantages regarding the lower number of trees per ha, limited vegetative growth, reduction of the central axe dominance (especially in some pear cultivar) etc.

a b

Fig. 5. Bi Baum® at apple, first leaf (a) and pear, second leaf (b)

Recently, the double budding method was introduced to sweet cherry too in order to produce Bi Baum® trees (Figure 6).

Fig. 6. Bi Baum® sweet cherry trees in high density planting at INRA Balandran, France

Besides the double budding, the trees for Parallel U, can be produced in nursery by early

scion tipping and by selection of two successive shoots. The last solution is to prune the tree after planting at 30-40 cm from the ground and to choose two successive and opposite shoots for the two axes. In all cases, the axes has to be trained and treated in order to have the same vigour (Figure 7).

a b

Fig. 7. Parallel U at peach (a) and apricot (b), second leaf

The training and pruning principles of Parallel U and Bi Baum® canopy, are:
- two axes as leaders;
- balanced vigour of axes;
- each axe has a high conical shape;
- the lateral shoots - to respect the 1: 3 ratio;
- fruiting shoots distributed in spiral along the axes;
- mixed shoots kept 2-3 years in apricot and plum, 1-2 years in peach;
- unnecessary shoots cut in stubs.

The planting distances and the trees densities for the two canopies are presented in Table 2.

Table 2. Planting distances and trees density for the Parallel U and Bi Baum® canopies

Species	Planting distances (m)	Planting density (trees/ha)
Apple, pear	3.5-4.0 x 1.2-1.5	1,666 - 2,380
Sweet cherry, peach, plum, apricot	4.0-5.0 x 2.0-2.5	800 - 1,250

Trident or Chandelier is a canopy that has been introduced recently for medium and high vigour trees in stone fruits, in order to distribute the growing vigour on three vertical axes. In this case, the total height of the tree is reduced and each vertical axe is managed respecting the principles already presented before. The trees can be pre formed in the nursery with three vertical shoots, preferably obtained from successive buds after the main scion shoot tipping. Another possibility is the formation of the tree shoots after planting following the scion pruning (Figure 8).

Fig. 8. Formation of Trident canopy at peach by pruning the scion after planting (beginning of second leaf)

The recommended planting distances, are influenced by the specie and variety/rootstock vigour. As one can see in the Table 3, the distance between row is correlated with the final tree height, while the one between trees on the row, needs to offer enough space for the growth of the vertical axes (Figure 9).

Table 3. Planting distances and trees density for the Trident canopy

Species	Planting distances (m)	Planting density (trees/ha)
Apple, pear	3.5-4.0 x 1.5-2.5	1,000-1,904
Sweet cherry, peach, plum, apricot	4.0-5.0 x 2.5-3.0	666-1,250

Fig. 9. Trident canopy at apricot, planted 2.5 m on the row (beginning of third leaf)

The three axes need to be parallel, balanced regarding the vigour and the general growth and need to be garnished with fruiting branches disposed in spiral in order to form a fruiting high cone/cylinder (Figure 10).

Fig. 10. Balanced Trident canopy at peach (beginning of third leaf)

One of the most common problem that appears is the unbalanced growth of the three axes (Figure 11).
In species/varieties with a dominant growth of the central leader (some pear varieties, sweet cherry etc.), this has to be replaced trough transfer on some low vigour lateral shoot (Figure 12).

Fig. 11. Trident canopy at pear with a too vigorous central axe (acrotony), Ferrara, Italy

Fig. 12. Replacement of the vigorous central axe at sweet cherry with a weak shoot.

The unbalanced growth of the three axes can be caused by the polarity, the upper located axe on the trunk being more vigorous than the other two (Figure 13).
Also in this case, some special measures has to be taken in order to increase the vigour of the less vigorous axe, and to decrease the vigour of the most vigorous one (incisions, increassing of the growth angle, etc).

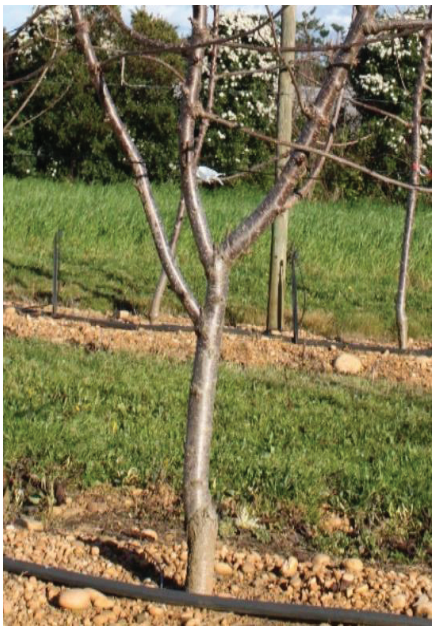


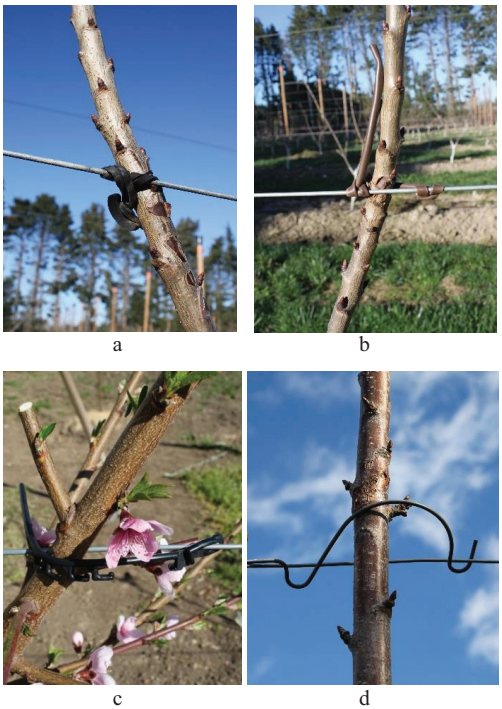
Fig. 13. Unbalanced axes vigour at sweet cherry Trident canopy, related to the polarity

Recently, the philosophy of the vertical axes canopies was transferred with great results to other fruit crops and especially to the fruit berries for fresh consumption (Asănică, 2017; Asănică et al., 2019).

During the formation for all the canopies with one, two or three vertical axes, some important principles has to be respected:

- by all the means, the vertical axe has to be stimulated to grow vertically;
- there are several technical possibilities to tied the axe on the trellising wire (Figure 14);
- in order to stimulate the circulation of the sap flow to the axe top, its annual growth (spear) has to be pruned each spring;
- the axe has to garnished with lateral fruiting shoots. Early spring incisions above vegetative buds or short branches can be executed, especially in sweet cherry (Figure 15);
- a ratio of 3: 1 has to be established between the diameter of the axe and of the lateral branches.

In all cases, after the pruning or the incisions of the axes, fungicides treatments against *Monilinia* has to be applied in order to prevent any wood infections, especially in stone fruits.



a b c d

Fig. 14. Vertical axe fixing on trellis wire with Tree-fix (a) and PVC tube (b), REMA (c) and Steel wire (d)

Fig. 15. Incision on the central axe at sweet cherry and the lateral shoots formation afterword

Pruning

The tree pruning was considerable simplified after the adoption of the vertical axe canopies, the same principles being applied in Vertical axe, Parallel U and Trident. Certainly, some differences are existing between species and sometimes between different fruiting types within the same species (spur or standard).

For **apple and pear** the main pruning principles refers to:
- all the shoots kept in the canopy need to have terminal flower (mixed) buds (Figure 16);
- the vegetative shoots will be eliminated;
- when the number of flowering spurs is too high, spur extinction has to be made, by keeping a distance of 10-12 cm between spurs (Figure 17);
- each lateral fruiting branch needs to have one direction of growth;
- old week branches have to be reduced in order to form new fruiting shoots;
- during the hand fruit thinning (end of May-June), the water shoots (suckers) need to be ripped off.

Fig. 16. Pruning of apple tree – Flowering rods and stubs

Fig. 17. Pruning of apple tree – spur extinction and stubs

In **stone fruits**, it is important to mention that all the branches that are removed from some reason have to be pruned in stubs. Their length is generally correlated to the branch vigour: higher the vigour, longer the stub (Figure 18). The stubs will stimulate the formation of good vigour mixed fruit shoots and will eliminate the risks of *Monilinia* infections.

a b c

Fig. 18. Effect of stub pruning on mixed shoot formation in apricot. Low vigour (a) medium vigour (b), high vigour (c)

An extremely important period for the canopy and fruiting management is summer pruning during May-June. With that occasion, after the fruit set, it is possible to evaluate the fruit load and to eliminate the empty branches. Besides the hand fruit thinning, a similar action can be made by pruning of the too loaded fruiting branches. A special attention has to be apply to the strong water shoots that started to form anticipates. They will be pruned at 4-5 leaves in order to block their initial growth and the stimulate the formation of 2-3 medium vigour mixed shoots (Stănică, 2019).

CONCLUSIONS

The vertical axe canopies, opened a new era in the fruit growing industry that can be defined trough few ideas:
- a moderate planting density between 1,000-3,000 trees/ha was generalized taking in consideration the species and the scion/rootstock vigour. Extremely high densities were banned both by farmers and specialists;
- the vertical axe canopies are in line with the fruit tree natural habit and growth tendency;
- besides the vertical axes trellising, no other expensive man labour activities are needed;
- the used poles for vertical axes trellising are generally utilised as hail net or rain protection supports;
- trees canopy is managed as vertical fruiting walls with all the advantages related to the excellent sun exposer and with positive influence on yield and fruit quality;
- soil mechanized management on the row and on intercrop is easily and efficient;
- for most of the species, the mechanical pruning is applicable;

- hand fruit thinning and picking and other manual activities as pruning can be made on moving platforms.

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FRUIT SENSORIAL EVALUATION OF SOME KIWI HYBRID CULTIVARS (*ACTINIDIA* SP.)

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Abstract

The aim of this paper is to present preliminary data regarding the sensorial analysis and customers' perception on some hybrid kiwifruit, in different moments after harvesting. The plants were grown in the Experimental Field of Faculty of Horticulture, at the USAMV of Bucharest. The fruits were harvested starting in the beginning of October and finished at beginning of November. They were stored in two different conditions. At the time of consumption maturity, fruit analyses regarding flesh firmness, soluble solids (SSC) and vitamin C content, were performed for each genotype. Sensorial analyses were realized using a panel of consumers selected by age, gender and origin. The fruit size and shape, fruit pulp colour, taste and flavour were evaluated by awarding grades from 1 to 5. The results showed that the customer's preferences are influenced by gender, age and origin. The new kiwi hybrid selections were highly appreciated, most of the kiwifruit interspecific hybrid genotypes Actinidia deliciosa x Actinidia chinensis receiving higher scores than the single species varieties.

Key words*: consumer preference, fruit characteristics, storage, questionnaire.*

INTRODUCTION

Actinidia (kiwifruit) is characterized by high content of biologically active compounds, including ascorbic acid (Plekhanova et al., 1940; Namestnikov et al., 1989).

According to Drummond (2013) kiwifruit is recognized as highly nutritious and low in calories with a potential to deliver a range of health benefits.

Over the past 20 years, there has been considerable interest in identifying new product opportunities for kiwifruit (Harker et al., 2007).

Harker (2007) mentions in a review study that consumer research was based on the identification of attributes that promote or inhibit kiwifruit consumption. Also Harker (2007) suggests that for kiwifruit sells increase it is necessary to take into account consumers' beliefs, attitudes and perceptions regarding this fruit and the impact of specific traits such as flavor, appearance and novelty on preferences and willingness.

Cangi (2011) points out that the maturity level, colors, sugar, solids, size and mechanical defects, firmness etc. are some of the important factors for kiwifruit marketing.

There are many studies focused on different varieties of *Actinidia* sp. concerning their health benefits, storage life and possibilities of maintaining and controlling postharvest kiwifruit ripening (Burdon et al., 2004; Stonehouse et al., 2013).

It was observed also that the postharvest preservation technologies are applied to reduce damage, prolong shelf life, and keep the nutritional quality of several fruits and vegetables (Liato et al., 2017).

In Romania the first kiwi orchards were planted in 1993 (Zuccherelli, 1994; Stănică & Cepoiu, 1996).

Harker (2007) suggests that in order to establish new flavor profiles for a wide range of kiwifruit genotypes and to map consumer preferences, different sensory panels can be used.

The studies illustrate that fruit breeding should target not only the elite fruit that are significantly more liked than the existing cultivars, but also special, unique fruit that

create major new flavor niches (Harker et al., 2017).

Based on these considerations, the aim of this study is to evaluate the customer's preferences regarding the sensorial qualities of some Romanian kiwifruit hybrids, in different moments after harvesting.

According to Harker (2007) barriers to kiwifruit consumption often relate to the difficulty to determine when the fruit is ready to eat, and the need to use utensils to cut and scoop out the flesh to be able to eat it. Novelty can be either an advantage or a disadvantage depending on the openness of the consumers toward new foods (i.e., whether are conservatory or seek variety in their diet).

MATERIALS AND METHODS

Fruits sampling and preparation

In the Experimental Field at the Faculty of Horticulture - 26°6'0" East longitude and 44°25'60" North latitude, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest an experimental field with kiwifruit hybrid genotypes, besides other varieties of *Actinidia deliciosa*, *A. chinensis* and *A. arguta*, was established.

For the study eight kiwifruit genotypes were used: R0P9, R0P10, R0P12, R1P9, R2P3, R2P6, R6P2 and R6P4. The plants were grown on a T - bar trellis system, a micro spray irrigation system was used and an organic orchard management was applied.

The fruits were harvested starting in the beginning of October for *A. chinensis* selections (R0P9, R0P10), continued in 24th of October 2017 for some interspecific hybrids (R6P2, R2P6) and finished at beginning of November for other hybrids of *A. deliciosa* (R0P12, R1P9, R2P3, R6P4).

Harvesting moment was considered when the fruit flesh firmness was below 7 kgf/cm².

After picking, the fruits were stored in two different conditions: in cold storage at 3ºC and 95% humidity and in controlled atmosphere with 1.5% oxygen, 1-2°C and 95% relative humidity.

At the beginning of consumption maturity, fruit analyses regarding flesh firmness, soluble solids (SSC) and ascorbic acid content, were analysed for each hybrid.

Sensorial analyses were realized using different respondent panels selected by age, gender and origin. Fruit size and shape, fruit pulp color, taste and flavor were noticed with grades from 1 to 5.

All the determinations and analyses were made in laboratories of Research Center for Studies of Food Quality and Agricultural Products.

Physico-chemical analysis

Fruit flesh firmness was determined by measuring the penetration force using an electronic penetrometer, equipped with a piston of 8 mm diameter. The results were expressed in kgf/cm² (Chen, 2015).

Soluble solids were determined from kiwifruit juice (Yoon, 2005; Saei, 2011; Mureşan, 2014; Oltenacu, 2015), with Krüss Refractometer DR301-95, the results being expressed in % Brix.

Ascorbic acid content of kiwifruit samples was determined with HPLC - Agilent Technologies 1200 Series equipment. A ZORBAX Eclipse XDB-C18 (4.6 x 50 mm, 1.8 µm) column with Rapid Resolution HT and a detector UV-DAD detection wavelength 220/30 nm, using reference as wavelength 400/100 nm. Mobile phases were A = 99% (ultrapure water with H_2SO_4 up to 2.1 pH) and B = 1% (acetonitrile with 10% A).

For each genotype, an average sample of 10 fruits was used and mixed into a Grindomix robot for a period of 10 seconds at a speed of 0.55 rpm 1 g of fruit pulp was extracted in centrifuge tubes with 10 ml of water acidified with sulphuric acid to a pH of 2.1.

Then the tube was incubated for 45 minutes at 4°C under dark conditions. After this operation, the tubes were centrifuged for 1 minute at 1000 rpm to sediment the coarse part of the preparation. The samples were filtered through a filter Agilent RC 0.2 µm.

The injection volume was 2 µl, with 4 minutes post time, flow rate at 0.5 ml/minute at 30^{o}C in column compartment.

The samples were analysed in duplicate and were expressed in mg/100 g.

In order to perform the quantitative analysis of samples a calibration curve was obtained through injection of known concentration of standards (from 12.5 to 1000 µg/ml).

Sensorial analysis

Sensorial assessment was carried out in a sensorial testing laboratory by consumer panellists of different age, gender and origin.

Fruit quality was evaluated by appearance and taste (fruit size and shape, fruit pulp colour, taste and flavor) and for the results it was used a 1-5 rating scale.

The tasting panel was formed by 168 respondents with ages from 14 to over 60 years old, both males and females, from rural and urban area.

In order to perform a representative study, eight of the questionnaires were rejected because the respondents skipped one or more questions.

Statistical evaluation of the experimental data was performed by simple comparisons of mean values and standard deviation, calculated using incorporated function of Microsoft Excel.

RESULTS AND DISCUSSIONS

The fruits were harvested when the fruit flesh firmness was less than 7 kgf/cm² (Figure 1). The initial physical and biochemical analysis, after harvesting time (average fruit weight, fruit flesh firmness, soluble solids content) are presented in Table 1. The size of green kiwifruits ranged from small (44.83 g at R6P4) to large (102.18 g at R1P9), while the yellowish fruit of the interspecific hybrid (R2P6 and R6P2) was rather small in size with only 11.22 g or 12.33 g (Table 1). For *A. chinensis* genotypes were registered 31.82 g for R0P9 and 37.03 g for R0P10.

The kiwifruits, after picking, during the post-harvest storage, continued the physiological development until they become suitable for consumption. At the beginning of consumption maturity, fruit analyses regarding soluble solids content (SSC), firmness and ascorbic acid were performed for each genotype (Table 2).

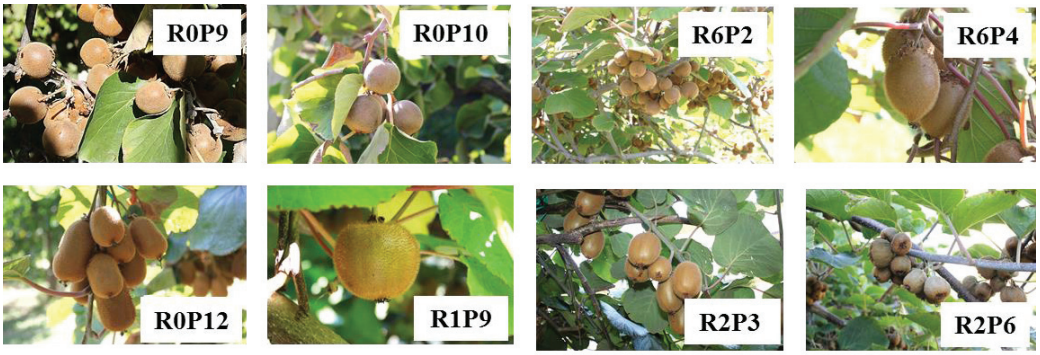

Figure 1. Studied kiwifruits hybrids photographed before harvesting time

Table 1. Physical and biochemical characteristics of kiwifruit genotypes before storage

Genotype	Time of harvesting	Average fruit weight (g)	Firmness (kg/cm^2)	Soluble solids content (% Brix)
R0P9	04.10.2017	31.82	6.05	10.16
R0P10	04.10.2017	37.03	6.45	10.16
R6P2	24.10.2017	12.33	6.08	11.34
R6P4	24.10.2017	44.83	7.10	12.65
R0P12	06.11.2017	46.21	2.28	11.33
R1P9	06.11.2017	102.18	1.32	13.54
R2P3	06.11.2017	88.66	2.27	10.96
R2P6	06.11.2017	11.22	0.99	15.34

Table 2. Physical and biochemical characteristics of kiwifruit genotypes at the beginning of ripening

Genotype	Time of maturity consumption	Firmness (kg/cm^2)	Soluble solids content (% Brix)	Ascorbic acid content (mg/100 g)
R0P9	30.01.2018	0.12	15.5	498.05 ±18.50
R0P10	15.01.2018	0.20	10.9	536.30 ± 4.42
R6P2	15.01.2018	0.45	14.2	100.23 ± 0.10
R6P4	14.11.2017	0.58	14.8	105.83 ± 1.78
R0P12	28.03.2018	0.54	15.7	45.04 ± 0.13
R1P9	28.03.2018	0.56	14.7	77.20 ± 0.85
R2P3	14.11.2017	0.59	12.5	56.07 ± 0.43
R2P6	14.11.2017	0.67	16.8	70.27 ± 3.72

For all genotypes throughout storage, a noticeable increase in soluble solids content can be observed (Figure 2). The fruits flesh firmness reduced constantly after harvesting (Figure 3).

The degree of flesh softening influences the life storage of kiwifruit. Krupa (2011) mentioned that kiwifruits were ready to eat when the flesh firmness reached less than 1.00 kg/cm^2.

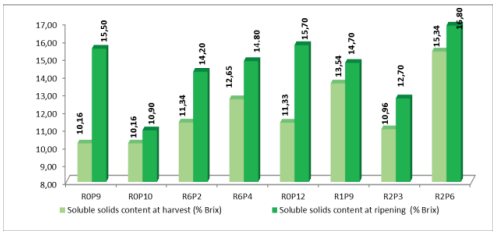

Figure 2. Evolution of soluble solids content (% Brix) for studied kiwifruit genotypes

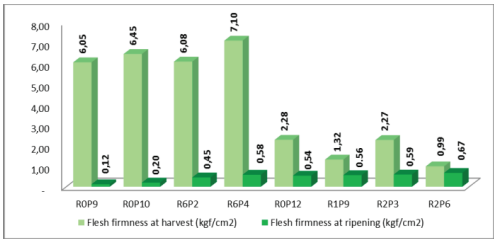

Figure 3. Evolution of fruit flesh firmness (kgf/cm^2) for studied kiwifruit genotypes

The fruit ascorbic acid content varies between the studied genotypes of *A. chinensis* from 498.05±18.50 mg/100 g (R0P9) to 536.30 ± 4.42 mg/100 g (R0P10) (Figure 4).

The measured values were higher than those reported in previous studies (Huang et. al., 1983; Kolbasina, 1986; Ferguson et al., 1988; Ferguson et al., 1992; Nishiyama, 2007).

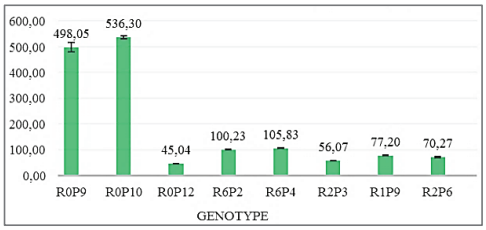

Figure 4. Ascorbic acid content (mg/100 g) at the beginning of ripening stage for studied genotypes

The ascorbic acid content of the interspecific hybrids genotypes R6P2 (100.23 ± 0.104 mg/100 g) and R6P4 (105.83 ± 1.783 mg/100 g) is also considered high.

For the hybrids genotypes of *A. deliciosa* the determined ascorbic acid content varied from 45.04 ± 0.13 mg/100 g (R0P12) to 77.20 ± 0.85 mg/100 g (R1P9).

Between the obtained values and the values reported in previous studies there were small differences (Huang et al., 1983; Visser et al., 1983; Nishiyama, 2004; Nishiyama et al., 2004; Nishiyama, 2007).

Kiwifruits quality was evaluated for appearance and taste by different consumers in different ripening stages.

For a better understanding of respondents' opinion and preferences on the Table 3 is presented their socio-demographic profile. The interviewed respondents were from 14 to more than 60 years old. 47% of them were women and 53% men. In 69.3% of the cases people were from the urban area and only 30.7% of them from the rural area.

Table 3. The socio-demographic profile of respondents

Criteria	Range	%
Age group (years)	<18	5.8
	18-24	37.4
	25-34	26.3
	35-44	14.2
	45-54	11.6
	55-64	4.7
Gender	female	47.0
	male	53.0
Origin area	urban	69.3
	rural	30.7

Sensorial analyses were organized for certain genotypes, monthly and the results are presented in the following figures (5-9) for each criterion: fruit size and shape, fruit flesh colour, taste and flavor.

In November, three fruit tastings were organized for R6P4, R2P3 and R2P6 genotypes. In January two fruit tastings were organized: on 15th for R0P10, R6P2, R2P3 and R2P6 genotypes and on 30th for R0P9, R0P10 and R2P3 genotypes.

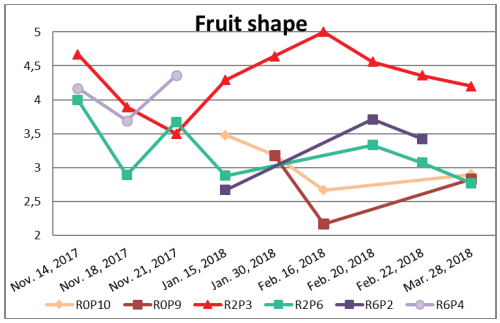

Figure 5. Evolution of customers' perception on fruit shape

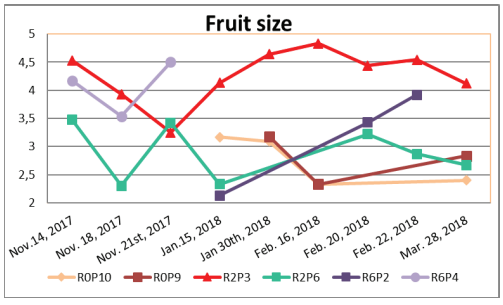

Figure 6. Evolution of customers' perception on fruit size

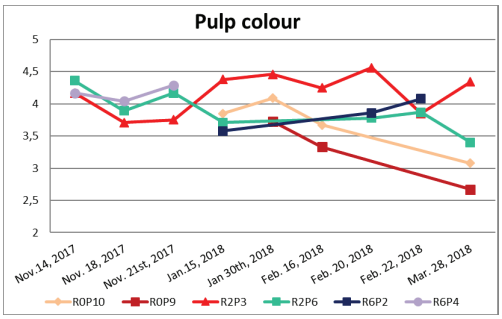

Figure 7. Evolution of customers' perception on fruit flesh colour

In February tree sensorial tastings were organized: on 16th for R0P9, R0P10 and R2P3 genotypes; on 20th and 22nd for R6P2, R2P3, R2P6 genotypes.

In March 28th the sensorial tasting was organized for R0P9, R0P10, R0P12, R1P9, R2P3 and R2P6 genotypes.

According to the fruit ripening phase, consumers have appreciated the different genotypes and the total score awarded for each is presented in Table 4.

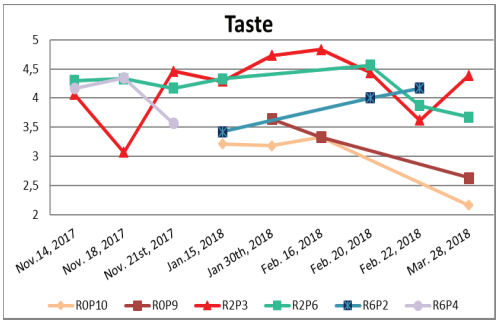

Figure 8. Evolution of customers' perception on fruit taste

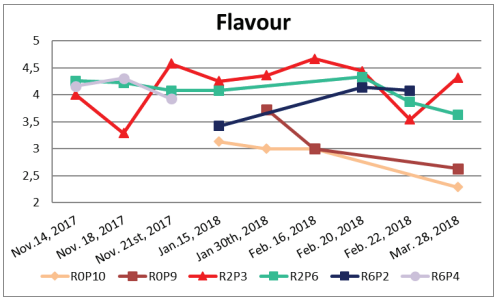

Figure 9. Evolution of customers' perception on fruit flavor

Table 4. Customers' perception on some kiwifruit hybrid genotypes in different ripening stages

Genotype	Date of sensorial analysis	Total score
R0P9	January 30th	**17.45**
	February 16th	14.17
	March 28th	13.63
R0P10	January 15th	**16.83**
	January 30th	16.55
	February 16th	15.00
	March 28th	12.82
R6P2	January 15th	15.21
	February 20th	19.14
	February 22nd	**19.67**
R6P4	November 14th	**20.97**
	November 18th	19.92
	November 21st	19.50
R0P12	March 28th	**18.21**
R1P9	March 28th	**19.34**
R2P3	November 14th	21.42
	November 18th	17.89
	November 21st	20.64
	January 15th	21.33
	January 30th	22.82
	February 16th	**23.58**
	February 20th	22.44
	February 22nd	19.92
	March 28th	21.37
R2P6	November 14th	**20.41**
	November 18th	17.63
	November 21st	19.54
	January 15th	17.33
	February 20th	19.22
	February 22nd	17.54
	March 28th	16.13

Genotypes rated with the highest score were R2P3 (23.583) - in January 30th, followed by R6P4 (20.965) and R2P6 (20.407) – in November 14th.

According to Table 2, R2P3, R6P4 and R2P6 obtained also good values regarding soluble solids content, at the beginning of ripening. That for the results of the analyses confirm the customers' preferences.

Genotype rated with the highest score for all evaluation criteria (fruit shape and size, pulp colour, taste, flavor) was R2P3. The total mean value was 21.62 (Table 5).

In Table 5 are presented the average evaluation values for each criterion and for each genotype.

Table 5. The average value of the evaluation for each criterion for each genotype

Genotype	Fruit shape 1-5	Fruit size 1-5	Pulp color 1-5	Taste 1-5	Flavor 1-5	Total 5-25
R0P9	2.73	2.78	3.24	3.20	3.13	15.08
R0P10	3.06	2.75	3.63	2.97	2.85	15.30
R6P2	3.26	3.16	3.84	3.86	3.88	18.01
R6P4	3.90	3.71	4.16	4.19	4.17	20.13
R0P12	3.37	3.21	4.03	3.79	3.82	18.21
R1P9	4.29	4.24	3.93	3.56	3.32	19.34
R2P3	**4.49**	**4.45**	**4.28**	**4.22**	**4.18**	**21.62**
R2P6	3.20	2.87	3.82	4.22	4.14	18.26

For R0P9 genotype total mean value was 15.084. The most appreciated criterion was the colour of the fruit pulp (Figure 10 - A).

The total mean value for R0P10 genotype was 15.30. The most appreciated criterion was the colour of the pulp (Figure 10 - B).

For R6P2 genotype total mean value was 18.01.

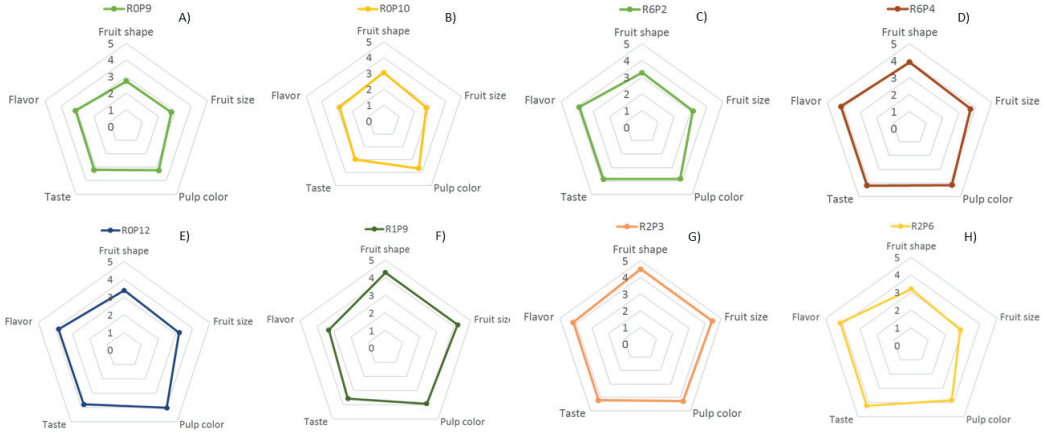

Figure 10. Kiwi fruits sensorial analysis - Distribution of characters for studied hybrids

The most appreciated criterion was the flavour (Figure 10 - C). For R6P4 genotype total mean value was 20.13.
The most appreciated criterion was the taste (Figure 10 - D). The content of SS, at the beginning of ripening (14.8 % Brix - Table 2) confirm the appreciation of customers for taste.
The total mean value for R0P12 genotype was 18.21. The most appreciated criterion was the colour of the pulp (Figure 10 - E).
For R1P9 genotype total mean value was 19.342. The most appreciated criterion was the fruit shape (Figure 10 - F).
For R2P3 genotype the total mean value was 21.62. The most appreciated criterion was the fruit shape (Figure 10 - G).
For R2P6 genotype total mean value was 18.26. The most appreciated criterion was the taste (Figure 10 - H). Also for this hybrid, the SS at the beginning of ripening was very high – 16.8 % Brix.

CONCLUSIONS

Genotype rated with the highest score for all evaluation criterias was R2P3 with a total mean value of 21.62.
The genotypes that were most appreciated for shape of the fruit were R1P9, with the total mean value of 19.34 and R2P3, with the total mean value of 21.62.
Average fruit weight (g) for R1P9 measures 102.18 g and the fruits of this hybrid have a nice spherical shape. Also for R2P3 average fruit weight (g) recorded a high value – 88.66 grams.
The genotype that were most appreciated for color of the pulp were – R0P9 (15.08 - total mean value), R0P10 (15.30) and R0P12 (18.210).
For taste, the genotype that were most appreciated were R6P4 (20.13) and R2P6 (18.26).
For flavor, the genotype that was most appreciated was R6P2 with the total mean value 18.01.
The highest amount of ascorbic acid content was determined for R0P10 - 536.30 ± 4.42 mg/100 g and for R0P9 - 498.05 ±18.50 mg/100 g (*A. chinensis* genotypes).
In the case of soluble solids content it was observed that R2P6, R0P9 and R0P12 have the higher content, 16.8, 15.5 and 15.7% Brix, respectively.
Following the obtained results, we can specify that kiwifruit hybrid genotypes received good appreciation from consumers and best of the tested hybrid genotypes (R2P3, R6P4 and R1P9) will be proposed to be propagated and introduced in the production test phase.

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INCIDENCE OF BACTERIAL DISEASE ON SOME APRICOT VARIETIES CULTIVATED IN BUCHAREST AREA

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Abstract

In the present study, the behaviour of some old and new apricot varieties, grown in an integrated orchard in Bucharest Area, is presented. The apricot orchard was planted in 2008 and in 2017using Romanian and foreign varieties grafted on Mirobolan, Myrobalan 29C, Saint Julien A and GF 677. The planting distances varied from 4.5 x 3.0 m to 3.5 x 2.0 m and several canopies were formed: Drapeau Marchand, Simple Palmette, Mikado, Parallel U and Trident. High concrete poles with 4 lines of wires, was made as trellis system for the trees planted in 2017. The land was maintained covered with a mixture of perennial grasses on the interrow and clean with herbicide on the row. Drip irrigation was applied for the 2008 orchard and micro sprinklers were used for the new orchard. Leaves were collected at the end of the growing season (late October) and the incidence of the bacterial disease was measured using a scanner and the WinFolia Software. In the same time the leaf are was measured. The results showed that the intensity of the bacterial attack was influenced by the variety, and surprisingly by the canopy.

Key words*: Armeniaca vulgaris, rootstocks, canopies, leaf area, frequency, attack intensity.*

INTRODUCTION

Bacterial spot caused by *Xanthomonas arboricola* pv. *Pruni* was described for the first time in the USA (Michigan) in 1903 on Japanese plum and is a serious disease that can affect apricot fruit quality and production worldwide.

In Romania is widespread in all fruit-growing areas on all stone-fruits. Due to the attack, significant damage was recorded in plum, peach or apricots through strong defoliation and fruit fall in varieties susceptible to the attack of this bacterium.

The disease is manifested on leaves, fruits, young shoots, branches and stems.

At leaves, appear from the first month, small circular or angular spots with a clear, translucent, green appearance, slightly darker than healthy tissue. At first, the spots appear around the stomata or are restricted to the tissues between the ribs. As a feature is that they are surrounded by a glassy and transparent area. They are often covered with an abundant, yellowish exudate.

In time, the tissue next to the spots will become necrotic, it will dry out, come off and fall and leaves remain drilled.

To a strong atack, the spots become joined together, are expanding especially at the edge of the leaves that will appear sharply. When spots appear on the petiole of the leaves, these will fall more and more.

Initially, on the fruit appear small brown spots, and then become blackish, circular with an aqueous appearance, spots that grow with the growth of the fruit.

These are found in the pulp of the fruit and have on their surface a yellow exudate, very rich in bacteria.

There are cases when on unripe fruits, you can fiind a dark green ring around the spots.

Instead of these spots, the fruit tissues react by glue secretion and by the appearance of a suber separator layer, that will separate the sick tissue from healthy tisuue. In time, the spots will become necrotic, will cracks.

The simptoms produced from the bacteria can be confunsed with the ones of the *Coryneum beijerin-ckii* Qud.

The difference is that the spots frum the leaves, produced by the fungus, are bigger, up to 3 mm in diameter, surrounded by a reddish-brown edge and the surface exudation is missing.

The spots from the fruits are more prominent and they are not covered by this exudate.

Leaves appear similar to those on the leaves and fruits with small differences. In front of these spots, the tissue will become necrotic, will crack and abundant glue leaks will occur, especially at the apricot and peach cultivars.
When the attack become powerful, the spots become expand, including the shell on certain portions.
This causes an interruption of the sew followed by bending and drying the top of the shell.
We can find the attack even on the branches and strain, looking like an open cancer, that becomes deeper in years. When it expands, the trees will dry.

MATERIALS AND METHODS

The apricots leaves were collected from the Experimental orchard of Faculty of Horticulture of USAMV Bucharest. The apricots varieties used were: 'Congat', 'Primando', 'Primaya', 'Rubista', 'Portici','Pisana', 'CMBU', 'Bergeron', 'Vitilo', 'Buccucco Liscia', 'Wonder Cot', 'Lady Cot', 'Delice', 'Lili Cot', 'Milord', 'Swired', 'Congat', 'Mikado', 'Lido', 'Med Flo', 'Flopria', 'Faralia', 'Farely', 'Fartoli', 'Farbali', 'Farbela', 'Anegat', 'Farlis', 'Farclo', 'San Castrese', 'Danubiu', 'Goldrich', 'Auras', 'Harcot', 'Dacia', 'Sweet Cot', 'Augustin', 'Fortuna', 'Cristal', 'Amiral', 'Olimp'.
In October, 2018 it was harvested 10 leaves of each tree, which were kept in the refrigerator at a temperature of 2°C and 85% humidity.
The observation was made for each tree and the leaves were analysed with *WinPholia* program, with whom the harvested leaves were scanned, photographed and then, the percentage of attack of bacteriosis was calculated. The leaves were scanned both individually and in groups (2-3 or even 10). For calculate the attack rate of the disease, the disease-specific colors for each leaf were selected as well as for the healthy foliar surface, in the end, the program calculate the damage degree. This indicator is represented by histogram type charts.
Statistical analyses were made using Anova test.

RESULTS AND DISCUSSIONS

The results showed that the 'CMBU' variety had the highest percentage of healthy foliar surface (Figure 4), the affected part being only 8.11%. At the opposite side, the variety 'Rubista' (Figure 2), with the most attacked surface, had a double percentage 19.37%, followed by the 'Primando' variety (Figure 3) with 18.36%.
The others varieties recorded values between 10.34% and 14.24% (Figure 1).

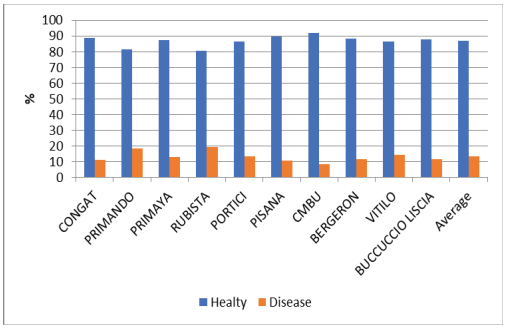

Figure 1. Comparison of bacterial disease attack on apricot varieties leaves

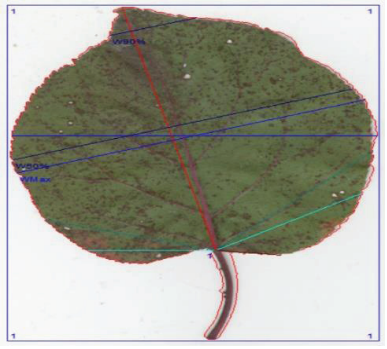

Figure 2. Symptoms of bacterial spot disease on a detached apricot leaf of 'Rubista' variety

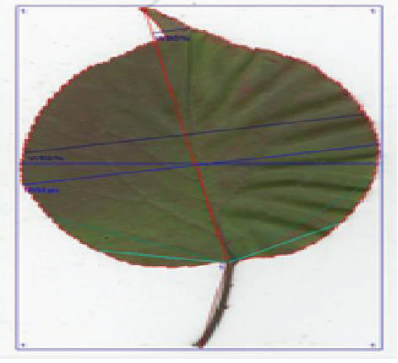

Figure 3. Symptoms of bacterial spot disease on a detached apricot leaf of 'Primando' variety

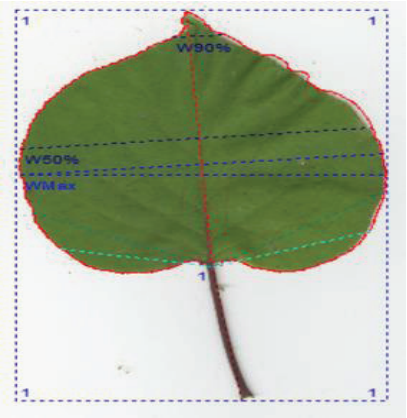

Figure 4. Symptoms of bacterial spot disease on a detached apricot leaf of 'CMBU' variety

The planting distance for 26 rows was 3.5 m x 2.0 m, the canopy was Bi-Baum, and the applied irrigation method being micro sprinklers.

The varieties on this row was: 'Wonder Cot', 'Lady Cot', 'Delice', 'Lili Cot', 'Milord', 'Swired', 'Congat', 'Mikado', 'Lido', 'Med Flo', 'Flopria'.

Under the condition of 2018, the 'Milord' variety (Figure 7) had the least attacked foliar surface with only 7.07%. On this row, the most attacked foliar surface was the variety 'Wonder Cot' (Figure 6) with 21.91% followed by 'Mikado' (Figure 8) with 21.06%. The percentages of attack of the other varieties reneged between 9.24% at 'Congat' and 19.50% at 'Med Flo' (Figure 5).

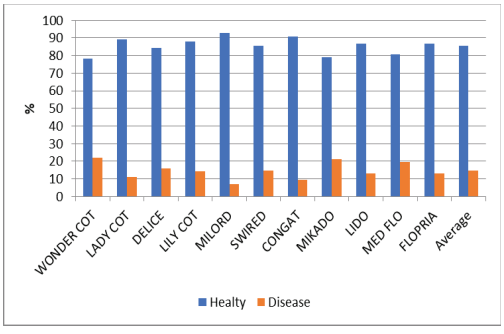

Figure 5. Comparison of bacterial disease attack on different apricot varieties (2018)

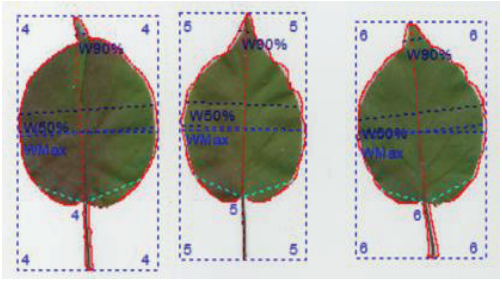

Figure 6. Symptoms of bacterial spot disease on apricot leaves of 'Wonder Cot'

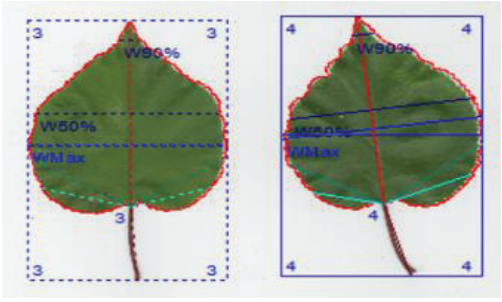

Figure 7 Symptoms of bacterial spot disease on detached apricot leaves of 'Mylord' variety

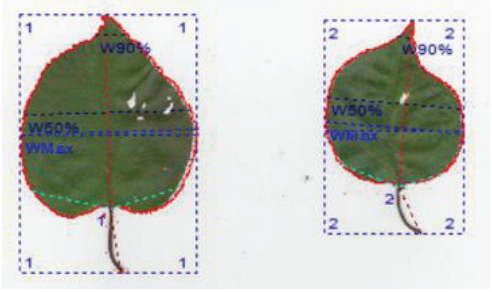

Figure 8. Symptoms of bacterial spot disease on detached apricot leaf of 'Mikado' variety

The row 27 as well as 26, the planting distance and irrigation form, are the same, the difference is that the canopy differs, on this we have Trident. As well as varieties on this row we have: 'Faralia', 'Farely', 'Fartoli', 'Farbali', 'Farbela', 'Anegat', 'Farlis', 'Farclo', 'Primaya'.

On this row, the 'Farbali' variety had the lowest percentage of the attack leaf, both in range and compared to the other varieties analysed, with only 4.98%. The highest values were recorded by the variety 'Farclo' grafted on Mirobolan 29C.

The values recorded by the remaining varieties ranged between 6.93% by the 'Fartoly' variety grafted on Saint Julien A and 13.93% by the 'Farclo' variety grafted by Saint Julien A. We have noticed that the differences between grafted varieties in different rootstocks, in this case, are not quite large (Figure 9).

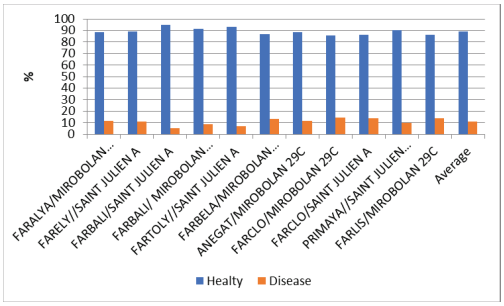

Figure 9. Comparison of bacterial disease attack on different apricot varieties grafted on Saint Julien A or Mirobolan 29C (2018).

Twelve old varieties have also been analysed and the results showed that the 'San Castrese' and 'Auras' variety, had the smallest values being amongst the most resistant to bacteriosis attack (Figure 10).

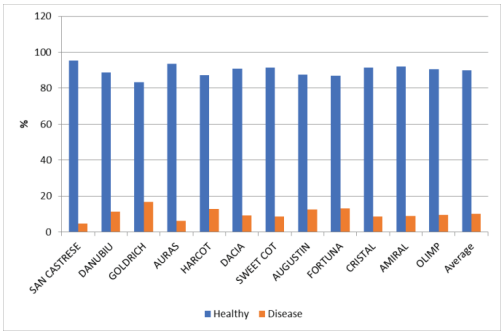

Figure 10. Comparison of bacterial disease attack on dome Romanian and foreign varieties of apricots (2019)

CONCLUSIONS

The most tolerant varieties on *Xanthomonas arboricola pv pruni* are 'San Castrese' and 'Farbali' grafted on Saint Julien A rootstock. 'Rubista', 'Primando' and 'Wonder Cot' shows sensitivity to bacterial attack and also 'Farclo' on Mirobolan 29C.

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DETERMINATION OF GROWING DEGREE-DAY (GDD) VALUES: PISTACHIO (*PISTACIA VERA* L.) CASE

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Abstract

Pistachio is a drought-tolerant fruit that can be grown in arid areas under limited water conditions. Therefore, in our country, most of the cultivation is done in Southeastern Anatolia. Therefore, The Southeastern Anatolia Region was determined as the research area. Growing Degree Day Method (GDD) was also used as the method in this study. GDD values: Long-term daily maximum and minimum temperature values of the provinces in our region are used. Growing degree-day values are calculated according to the recommended temperature values for phenological periods of pistachio. According to GDD values, Kilis and Adıyaman were determined as the second most proper growth area after Şanlıurfa. It may be suggested due to the similarity between the geographical, soil, topographical and ecological structures of Kilis with those of Gaziantep that studies should be carried out for improving pistachio cultivation and that projects should be carried out for increasing yield per tree based on the consideration that it is not possible to increase areas of agriculture. Therefore, this and other similar studies will result in attaining higher yields by carrying out production activities in suitable areas while also contributing to the country economy.

Key words*: Growing degree-day, Southeastern Anatolia Region, temperature.*

INTRODUCTION

Pistachio grows in the Near East, Mediterranean Region and western parts of Asia in the world and its production is carried out in 56 provinces in our country the most predominant of which are Gaziantep and Şanlıurfa. These provinces are located in the Southeastern, Mediterranean, Aegean and even the Central Anatolia regions of our country with Gaziantep, Kahramanmaraş, Adıyaman, Şanlıurfa, Mardin, Kilis, Diyarbakır and Siirt standing out. Pistachio is widely grown in the aforementioned areas since they have suitable climate and ecological conditions (Anonymous, 2001). This agricultural product that is of significant importance for the economy of the region and the country has high potential with regard to domestic consumption and export incomes.

Climate is the most important factor that has an impact on the biological development and growth of plants as well as their biological distributions. Temperature and precipitation subject to standard climate data are among the most important climate parameters. However, temperature is an important limiting factor for plant growth at regions with a temperate climate as well as high latitudes (Forland et al., 2004).

Environmental variables and especially temperature are of significant importance with regard to plant growth, development and yield (Kaleem et al., 2011). Combined effects of environmental factors not only change plant phenologies but also result in significant changes in many physical and qualitative characteristics. Temperature is also among the critical factors that determines the growth areas for pistachio cultivars. Longer periods of high temperature is required in summer months for the proper development and maturing of the plant while lower temperature values for certain periods of time are required in winter months

(Anonymous, 2001). Temperature has four different effects on the distribution areas of Pistachio cultivars. These are winter frosts, late spring frosts, chilling requirement and total temperature demands. Southeastern Anatolia Region has suitable conditions for pistachio production with regard to summer temperature sum. In this regard, the long term mean summer temperatures (June-July-August) are above 30°C in Southeastern Anatolia. In case temperature sum is insufficient, the fruit cannot ripen well and the splitting percentage is quite low (Anonymous, 2001).

Growing degree-day method (GDD) is a very useful climate indicator for users that need to manage climate related risks and opportunities. Moreover, GDD provides information on temporal temperature trends and helps in estimating the impacts of climate fluctuations or changes (Grigoriva et al., 2010). Tekin (1993) carried out a study for determining the chilling requirements and Growing-Degree Day hours of pistachio cultivars. It has been determined with regard to the heat requirement among pistachio cultivars that Ohadi has the highest value with 11500 growing degree day hour (GDDH), while Kırmızı variety has the lowest temperature value with 10700 GDDH. Siirt variety is ranked in between these two values. Hence, Siirt region is suitable due to its climate conditions for the growth of the Siirt variety among pistachio cultivars.

The GDD method calculated using daily average maximum and minimum temperatures is used frequently in agricultural and phenologic studies (Grigorieva et al., 2010). GDD method is used for determining the crop maturity and flowering times, classification of plants and estimation of the time between two growth periods (Kaleem et al., 2011). It is known that there is a relationship between temperature and living organism (Birgücü & Karsavuran, 2009), as well as a linear relationship between GDD and plant growth ratio (Lu et al., 2001). Different methods such as average temperature method, sinusoidal method and integration (use of continuous data) are used for calculating GDD (Kaya, 2010).

In this study, climate data were used for examining the temperature limit values in phenological stages, the acquisition of related GDD values as well as the relationships between temperature and GDD for the Southeastern Anatolia Region, a popular pistachio production area with significant potential.

MATERIALS AND METHODS

Pistachio (*Pistacia vera* L.) is a fruit of the gum tree family. Pistachio is used as an ornamental tree in parks and gardens and is also used in many sectors including pharmacy, pastry, confectionery, ice cream industry. In our country, Pistachio is called "*golden tree"* or "*green gold*" (Anonymous, 2010). Due to these and similar features, pistachios were chosen as the research subject. In our country, the Southeastern Anatolia Region where pistachio is the most produced, and here, especially in Gaziantep and Şanlıurfa provinces are heavily cultivated (Aslan, 2017). Therefore, the Southeastern Anatolia Region was determined as the research area (Figure 1). Long-period daily maximum and minimum temperature (1950-2018) values, which are measured in meteorological stations belonging to 9 provinces in the study area and have different recording lengths, are used. Characteristics of meteorological stations in provinces are given in Table 1 (Anonymous, 2018a).

Figure 1. Map of Southeast Anatolia Region (Anonymous, 2019)

Table 1. Characteristics of Meteorological Stations

Meteorological stations	Period duration (years) (n)	Height (H, m)	Latitude-North	Longitude-East
Adıyaman	1962–2018 (57)	37°45	38°16	679
Batman	1952–2018 (67)	37°53	41°07	610
Diyarbakır	1960–2018 (59)	37°54	40°13	649
Gaziantep	1950–2018 (69)	37°04	37°29	854
Kilis	1959–2018 (60)	36°43	37°05	638
Mardin	1950–2018 (69)	37°18	40°46	1050
Siirt	1950–2018 (69)	37°55	41°56	896
Şanlıurfa	1950–2018 (69)	37°09	38°47	547
Şırnak	1970–2018 (49)	37°31	42°28	1381

The optimum temperature values used in the calculation of GDD values in different phenological periods of pistachio plant are given in Table 2 (Aktuğ et al., 2007; Anonymous, 2018a; Anonymous, 2018b).

Table 2. Optimum Temperature Values for Different Phenological Periods of Pistachio

Phenological period	Temperature (T_b, ^oC)	Date	Period Duration (Day)
Pre-flowering period	8–15	21 March – 1 April	11
Flowering period	9–19	1 April – 29 April	29
Leaf formation period	10–20	16 April – 30 April	15
Outer Shell development period	11–23	1 May – 15 May	15
Shell hardening period	16–30	16 May – 30 June	46
Shell splitting period	24–32	1 July – 31 August	62
Harvest period	17–26	1 Sept. – 30 Sept.	30
Total			208

Growing Degree-Day (GDD) Method: Growing-degree-days are defined as the integration of excess temperatures in days with maximum and minimum values limited by temperatures (Kaya, 2010). In order for plants to continue to develop in each phenological period, the sum of temperatures above the base temperature is defined as GDD (Kadıoğlu & Şaylan, 2001; Parthasarathi et al., 2013; Payero, 2017; Miller et al., 2018). GDD values can be calculated with the following equations:

$$Tave = \frac{T\max + T\min}{2} \quad (1)$$

$$GDD = \sum_{i=1}^{n} (Tave - Tb) \quad (2)$$

Where: T_{ave} stands daily average temperature (°C); T_{max} stands daily maximum temperature (°C); T_{min} stands daily minimum temperature (°C); T_b stands optimum temperature value recommended for different phenological periods of pistachio plant (°C) and n represents the yearly number of days. In the case of $T_{ave} > T_b$ in Equation 2, the GDD value is calculated (McMaster & Wilhelm, 1997; Rulm et al., 2010; de Souza, et al., 2011; Elnesr & Alazba, 2016).

Statistical Methods: The relationships between GDD values calculated for different phenological periods and optimum temperature values of pistachios were tested by regression analysis.

Regression Analysis: It is a statistical method used to determine the direction and amount of the relationship between two variables. In this method, one of the variables is considered as dependent and the other as independent. In trend studies, time (years) is taken as dependent variable and values such as temperature and precipitation are used as independent variables. Regression constant and regression coefficient are obtained from the analysis.

$$Y = a + b.x \quad (3)$$

Where: Y is the dependent variable, x is the independent variable, whereas a and b are equation regression coefficients (Gürtan, 1979; Helsen & Hirsch, 1993; Şahinler, 2000; Xu, 2002; Alpar, 2003; Shammugasundram, 2012; Kılıç, 2013).

RESULTS AND DISCUSSIONS

Long-term maximum (T_{max}), average (T_{ave}) and minimum (T_{min}) temperature values of the meteorological station in the research area are given in Figure 2.

Temperature is one of the important factors for determining the pistachio production locations. It requires longer periods of high temperature for growth and maturity during summer months, while lower temperature values for certain periods of time are required in winter months (Anonymous, 2001; Anonymous, 2010). It was determined upon examining Figure 2 that long term Tmax values are not below 30°C, while the T_{ave} values did not go below 15°C.

Thus, it can be stated that the Southeastern Anatolia region meets these temperature values. Figure 3 shows the GDD values calculated according to the optimum temperature values suggested for the different growth periods (showed in Table 2) of the pistachio plant as well as the changes in the daily maximum, minimum and mean temperature values throughout the growth periods.

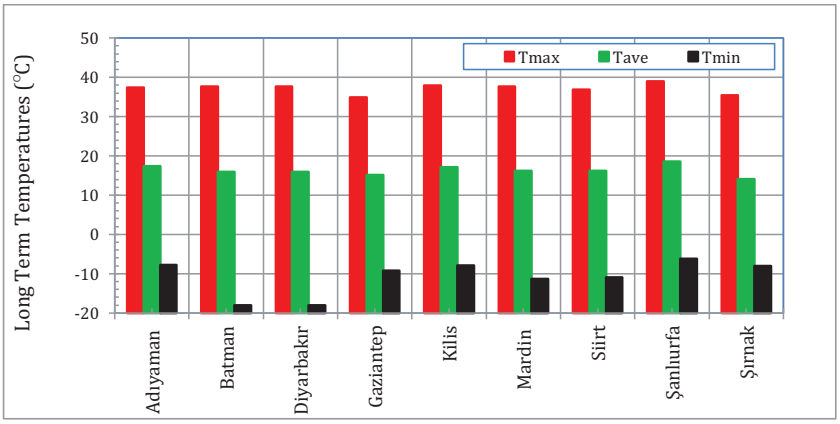
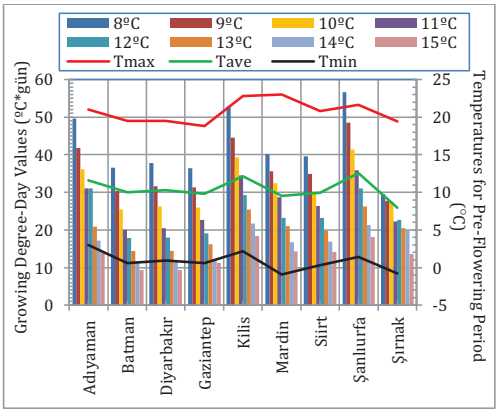
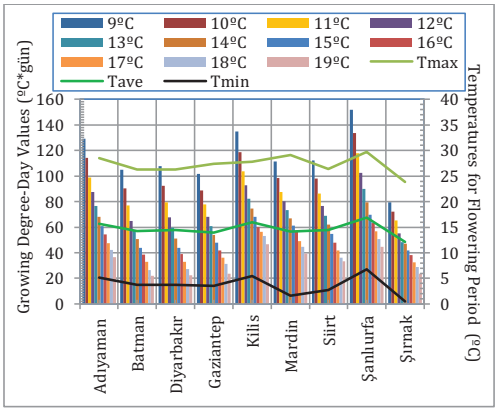


Figure 2. Long-term Period Temperature Values of Provinces





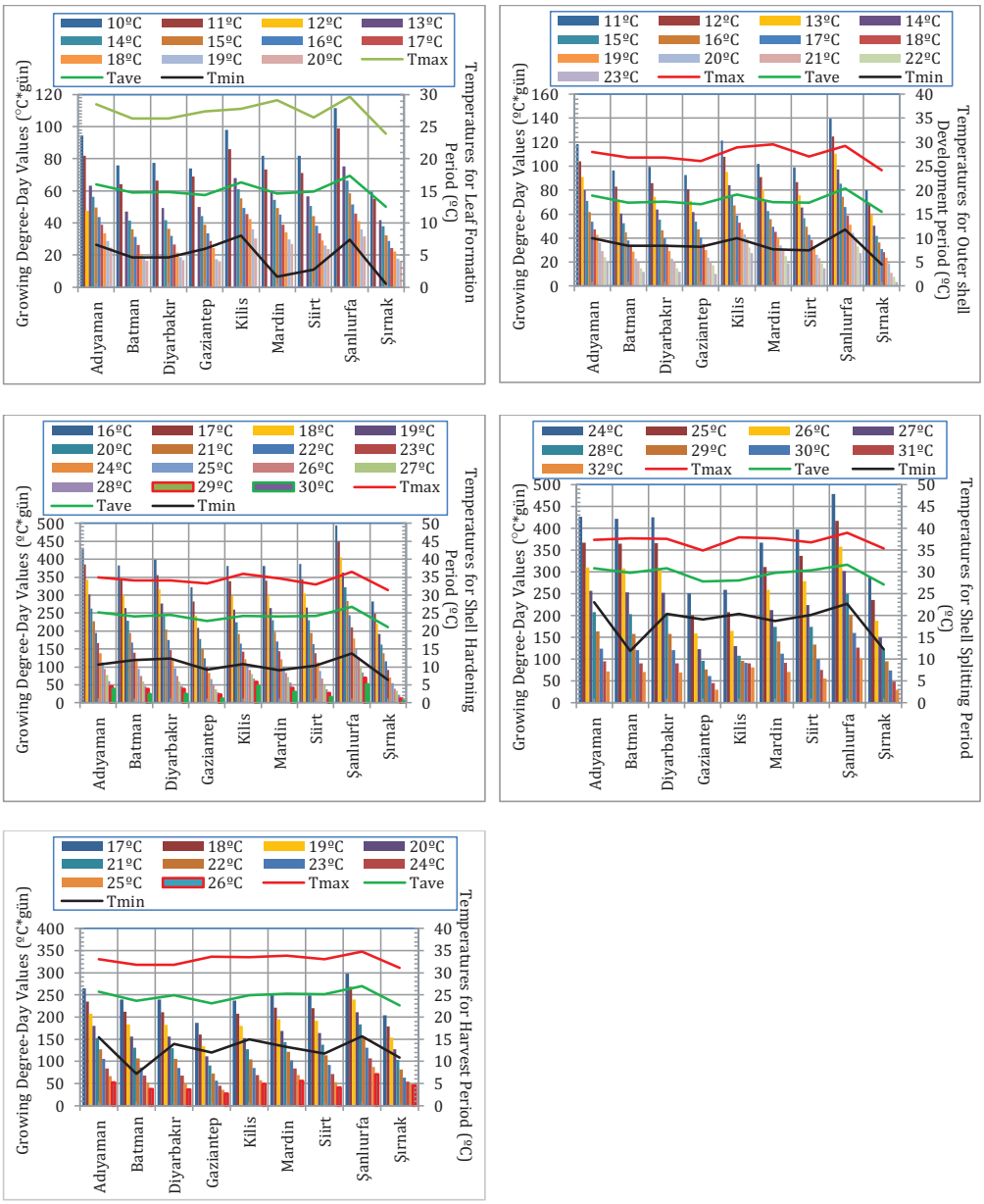


Figure 3. GDD values according to recommended optimum temperature values of pistachio

The GDD values for each development period of pistachio were calculated for each province. Highest GDD values during the pre-flowering phenological stage were observed in the provinces of Şanlıurfa, Kilis and Adıyaman while the lowest GDD values were determined in the Şırnak province. Şanlıurfa and Kilis were again determined as the provinces with the highest GDD values during the flowering period. Lowest GDD values were observed in the Şırnak province during this period. It can be seen from Figure 3 that the Şanlıurfa province had the highest GDD values in each phenological period of pistachio followed by Kilis. Highest GDD values were obtained in the Kilis province excluding only the shell splitting and harvest periods. According to TUIK (2019) data; Şanlıurfa 139216 ha, Gaziantep 136347.3

ha and Siirt 28207.1 ha were determined as the provinces with highest production. Based on GDD values, the locations that are most suited for growth were determined as Şanlıurfa, Kilis and Adıyaman with indicated as the city with the highest production which is also in accordance with our findings (TUIK, 2019). Therefore, Şanlıurfa province was determined as the best location with the largest area for growth.

The fact that Gaziantep followed Şanlıurfa as the province with the second highest production is not in accordance with our findings. Kilis comes after Şanlıurfa as the second most suited province for growth and Gaziantep is way back in this evaluation according to its GDD values. However, Kilis and Gaziantep have many similarities with regard to geographical location, topography, soil and vegetation, climate properties and elevations from sea level with no apparent differences between them. When the proximity of these provinces, similar ecologies and size of growth areas as well as their differences are taken into consideration, Gaziantep has higher potential with due to its production area and amount. On the other hand, if we consider that Kilis was a district of Gaziantep until June 10, 1995 it can be observed that the climate parameters used for evaluation are in accordance with the ecology of Gaziantep. Hence, the rise of Kilis in GDD rankings actually points out Gaziantep. Furthermore, Aslan (2017) states that the two most important cities in the production of pistachio in the GAP region are Gaziantep and Şanlıurfa. This is also support our comment on this issue. The production area of Kilis was determined as 6482.9 ha. According to TUIK (2019) data, pistachio yield for the provinces of Şanlıurfa, Gaziantep and Siirt was determined as 5 kg tree^{-1}. It was concluded due to the high yield of the pistachio trees in the Kilis province (6 kg tree^{-1}) and the fact that it is the second most suited location for pistachio growth that the production areas in the city of Kilis should be increased and that investments should be made for pistachio production in the region with opinions on increasing related works in the region. Determining proper locations for plant growth and carrying out plant production in these areas will increase the income of the producers while also contributing to the country's economy.

Statistical Method Results: The relationships between the optimum temperature (Tb) suggested for different phenological periods and the GDD values were examined via regression analysis after which a two-parameter parabolic equation was developed ($GDD=a+b.T+c.T^2$) with statistical values shown in Table 3. It was concluded based on the developed equality that temperature may be an effective factor during the growth periods of the pistachio plant.

Table 3. Statistical Results Between Calculated GDD Values and Recommended Temperature Values

Meteorological Stations	Equation Coefficients			r	s	$F_{calculated}$	p
	a	b	c				
Pre-flowering period							
Adıyaman	103,9	- 7,945	0,1284	0,989	2,16888	111,53	0,001
Batman	105,6	- 11,26	0,3242	0,998	0,582466	922,97	0,001
Diyarbakır	111,7	- 12,05	0,3496	0,999	0,557938	1119,18	0,001
Gaziantep	95,52	- 9,415	0,2519	0,998	0,596577	789,07	0,001
Kilis	126,6	- 11,74	0,3027	0,999	0,586796	1414,95	0,001
Mardin	79,56	- 5,542	0,07777	0,998	0,673076	653,98	0,001
Siirt	90,34	- 7,821	0,1833	0,9999	0,234432	5016,25	0,0001
Şanlıurfa	139,2	- 13,00	0,3290	0,9999	0,538335	2169,53	0,0001
Şırnak	32,40	0,478	- 0,1086	0,962	1,66383	31,32	0,001
Flowering period							
Adıyaman	311,4	- 25,77	0,6002	0,999	1,385290	2384,26	0,001
Batman	264,8	- 22,77	0,5305	0,998	1,96915	918,91	0,001
Diyarbakır	274,4	- 23,70	0,5541	0,998	1,55384	1581,80	0,001
Gaziantep	227,3	- 17,42	0,3598	0,998	1,59960	1182,77	0,001
Kilis	324,6	- 27,37	0,6772	0,998	1,62790	1540,36	0,001
Mardin	226,4	- 16,02	0,3292	0,998	1,62599	980,58	0,001
Siirt	256,6	- 20,27	0,4489	0,999	1,22021	2248,80	0,001
Şanlıurfa	375,7	- 31,84	0,7643	0,9999	1,18645	4440,06	0,0001
Şırnak	168,9	- 12,22	0,2455	0,995	1,94241	423,98	0,001

Leaf formation period							
Adıyaman	238,6	- 18,49	0,3922	0,999	1,06890	2236,67	0,001
Batman	213,5	- 18,04	0,4098	0,997	1,47747	864,91	0,001
Diyarbakır	220,5	- 18,65	0,4234	0,999	0,954591	2217,90	0,001
Gaziantep	186,7	- 13,90	0,2679	0,997	1,53238	817,79	0,001
Kilis	213,9	- 17,21	0,3628	0,997	1,66921	810,78	0,001
Mardin	174,9	- 11,38	0,1987	0,999	0,838783	2320,23	0,001
Siirt	206,2	- 15,89	0,3375	0,9999	0,610116	5033,59	0,0001
Şanlıurfa	295,3	- 23,76	0,5319	0,9999	1,00330	3447,50	0,0001
Şırnak	146,0	- 10,69	0,2094	0,999	0,745503	1955,08	0,001
Outer Shell development period							
Adıyaman	306,7	- 21,81	0,4094	0,999	1,52405	2511,82	0,001
Batman	275,0	- 21,12	0,4236	0,998	1,73218	1427,14	0,001
Diyarbakır	283,2	- 21,54	0,4256	0,999	1,23754	3088,05	0,001
Gaziantep	241,4	- 17,09	0,3105	0,999	1,31797	2293,65	0,001
Kilis	308,4	- 21,79	0,4212	0,998	1,66859	1888,57	0,001
Mardin	249,1	- 16,84	0,3019	0,998	1,59413	1585,78	0,001
Siirt	269,7	- 19,90	0,3859	0,999	1,22771	2869,73	0,001
Şanlıurfa	362,6	- 25,74	0,4889	0,9999	1,17687	5631,28	0,0001
Şırnak	211,4	- 15,09	0,2661	0,996	2,24744	672,82	0,001
Shell hardening period							
Adıyaman	1552,0	- 92,53	1,402	0,9999	1,55258	47378,53	0,0001
Batman	1432,0	- 86,91	1,336	0,9999	1,44959	44956,93	0,0001
Diyarbakır	1490,0	- 90,06	1,375	0,9999	2,03097	25346,93	0,0001
Gaziantep	1317,0	- 84,05	1,357	0,9999	2,35354	12499,78	0,0001
Kilis	1465,0	- 91,61	1,487	0,9999	3,55590	6335,41	0,0001
Mardin	1390,0	- 83,48	1,277	0,9999	2,39007	15579,06	0,0001
Siirt	1454,0	- 87,96	1,334	0,9999	1,52444	44180,97	0,0001
Şanlıurfa	1631,0	- 91,48	1,294	0,9999	2,28466	28010,45	0,0001
Şırnak	1113,0	- 68,86	1,066	0,9999	2,40053	9970,45	0,0001
Shell splitting period							
Adıyaman	3506,0	- 190,6	2,600	0,9999	2,62222	8983,41	0,0001
Batman	3572,0	- 195,6	2,689	0,9999	3,62241	4719,31	0,0001
Diyarbakır	3682,0	- 203,1	2,816	0,9999	3,19117	6191,44	0,0001
Gaziantep	2981,0	- 179,4	2,729	0,999	4,20877	1259,20	0,001
Kilis	3725,0	- 237,4	3,868	0,996	6,15797	402,09	0,001
Mardin	3452,0	- 197,2	2,862	0,9999	1,38126	22075,50	0,0001
Siirt	3892,0	- 222,1	3,193	0,9999	2,77728	7501,83	0,0001
Şanlıurfa	3581,0	- 190,1	2,541	0,9999	2,97382	7874,91	0,0001
Şırnak	2881,0	- 165,9	2,404	0,999	4,59335	1430,93	0,001
Harvest period							
Adıyaman	1076,0	- 63,15	0,9119	0,9999	1,48783	10775,14	0,0001
Batman	1057,0	- 64,52	0,9716	0,9999	1,19285	15171,46	0,0001
Diyarbakır	1072,0	- 66,00	1,005	0,9999	0,952306	23888,39	0,0001
Gaziantep	1065,0	- 73,79	1,303	0,9999	0,900429	16623,76	0,0001
Kilis	1210,0	- 80,47	1,376	0,9999	1,95349	5041,50	0,0001
Mardin	1050,0	- 63,55	0,9722	0,9999	0,762851	33946,11	0,0001
Siirt	1048,0	- 62,24	0,9007	0,9999	1,59249	9079,12	0,0001
Şanlıurfa	1065,0	- 57,63	0,7416	0,9999	1,58823	10894,57	0,0001
Şırnak	1248,0	- 89,15	1,646	0,998	3,85931	983,82	0,001

An equation was obtained which reflects the relationship between the suggested temperature for each growth period and GDD values after which correlation analysis at a significance level of 5%, F test and probability values were determined and presented in Table 3.

A statistically significant relationship was determined between the GDD and temperature values for the Şanlıurfa province during each phenological stage.

The fact that the city of Şanlıurfa is suited for pistachio production due to its GDD values is statistical proof that it matches our data with Şanlıurfa being the city with highest production in 2018.

CONCLUSIONS

The purpose of this study was to determine the best growth area for pistachio. For this purpose, GDD values were calculated for the temperature values suggested for each phenological stage and it was determined that the cities of Şanlıurfa, Kilis and Adıyaman are the best production areas. It was indicated based on 2018 data that Şanlıurfa, Gaziantep and Siirt are the locations with highest production. Our findings are not in full accordance with data on the cities with highest production. According to GDD values, Kilis and Adıyaman were determined as the second most proper growth area after Şanlıurfa. It may be suggested due to the similarity between the geographical, soil, topographical and ecological structures of Kilis with those of Gaziantep that studies should be carried out for improving pistachio cultivation and that projects should be carried out for increasing yield per tree based on the consideration that it is not possible to increase areas of agriculture. Therefore, this and other similar studies will result in attaining higher yields by carrying out production activities in suitable areas while also contributing to the country economy. In this way, it will be possible for the producers to use technological agriculture inputs for highly efficient and planned production while also carrying out more productive and planned agricultural applications with full knowledge on which products can be grown at which location.

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PRELIMINARY RESULTS REGARDING THE INFLUENCE OF SOME FOLIAR BIOLOGICAL TREATMENTS ON THE PRESERVING CAPACITY AFTER HARVESTING OF THE APRICOTS

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Abstract

The paper presents the results obtained in 2018 on quality maintaining of the apricots varietiy 'Orizont'. The apricot trees were fertilized with the biological fertilizer Cropmax wich was applied together with some biological fungicides, insecticides and/or acaricides in three variants: v1= Cropmax + Konflic + Funres; v2= Cropmax + Oleorgan + Canelys; v3= Cropmax + Canelys + Mimoten. Apricots were kept in different technological conditions: room temperature (20-22ºC), temperature of 10-12°C (refrigerant conditions) and temperature of 3-5ºC, with and without modified atmosphere (cold storage), for 7, 15 and 30 days, respectively. The initial level and the evolution during storage of the firmness of the fruits were determined. After storage determinations were performed on the total losses, quantitative losses and qualitative depreciations. It was found that the quality of the apricots and their storage capacity vary according to the foliar biological treatments applied to the apricot trees and to the conditions in the storage environment. The v1 variant induces the best quality and storage capacity. Of the four studied storage methods, the best results were obtained within the cold storage with modified atmosphere, which recorded the smallest losses during storage.

Key words*: biological treatments, storage conditions, firmness, quantitative and the qualitative losses.*

INTRODUCTION

The apricots are very popular with consumers, both as a dessert fruit and, as well as processed in various ways. The high demand for fruits is determinate by their qualitative and technological attributes, by the complex biochemical composition and by the very pleasant taste and specific flavour etc. (Akin et al., 2008).

The aapricots obtained through the biological agriculture variant have a higher nutritional, hygienic and tasteful value compared to conventional products (Davidescu & Davidescu, 1994; Ion, 2004).

The biological fungicides, insecticides, acaricides and fertilizers have a lower cost and other advantages, such as: low level of the poluation for people and environment, small quantities of residues on the fruits, high selectivity to useful ecosystem fauna etc.

In Romania, it was tested a large range of foliar fertilizer for cultivated plent's and among fruit trees species (Platon & Soare, 2002; Rusu et al., 2002; Dinu et al., 2009; Bochis & Ropan, 2011; Petrisor et al., 2014; Singh, 2017) with special regarding on tree growing and increasing of fruit yield.

The question that arises here is about the correlation that may be carried between foliar biological treatments applied to the apricot trees and the preserving quality after harvesting of the apricots (Hitka, 2011).

Apricots are extremely perishable, which raises serious problems concerning the maintaining of their quality, from the moment they are harvested until they reach the consumer. Being highly perishable, the apricots are typically consumed or canned within two weeks of harvest. For extending this time, they must be

storage under certain temperature and humidity conditions which slow down the normal processes of senescence, characterized by altering the physiological, biochemical and morphological attributes of the fruits, which ultimately cause their depreciation.

In our country, doesn't exist enough information on the apricots storage in refrigerant and controlled atmosphere (CA) but, this type information is available in the literature, especially concerning the effect of CO2 level in CA. Veringa et al. tried to establish the influence of the variety and storage conditions on the preserving capacity after harvesting of the apricots belonging to two cultivars: Mamaia and Olimp, obtained from conventional agriculture.

In Italy, Europe's main producer of apricots (Andrich & Fiorentin, 1986) studied two varieties of apricots to determine their storage stability and to examine the effect of the CO2 level on weight decrease, firmness, total titrated acidity, pH, refractometric degree, physiological and pathological modifications. In other countries, the researchers determined the effect of controlled atmosphere storage of the ethylene relating different biochemical changes in apricot fruit (Palou & Crisosto, 2003).

The aim of this work is to establish the influence of some foliar biological treatments applied to the apricot trees on the obtaining and preserving the quality after apricots harvesting, in different storage conditions.

MATERIALS AND METHODS

The analysed fruits were obtained from the orchard of the experimental area of the Research Station for Fruit Growing Constanta. The apricots, variety 'Orizont', were harvested and tested in 2018.

In the technical literature (Stefan et al., 2018) this variety is described as follows: The tree is of medium vigour, fruiting mostly on May bouquets. Variety is self-fertile with very medium blooming period. The fruit (Figure 1) is of medium to large size (45-62 g), orange skin covered with red carmine on the sunny side, orange pulp, fine texture, juicy, sweet and pleasant flavored, with stone of medium sized, non adherent to the pulp (freestone). It yields in the 3rd-4th year after planting, and has a production capacity of 16.5 t/ha (635 trees/ha). It is tolerant to frost and drought, fruit destination being for fresh consumption and industrial pprocessing as jam, compote, dried fruit.

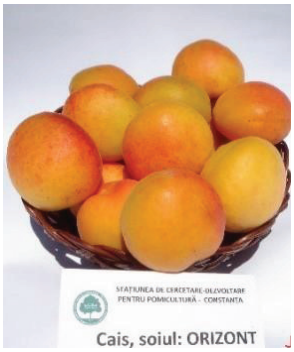

Figure 1. Apricots, 'Orizont' variety

The apricot trees were fertilized with the biological fertilizer Cropmax which was applied together with some biological fungicides, insecticides and/or acaricides, with the STIHL 400 SR atomizer (Figure 2).

Figure 2. Aplication of biological products to apricot trees

The experimental variants are:

- V1 - Cropmax 0.15% + Konflic 0.3% + Funres 0.3%;
- V2 - Cropmax 0.15% + Oleorgan 0.3% + Canelys 0.3%;
- V3 - Cropmax 0.15% + Canelys 0.3% + Mimoten 0.3%;
- V4 - control –untreated.
- The products which were used are described below:
- Cropmax - super-concentrated, foliar fertilizer (100% natural).

- Konflic - organic insecticide; it is a natural product used against the pest population (white fly, trips, aphids).
- Oleorgan - insecticide; it is a natural product which contains vegetable oils, used to combat and reduce the population of pests (white fly, trips, aphids) from horticultural crops.
- Funres - fungicide; it is a natural herbaeous extract intended to combat diseases of the horticultural crops, such as *Botrytis.* sp., *Sclerotinia* sp., *Peronospora* sp., *Phytophthora* sp.
- Mimoten - fungicide, with a preventive and curative effect on most fungi and bacteria that attack the crops and fruit trees, by inhibiting their growth and development (*Botrytis* sp., *Septoria* sp., *Sphaeroteca* sp.).
- Canelys - acaricide, a natural product successfully used to control the population of mite spiders *(Tetranichus* sp.) and of some pathogen fungi, as *Oidium* sp.

The apricots were kept at the Research and Development Institute for Processing and Marketing of the Horticultural Products Bucharest, in different technological conditions:
- room temperature (T = 24-27°C, RH = 69-71%), in 1 kg packaging - warm;
- fridge storage (T =10-12°C, RH = 75-78%), in packs of 1 kg (Figure 3);
- cold storage (T = 3-5°C, RH = 82-86%), in packs of 1 kg;
- cold + MA storage (T = 3-5°C, RH = 92-96%), in 1 kg hermetic packages, so that the composition of the atmosphere inside was modified, by the reducing of the O_2 amount and the increasing the CO_2 amount and also of air relative humidity - storage in modified atmosphere - MA.

Figure 3. Fridge storage of apricot

The storage period (days) varied according to the technological storage conditions, as follows:
- warm storage: 7;
- fridge storage: 15;
- cold storage: 30;
- MA storage: 30.

Before introducing to storage, biometric measurements were made, relating to: weight, height, high and small diameter.

The initial level and the evolution during storage of the firmness of the fruits were determined, with OF penetrometer (firmness tester), measuring the depth of penetration of the needle with diameter = 4 mm and length = 24 mm.

After storage, the determinations were performed on the total losses, quantitative losses (expressed by evaporate-transpiration) and qualitative depreciations.

RESULTS AND DISCUSSIONS

1. Biometric data

The results regarding the biometric data of the apricots are presented in Table 1, which shows that they are relatively close, as size and weight of the fruits, for variants which were fertilised with foliar fertiliser.

Table 1. Biometric data of the apricots

Variant	Height (mm)	Diameter (mm)		Average weight (g)
		high	small	
V1	43.2	40.7	33.9	52.70
V2	41.8	40.6	33.2	51.59
V3	42.5	41.6	34.2	51.96
V4	36.2	35.8	28.7	44.54

The apricots belonging variant V4 (unfertilised and untreated) are smaller, the difference between this variant and variant V1 being of 8.16 g.

2. Firmness

The results presented in Table 2 reveal the fact that at harvest the firmness of the apricots ranges from 100.53 PU at the V1 variant to 135.44 PU at the V4 variant, the average per cultivar being of 119.52 PU.

Table 2. The firmness of the apricots of the 'Orizont' cultivar upon harvesting and after storage

MU = PU

Moment of evaluation	Variant				Average/cultivar
	V1	V2	V3	V4	
At harvest	100.53	125.26	115.86	135.44	119.52
After warm storage	145.44	169.33	168.89	181.44	166.27
After refrigerated storage (10-12ºC)	144.22	145.67	145.11	146.66	145.41
After cold storage (3-5ºC)	105.66	128.44	129.22	144.22	126.89
After AM storage	101.08	126.21	116.66	136.98	120.41

* PU-Penetrometer Unit = 0.1 mm

During the warm storage (ambient temperature), the apricots lose easy their firmness, because of the quick ripening. Their firmness decreased to 166.27 PU at the level of variety, with the lesser value at the V4 variant (181.44 PU) and higher value at the V1 variant (145.44 PU).

It was observed that, by refrigerating storage, the structural-cellular degradation process of the apricots become slow-up, average firmness being, after 15 days, of 145.41 PU for variety, with close values between variants (144.22 PU for V1 variant, 145.11 UP for V3 variant, 145.67 PU for V2 variant and 146.66 PU for V4 variant).

When the apricots are stored in cold conditions, the intensity of the ripening process diminishes, so that the fruit maintain their structural and textural firmness for a longer period of time (126.89 PU for variety, after 30 days of storing). In the same time, the differences between variants become larger: 105.66 PU for V1 variant, 128.44 PU for V2 variant, 129.22 PU for V3 variant, 144.22 PU for V4 variant).

The enrichment of the atmosphere in carbon dioxide allowed the storage of apricots for 30 days with the maintenance of the firmness at 120.41 PU at variety level, with variations between 101.08 PU for V1 variant and 136.98 PU for V4 variant.

3. Quantitative and qualitative losses

The losses recorded during the warm storage for 7days are presented in Table 3.

It found that in case of storage in ambient temperature - warm for 7 days, total losses are higher in all 4 types of treatment, due to both by depreciation losses, but especially those mass (weight) losses. At the V4 variant - control we meet the highest losses (37.24%), and at the V1 variant - the lowest (26.53%).

Table 3. Losses recorded during warm storage of the apricots

Variant	Losses - %		
	Total	weight	depreciation
V1	26.53	14.22	12.31
V2	33.86	17.70	16.16
V3	31.70	16.49	15.21
V4	37.24	18.23	19.01
Average per cultivar	**32.33**	**16.66**	**15.67**

But skipping the variant of the treatment, the total losses recorded during the warm apricots storage of the 'Orizont' variety are 32.23% (16.66% weight losses and 15.67% by depreciation losses).

The impairment of the fruit is due to late infections caused by fungi *Monilinia laxa* and *M. fructigena* before harvesting, when they are barely visible. After harvesting, during transport and storage, their attack is rapidly evolving (depending on temperature) and the entire fruit rots. Moreover, during storage, it can lead to the rotting of the healthy, surrounding fruit, the mycelium penetrating directly or through almost invisible lesions. Also, fruit can be infected through wounds, blows or compression produced during harvesting and handling, by the molds *Botrytis cinerea* and *Rhizopus stolonifera*.

At temperature of 10-12ºC the apricots were recorded, after 15 days of storing, from 12.33% at V1 variant, till 14.36% at V4 variant - mass losses, from 11.10% for the V1 variant till 17.43% for V4 variant - depreciation losses, and from 23.43% (V1 variant) to 31.79% (V4 variant) - total losses (Table 4).

The level of the losses due to damage made mainly the difference between the four variants in terms of storage resistance under given conditions.

Table 4. Losses recorded during refrigerated storage (10-12ºC) of the apricots

Variant	Losses - %		
	total	weight	depreciation
V1	23.43	12.33	11.10
V2	26.49	13.45	13.04
V3	25.98	13.66	12.32
V4	31.79	14.36	17,43
Average per cultivar	26.92	13.45	13.47

By using the cold storage of the apricots, for 30 days, losses were recorded, both quantitative (weight) and qualitative (depreciation), much smaller than warm keeping (Table 5). Thus, the values found at the 'Orizont' variety, were: mass losses = 10.52%, depreciation losses = 10.29% and total losses = 20.81%.

Table 5. Losses recorded during cold storage (3-5ºC) of the apricots

Variant	Losses - %		
	total	weight	depreciation
V1	16.51	8.42	8.09
V2	20.97	10.85	10.12
V3	19.94	10.14	9.80
V4	25.80	12.67	13.13
Average per cultivar	**20.81**	10.52	10.29

V1 variant is also remarkable, with 16.51% total losses (8.42% quantitative losses + 8.09% qualitative losses), followed by V3 variant (losses: 19.94%, 10.14% and 9.80%, respectively). On the last place, with total losses of 25.80%, ranks V4 variant.

Very good results were obtained through the option of cold + MA storage, in which case the losses during preservation for 30 days were much lower compared to storage in cold conditions, without modified atmosphere (Table 6).

Table 6. Losses recorded during AM storage of the apricots

Variant	Losses - %		
	total	weight	depreciation
V1	0.23	0.23	-
V2	6.99	0.31	6.68
V3	6.47	0.33	6.14
V4	12.17	0.31	11.86
Average per cultivar	**6.47**	**0.29**	**6.18**

Thus, the level of the total losses, for 'Orizont' variety, was of 6.47% and those of weight losses was of 0.29%.

The V1 variant was not reported losses of the quality, and the quantitative losses were almost non-existent (0.30%). Even in the V4 variant the losses were significantly reduced, those being of 12.17%, 0.31% and 11.86%, respectively.

From the experimental data results that from the biological fertilization + treatments variants of orchard apricots, the best results in terms of losses during storage, from all the four technological methods, is V1 variant, followed by V3 variant. The worst results were obtained in V4 variant.

CONCLUSIONS

The results revealed the fact that, in general the apricots were sensitive to storage, the maximum storage duration being 7-30 days, depending on the storage conditions.

From the point of view of the firmness, the best results were obtained by the apricots of the V1 variant (Cropmax 0.15% - fertiliser + Konflic 0.3% - insecticide + Funres 0.3%- fungicide) which has the fruits with the best structural firmness. It was noticed that the speed of metabolising pectin substances and the decrease of the fruit's firmness differs according to the culture's treatment scheme but especially to the temperature and air composition in the storage room. During warm storage, the apricots rapidly lose their firmness because of the rapid ripening of the fruit. In the case of cold storage, the intensity of the ripening process is reduced, the fruit maintaining their structural and textural firmness for a longer period of time (30 days). By enriching the atmosphere in the storage room with carbon dioxide, the metabolic processes become even slower and the apricots' firmness is maintained for a longer period of time.

The ability to maintain the quality of the apricots fruit varies also depending by the scheme of treatment and the storage conditions of the environment and especially by temperature and gaseous air composition. The V1 variant induces the best storage capacity, with the lowest quantitative (weight) and

qualitative (depreciation) losses. Foliar fertilizers provide, besides the contribution of macro-and micro-nutrients and other organic substances that stimulate the metabolism of chlorophyll assimilation, energy efficiency and ultimately the quality of the fruits. The fungicide Furnes and insecticide Konflic used to combat diseases and pests, along with the Crompax fertilizer, contributes to obtaining healthier, better quality fruits, with better storage capacity. The most efficient methods of the fruits storage is cold room + modified atmosphere in which were recorded the lowest losses during storage.

Very good results were also obtained through the option of cold storage, in which case the losses during preservation for 30 days were much lower compared to storage at room temperature for 7 days.

Because the experiments have been performed for only one year, the results are provisional. In order to obtain definitive results, we will continue the research, trying higher concentrations of the used substances, in which case the differences between the variants will be sure more conclusive.

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THE EFFECT OF ABIOTIC FACTORS ON THE *IN VITRO* DEVELOPMENT OF *MONILINIA FRUCTIGENA* FUNGUS (ADERH. & RUHL.) HONEY, ISOLATED FROM THE APPLE

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Abstract

Monilinia fructigena (Aderh. & Ruhl.) Honey, the pathogenic agent of the apple rot, is common in apple orchards every year, causing important harvest losses that could continue in storage. In vitro studies have been carried on the impact of temperature, light and culture medium on the development of Monilinia fructigena fungus, isolated from apple fruits from 'Topaz', 'Florina', 'Goldrush', 'Idared' and 'Generos'. The minimum pathogen development threshold was +4°C and the optimal range was between 24-28°C. At higher 36°C temperatures, development has significantly decreased. The pathogenic agent developed well in light and continuous darkness (24 h). Fungal isolates preferred oat and potato-dextrose-agar culture media to form well-developed colonies.

Key words*: fungus, varieties, apple, abiotic factors.*

INTRODUCTION

Monilinia fructigena (Aderh. & Ruhl.) is the pathogenic agent of the apple brown rot or Monilinia (Agrios, 2005). This is one of the most spread apple diseases which could lead to significant losses in the orchards and storehouses (Byrde & Willettts, 1977). Generally, apple varieties are affected by *Monilinia fructigena* fungus and its severity is associated to the environment, the presence of damages caused by biotic and abiotic factors (Holb, 2004), the age of wounds affecting the fungus incubation period (X. M. Xu & J. D. Robinson, 2000).

Controlling the disease by applying treatments and cultural hygiene of orchards contributes to the significant reduction of brown rot (Helmann, 1998; Chițulescu & Cristea, 2017). Research has also been carried out on the control of *Monilinia* spp. pathogens by using the antifungal effect of some plant bio – complexes (Cristea et al., 2017; Cristea et al., 2017). Knowing the biological parameters of pathogens under laboratory conditions may support studies on the evolution of pathogen attack in field conditions, attack prediction and treatment provenience.

MATERIALS AND METHODS

The biological material used consisted of apple fruits from storage conditions attacked by *Monilinia fructigena*. Harvested isolates were used from five apple varieties: 'Topaz', 'Florina', 'Goldrush', 'Idared' and 'Generos'. Isolation of the pathogen was carried out after storage sampling on the potato-dextrose-agar culture medium, divided into 90 mm diameter Petri dishes. Isolates were identified based on spore morphology then pricked out and stored in pure cultures at the thermostat, at 22°C. Isolated cultures were used after a 15 days culture.

The effect of temperature, light and culture media on the development of the *Monilinia fructigena* pathogen was studied. Observations were made on the development of the fungus by measuring the average diameter of the colonies at 3, 6, 9 days after inoculation. Each variant was placed in three repetition ratios. Observations were made on the biological development thresholds of the fungus *in vitro*.

RESULTS AND DISCUSSIONS

Abiotic factors play an important role in the development of *Monilinia fructigena* fungus.

Laboratory studies were carried out on the effect of abiotic factors on the temperature, light, culture medium on the development of seed pathogens whose development continues in storage conditions, as well (Mardare et al., 2015; Dudoiu et al., 2015; Dudoiu et al., 2016). The data shows the fungus evolution at different temperature values (Table 1).

Table 1. Effect of temperature on the *in vitro* development of *Monilinia fructigena* fungus

Temp. °C	Colony diameter (mm)														
	3 days					6 days					9 days				
Isolated symbol M	TO	GE	GO	ID	FL	TO	GE	GO	ID	FL	TO	GE	GO	ID	FL
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	1	0	0	1	0	1	1	2	3	3	4
6	0	0	0	0	1	0	0	1	0	2	2	2	4	4	5
8	1	1	1	1	2	3	1	2	2	2	5	7	8	6	10
10	1	1	1	1	2	4	2	3	2	3	8	10	18	10	15
12	2	2	1	2	3	6	2	8	3	4	10	22	30	25	29
14	3	3	2	3	3	10	3	15	9	8	25	30	70	35	45
16	4	5	3	6	7	18	18	20	14	17	45	40	80	55	80
18	5	7	6	10	15	27	30	45	20	29	63	65	85	59	75
20	7	20	9	11	25	43	55	70	26	55	85	85	90	65	75
22	7	22	23	20	30	43	50	70	40	55	85	60	88	68	80
24	8	15	24	22	30	38	55	65	45	48	80	70	90	70	90
26	7	12	15	23	28	36	50	60	48	43	78	65	90	65	85
28	6	10	12	25	28	30	45	48	49	42	73	60	70	75	85
30	5	9	10	25	27	30	42	40	40	39	72	60	62	60	80
32	5	8	7	20	25	28	40	25	35	32	68	59	45	55	70
34	4	6	5	16	23	27	18	20	30	30	65	40	38	50	62
36	2	2	2	14	22	10	14	18	25	21	50	42	39	40	45
38	2	2	1	8	8	8	9	10	12	18	40	38	20	18	20
40	0	0	1	2	5	2	3	5	7	6	18	15	10	12	15

TO - 'Topaz', GE - 'Generos', GO - 'Goldrush', ID - 'Idared', FL - 'Florina'

Analyzing the data on temperature effect on *Monilinia fructigena* fungus development, it was found that the fungus developed at 4^0C after 9 days of incubation, forming 4 mm diameter colonies in the case of 'Florina' isolate.

The optimal developmental threshold of the fungus ranged between 24-28^{0}C (Figures 1 and 2).

The fungus formed sporulated colonies with an an average diameter ranging from 70-90 mm to the studied isolates.

The development rate of the colony was slower after 28^0C, and after 34^0C the fungus was no longer frustrated.

At temperatures higher than 36^0C the fungal development was significantly reduced (Table 1).

Figure 1. *Monilinia fructigena* (MGO) - 24^{0}C - PDA (3 days)

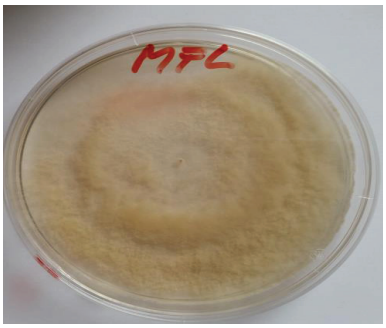

Figure 2. *Monilinia fructigena* (MFL) - 24^{0}C - PDA (9 days)

In order to analyze the effect of the culture medium on the development of *Monilinia fructigena* colonies isolated from the studied varieties, studies on the culture natural media were considered: prune agar, rice extract agar (REA), oat flakes agar, semi-dextrose agar (PDA) semi-synthetic media, 2% agarized water medium.

The data show that the fungus preferred the PDA semi-synthetic medium and the natural medium with oatmeal flakes (Table 2). *Monilinia fructigena* fungus grew best on PDA and oatmeal media (Figure 3).

Monilinia fructigena ('Florina') recorded a rapid growth rate reaching 90 mm after 9 days of observation (Figure 4).

Table 2. The effect of culture medium on the development of *Monilinia fructigena*

Monilinia fructigena		Diameter (mm)/days														
Varieties	Isolated medium	PDA			Prune-agar			REA			Oat flakes agar			2% agarized water		
		3	6	9	3	6	9	3	6	9	3	6	9	3	6	9
'Topaz'	MTO	7	40	85	6	28	40	0	0	0	20	60	80	6	7	9
'Florina'	MFL	25	70	90	12	40	65	20	45	70	18	75	90	16	60	75
'Goldrush'	MGO	9	55	75	0	0	0	0	25	40	18	65	83	0	0	0
'Idared'	MID	11	26	65	8	16	30	10	42	70	10	50	85	8	32	65
'Generos'	MGE	20	55	85	0	0	0	10	47	63	10	65	80	6	44	70

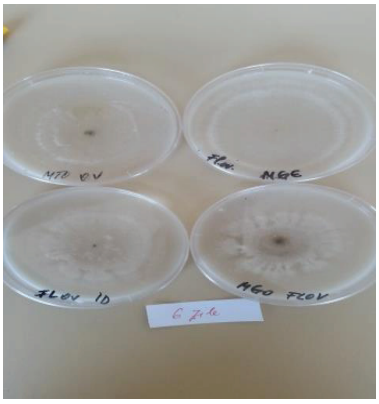

Figure 3. *Monilinia fructigena* - oatmeal flakes medium (6 days)

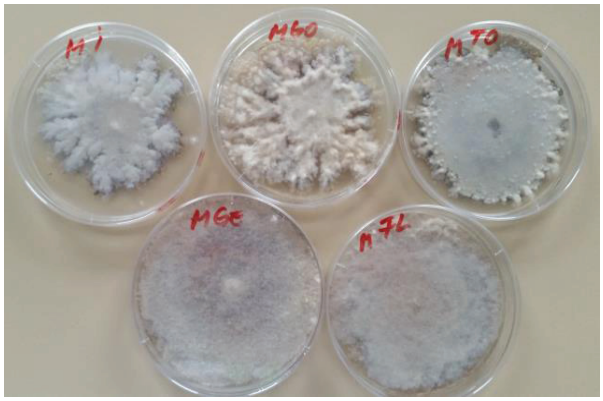

Figure 4. *Monilinia fructigena* - PDA medium (9 days)

Effect of light on the fungus development has also been studied in the following variances: continuous light (24 h), continuous dark (24 h), light/dark alternation (12 h/12 h and 16 h light/8 h dark) (Table 3).

The data exhibited *Monilinia fructigena* fungus preference to continuous light and dark whereupon the highest values of the colony diameter and abundant sporulated were registered (Table 3) (Figures 5 and 6).

Also, for the alternant variance 12 h light/12 h dark, colonies developed between 75 mm in diameter at MFL and 90 mm in MGO. *Monilinia fructigena*, isolated from the 'Idared' variety, developed more slowly, forming colonies with a diameter of 60-65 mm in the complete light and dark variances, as well as the light/dark alternation.

In 16 hours of light/darkness alternation, *Monilinia fructigena* fungus colonies developed more slowly, reaching 78 mm in diameter in the case of the 'Florina' isolate.

Table 3. Effect of light on *Monilinia fructigena* development

Monilinia fructigena		Diameter (mm)/days - continuous light 24 h		
Varieties	Isolated symbol	3 days	6 days	9 days
'Topaz'	MTO	30	55	85
'Florina'	MFL	45	60	90
Goldrusk	MGO	50	70	90
'Idared'	MID	35	40	65
'Generos'	MGE	37	65	90
Monilinia fructigena		Diameter (mm)/days - dark - 24 h		
Varieties	Isolated symbol	3 days	6 days	9 days
'Topaz'	MTO	32	60	80
'Florina'	MFL	50	90	90
Goldrusk	MGO	55	90	90
'Idared'	MID	15	30	60
'Generos'	MGE	35	89	90
Monilinia fructigena		Diameter (mm)/days - 12h light/12h dark		
Varieties	Isolated symbol	3 days	6 days	9 days
'Topaz'	MTO	7	40	85
'Florina'	MFL	25	55	75
Goldrusk	MGO	9	70	90
'Idared'	MID	11	26	65
'Generos'	MGE	20	55	85
Monilinia fructigena		Diameter (mm)/days - 16h light/8 h dark		
Varieties	Isolated symbol	3 days	6 days	9 days
'Topaz'	MTO	8	30	42
'Florina'	MFL	30	56	78
Goldrusk	MGO	15	35	60
'Idared'	MID	10	28	40
'Generos'	MGE	12	38	43

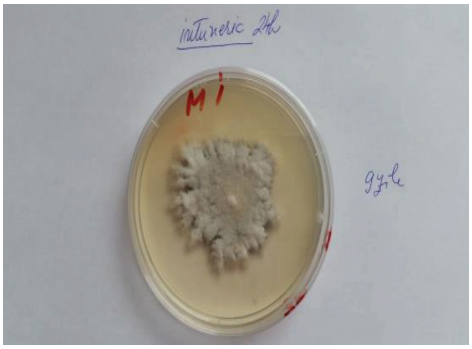

Figure 5. *Monilinia fructigena* (MID) - 24h dark (9 days)

Figure 6. *Monilinia fructigena* (MFL) - 24h dark (9 days)

CONCLUSIONS

The following conclusions can be drawn from the present research: *Monilinia fructigena* fungus (Aderh. & Ruhl.) Honey develops well at temperatures of 22-28^{0}C, with a minimum development threshold starting at 4^{0}C; the pathogen prefers semisynthetic and natural oat flakes media, forming well developed colonies in continuous total light and dark.

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ANTIOXIDANT CAPACITY, TOTAL PHENOLIC AND FLAVONOID CONTENT OF EDIBLE ROSE PETALS FROM ORGANIC PRODUCTION

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Abstract

In 2015, in the Experimental Field of Faculty of Horticulture within USAMV Bucharest an organic edible rose plantation with three varieties from David Austin collection: 'Crown Princess Margareta', 'Falstaff' and 'Brother Cadfael' was established. Before and after planting, three ameliorative species were sown in seven variants for increasing the soil biological activity. Two types of mulches were applied on the rose rows with special results on the agrochemical parameters. Organic plant protection and fertiliser schemes were used. The petal antioxidant capacity, total phenol and flavonoid content were determined from fresh petals. Part of them were dried at 45°C during four hours and analysed. Three variants of jam were made from each variety, using different ingredients as lemon, sea buckthorn and ginger and analysed. The extracts were subjected to analysis using spectrophotometric methods. Antioxidant capacity was similar in all the three variants. Total phenolic and flavonoid content varied according to the experimental variants, 'Falstaff' registering the highest amount. Dried petals registered similar biochemical parameters or higher than the fresh or processed ones, drying being recommended as an efficient preserving method.

Key words*: Rosa sp., varieties, drying, jam, spectrophotometry, free radicals.*

INTRODUCTION

Rose is a complex plant. Considered the Queen of plants, the symbol of love, purity, victory, beauty, friendship and respect among men (Milică et al., 2010), nobility and refinement (Bojor & Dumitru, 2007), the edible rose has special nutraceutic properties.

This is due to its content in carotenoids, anthocyanins, flavon detivatives, tannins, vitamins, ethereal oils etc (Milică et al., 2010) with anticancerous, slightly laxative, aphrodisiac, decongestant, disinfectant, antiseptic, hemostatic properties (Bojor & Răducanu, 2010), antimicrobial (Park et al., 2016; Sengul et al., 2017).

The rose has been used since ancient times as a medicinal plant (Lambraki, 2001; Hessayon, 2005; Milică et al., 2010; Lia et al., 2014; Dong et al., 2017; Fernandes et al., 2017). Milică et al. (2010) mentioned the role of rose jam in the treatment of lung and oral cavities.

The nutritional and biochemical parameters of rose petals can become a source of bioactive compounds along with other edible flowers (Pires et al., 2017; Santos et al., 2018). Riffault-Valois recalls the role of polyphenols in plant defense mechanisms, attracting pollinators and color shades. Ouerghemmi et al. (2016) analyzed the antioxidant activity and phenolic profile of the organic extract from the leaves of several varieties of roses, also mentioning the important role of polyphenols both as a source of antioxidants in the human diet but also as an important factor of the plant's immune system, indicating the adaptability of the plant of adaptation to the environment, also mentioned by Hashidoko (1996); Coruh and Ercisli (2010).

The antioxidant activity of three species of roses *Rosa damascena* Mill., *Rosa bourboniana* Desp. and *Rosa Brownies* Lindl., from fresh petal extracts was determined by Kumar et al. (2009). *Rosa rugosa* Thunb. at Lia et al. (2014), who studied phenolic content and antioxidant activity in 51 species of edible flowers, ranks first in terms of antioxidant capacity.

Ge and Ma (2013), studying the content of anthocyanins and polyphenols in edible roses, suggested that the varieties studied should be used to replace artificial colors in food.

MATERIALS AND METHODS

In 2015, in the Experimental Field of Faculty of Horticulture within USAMV Bucharest an organic edible rose plantation with three varieties from David Austin collection: 'Crown Princess Margareta', 'Falstaff' and 'Brother Cadfael' was established. Before and after planting, three ameliorative species were sown in seven variants for increasing the soil biological activity (V1-V8). Two types of mulches (wool and wood chips) were applied on the rose rows with special results on the agrochemical parameters (Vn.1. wood chips and Vn.2. wool, while the control Vn.3., was represented by unmulched soil) (Butcaru et al, 2017). Organic plant protection and fertiliser schemes were used. The antioxidant capacity, total phenol and flavonoid content were determined from fresh and dry petals and from jam rose petal. The extracts were subjected to analysis using spectrophotometric methods.

Total content in polyphenols.
The total content in polyphenols was determined spectrophotometrically with the Folin-Ciocalteu method after Skupień (2006), Khanizadeh (2008), Delian (2011), Mureşan (2014), Bezdadea Catuneanu et al. (2017), with some modifications. 25 µl sample (hydro-alcoholic extract) + 1.975 H_20 + 125 µl Folin-Ciocalteu + 375 µl Na_2CO_3 (20%) were homogenized, followed by incubation for 2 hours at ambient and dark temperature. Then the samples were read on the spectrophotometer to the blank at wavelength λ = 750 nm. In the case of hydroalcoholic extracts obtained from rose petals, it was necessary to make 1:10 dilutions in order to fit the results on the calibration curve. The total polyphenol content was expressed in M / ml, and the calculation was performed using the calibration curve: y = 0.0008x + 0.0003.

Total content in flavonoids.
The total flavonoid content was determined spectrophotometrically after a method adapted after Zilić (2011), Shen (2016), Bezdadea Cătuneanu et al. (2017). 250 µl of sample (hydro-alcoholic extract) + 1.25 ml H_2O + 75 µl $NaNO_2$ (5%) were homogenized, after which incubation was carried out for 5 minutes at ambient temperature. 75 µl of $AlCl_3$ (10%) was added and incubated for 6 minutes, after which 500 µl of NaOH (1M) was added. Samples were read on the spectrophotometer relative to the blank at wavelength λ = 510 nm. Dilutions of 1: 2 were used to align the results on the calibration curve. The total flavonoid content was expressed in M/ml, and the calculation was performed using the calibration curve:

$$y = 0.000627455 * x + 0.0068.$$

Antioxidant capacity.
The evaluation of antioxidant activity was performed according to the spectrophotometric method after Khanizadeh (2008), Mureşan (2014), Drogoudi (2016), Bezdadea Catuneanu et al. (2017).
500 µl sample + 1 ml DPPH (0.1 mM) was homogenized and incubated for 30 minutes at ambient temperature and dark. Samples were read on the spectrophotometer relative to the blank at the wavelength λ = 515 nm. The results were calculated according to the formula:
AADPPH (%) = (Acontrol-Asample)/(A control) x 100, where Acontrol is the absorbance of the control sample (not containing the hydroalcoholic extract) and Asample is the sample absorbance. Dilutions of 1: 10 were used. Expression of results was made in percentages.

Rose jam variants.
Three variants of jam were made from each variety, using different ingredients as lemon, sea buckthorn and ginger and analysed (Table 1).

Table 1. Rose petal jam variants

Variant	Description
D1	'Crown Princess Margareta' + lemon
D2	'Falstaff' + lemon
D3	'Brother Cadfael' + lemon
D4	'Crown Princess Margareta' + lemon
D5	'Falstaff' + ginger
D6	'Brother Cadfael' + ginger
D7	'Crown Princess Margareta' + sea buckthorn juice
D8	'Falstaff' + sea buckthorn juice
D9	'Brother Cadfael' + sea buckthorn juice

Dry rose petals
Part of the petals were dried at 45°C during four hours and analysed.

RESULTS AND DISCUSSIONS

Total polyphenols and flavonoid content, antioxidant capacity of fresh petals

Total content in polyphenols.

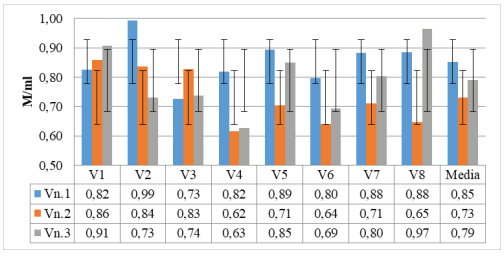
In 'Crown Princess Margareta' variety there are significant differences between the wool mulched variants and the others.

In the wood chips mulched variants, values between variants do not differ significantly being from 0.73 M/ml (V3.1) to 0.99 M/ml (V2.1).

In the wool mulched variants, there are significant differences between the variants, the lowest values being present in variants V4.2 (0.62 M/ml), V6.2 (0.64 M/ml) and V8.2 (0.65 M/ml) and the highest values being V1.2 (0.86 M/ml), V2.2 (0.84 M/ml) and V3.2 (0.83 M/ml).

There are also significant differences in the unmulched rows, ranging from 0.63 (V4.3 M/ml) to 0.97 (V8.3 M/ml) and 0.91 (V1.3 M/ml) (Figure 1).

Comparing experimental variants V1-V8, there are significant differences, the lowest values being found in V4, V6, V3 variants and the highest in variants V1, V2, V5 and V8.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	0,82	0,99	0,73	0,82	0,89	0,80	0,88	0,88	0,85
Vn.2	0,86	0,84	0,83	0,62	0,71	0,64	0,71	0,65	0,73
Vn.3	0,91	0,73	0,74	0,63	0,85	0,69	0,80	0,97	0,79

Figure 1. Total polyphenols content in petals at 'Crown Princess Margareta' variey

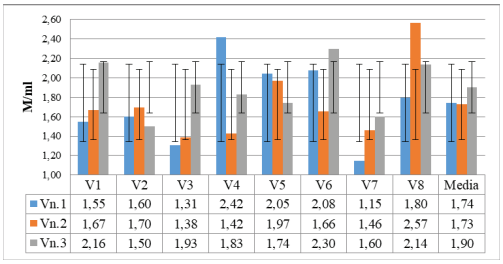
At the 'Falstaff' variety there are significant differences between variants.

In the wood chips mulched variants, the values range from 1.15 M/ml (V7.1) to 2.42 M/ml (V4.1).

In the wool mulched variants, the lowest values are present in variants V3.2 (1.38 M/ml), V4.2 (1.42 M/ml) and V7.2 (1.46 M/ml) and the highest values at V8.2 (2.57 M/ml).

In the non-mulched raws, the lowest values are in variants V2.3 (1.50 M/ml), V5.3 (1.74 M/ml) and V7.3 (1.60 M/ml) values in V6.3 (2.30 M/ml), V1.3 (2.16 M/ml) and V8.3 (2.14) (Figure 2).

Comparing the variants, there are significant differences, the smallest values being found in V7 and the highest in the V8.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	1,55	1,60	1,31	2,42	2,05	2,08	1,15	1,80	1,74
Vn.2	1,67	1,70	1,38	1,42	1,97	1,66	1,46	2,57	1,73
Vn.3	2,16	1,50	1,93	1,83	1,74	2,30	1,60	2,14	1,90

Figure 2. Total polifenols content in petals at 'Falstaff' variety

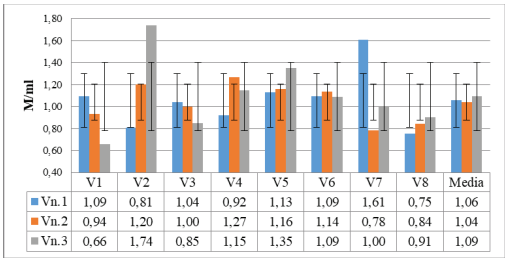
At the 'Brother Cadfael' variety there are also significant differences between variants.

In the wood chips mulched variants, the values range from 0.75 M/ml (V8.1) and 0.81 M/ml (V2.1) to 1.61 M/ml (V7.1), respectively.

In the wool mulched variants, the smallest values are present in variants V7.2 (0.78 M/ml) and V8.2 (0.84 M/ml) and the highest values at V4.2 (1.27 M/ml), V2.2 (1.20 M/ml), V5.2 (1.16 M/ml) and V6.2 (1.14 M/ml).

In the unmulched rows, the lowest value is present in variant V1.3 (0.66 M/ml) and the highest value at V2.3 (1.74 M/ml) (Figure 3).

By comparing the variants, the smallest values are found in the V8 variant and the highest in the variants V2 and V5.

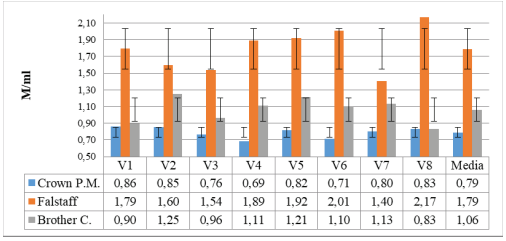


	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	1,09	0,81	1,04	0,92	1,13	1,09	1,61	0,75	1,06
Vn.2	0,94	1,20	1,00	1,27	1,16	1,14	0,78	0,84	1,04
Vn.3	0,66	1,74	0,85	1,15	1,35	1,09	1,00	0,91	1,09

Figure 3. Total polyphenols content in petals at 'Brother Cadfael' variety

There are significant differences between the total content of polyphenols in the three varieties of roses (Figure 4). 'Falstaff' variety has almost double values (1.79 M / ml) than 'Crown Princess Margareta' (0.79 M / ml) and

significantly higher than 'Brother Cadfael' (1.06 M / ml).



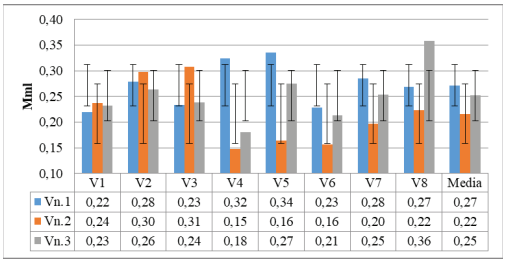
	V1	V2	V3	V4	V5	V6	V7	V8	Media
Crown P.M.	0,86	0,85	0,76	0,69	0,82	0,71	0,80	0,83	0,79
Falstaff	1,79	1,60	1,54	1,89	1,92	2,01	1,40	2,17	1,79
Brother C.	0,90	1,25	0,96	1,11	1,21	1,10	1,13	0,83	1,06

Figure 4. Total polyphenols content in petals at the three rose varieties

Total content in flavonoids.

In the 'Crown Princess Margareta' variety, in the wood chips mulched rows, the smallest values are V1.1 (0.22 M/ml), V3.1 (0.23 M/ml) and V6.1 (0.23 M/ml) M / ml) and the highest values are at V5.1 (0.34 M/ml) and V4.1 (0.32 M/ml). In the wool mulched rows, the lowest values are V4.2 (0.15 M/ml), V6.2 (0.16 M/ml) and V5.2 (0.16 M/ml), and the higher values at V3.2 (0.31 M/ml) and V2.2 (0.30 M/ml). There are also significant differences in the unmulched variants, ranging from 0.18 M/ml (V4.3) and 0.21 M/ml (V6.3) to 0.36 M/ml (V8.3).

By comparing the variants, the smallest values are in V6 and V4 variants and the highest in V8 and V2 variants (figure 5).



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	0,22	0,28	0,23	0,32	0,34	0,23	0,28	0,27	0,27
Vn.2	0,24	0,30	0,31	0,15	0,16	0,16	0,20	0,22	0,22
Vn.3	0,23	0,26	0,24	0,18	0,27	0,21	0,25	0,36	0,25

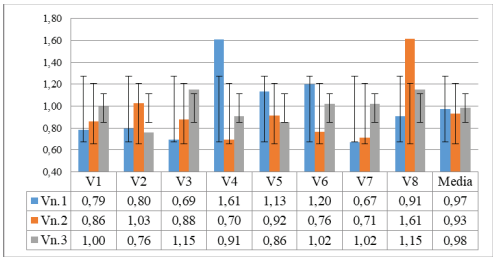
Figure 5. Total content of flavonoid in petals at 'Crown Princess Margareta' variety

At the 'Falstaff' variety there are significant differences between variants. In the wood chips mulched rows, the values range from 0.67 M/ml (V7.1) and 0.69 M/ml (V3.1) to 1.61 M/ml (V4.1), respectively.

On the wool mulched rows, the smallest values are present in variants V4.2 (0.70 M/ml) and V7.2 (0.71 M/ml) and the highest values at V8.2 (1.61 M/ml).

In the unmulched variants, the lowest value is present in variant V2.3 (0.76 M/ml) and the highest values are at V8.3 (1.15 M/ml) and V3.3 (1.15 M/ml) (Figure 6).

By comparing the variants, there are significant differences, the smallest values being found in the V7 and V1 variants, and the largest in the V8 variant.

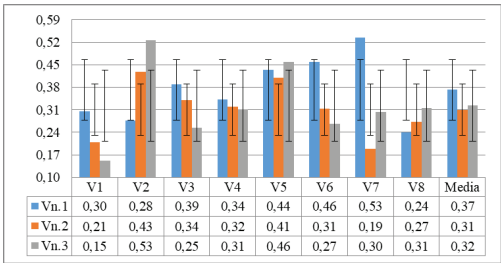


	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	0,79	0,80	0,69	1,61	1,13	1,20	0,67	0,91	0,97
Vn.2	0,86	1,03	0,88	0,70	0,92	0,76	0,71	1,61	0,93
Vn.3	1,00	0,76	1,15	0,91	0,86	1,02	1,02	1,15	0,98

Figure 6. Total content of flavonoid in petals at 'Falstaff' variey

There are also significant variations at the 'Brother Cadfael' variety. In the wood chips variants, the values range from 0.24 M/ml (V8.1) to 0.53 M/ml (V7.1). On the wool mulched rows, the smallest value is present at V7.2 (0.19 M/ml) and highest at V2.2 (0.43 M/ml). In the unmulched rows, the lowest value is present in variant V1.3 (0.15 M/ml) and the highest value at V2.3 (0.53 M/ml). (Figure 7)

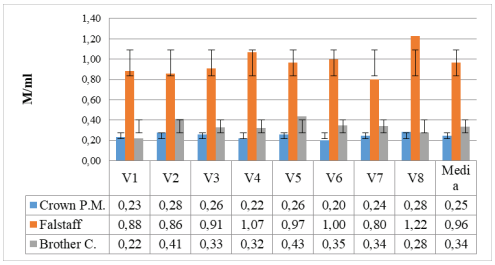
By comparing the variants, the smallest values are in V1 and the highest in V5.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	0,30	0,28	0,39	0,34	0,44	0,46	0,53	0,24	0,37
Vn.2	0,21	0,43	0,34	0,32	0,41	0,31	0,19	0,27	0,31
Vn.3	0,15	0,53	0,25	0,31	0,46	0,27	0,30	0,31	0,32

Figure 7. Total content of flavonoid in petals at 'Brother Cadfael' variety

By comparing the flavonoid content to the three varieties, there are significant differences between them (Figure 8). 'Falstaff' variety has values four times bigger (0.96 M/ml) than 'Crown Princess Margareta' (0.25 M/ml) and almost three times bigger than 'Brother Cadfael' (0.34 M/ml).



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Crown P.M.	0,23	0,28	0,26	0,22	0,26	0,20	0,24	0,28	0,25
Falstaff	0,88	0,86	0,91	1,07	0,97	1,00	0,80	1,22	0,96
Brother C.	0,22	0,41	0,33	0,32	0,43	0,35	0,34	0,28	0,34

Figure 8. Total content of flavonoid in petals at the three rose varieties

In general, there are significant positive correlations between the content of polyphenols and flavonoids in petals in all varieties of roses, in the experimental variants (Figures 9 and 10).

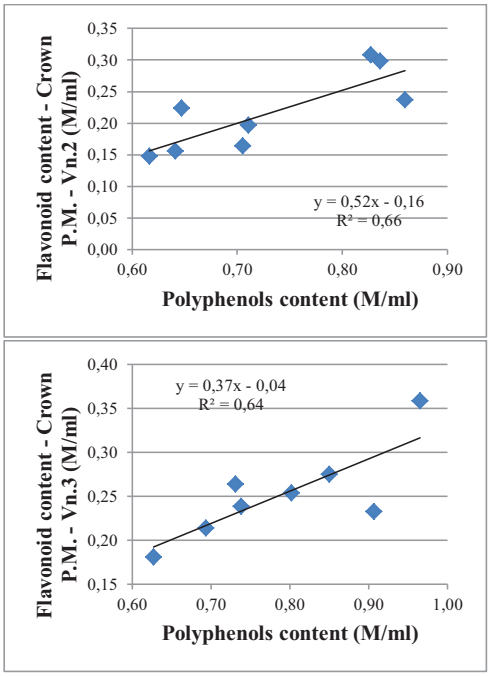


Figure 9. Correlations between flavonoid and polifenols content in fresh petals (Vn.2 and Vn.3)

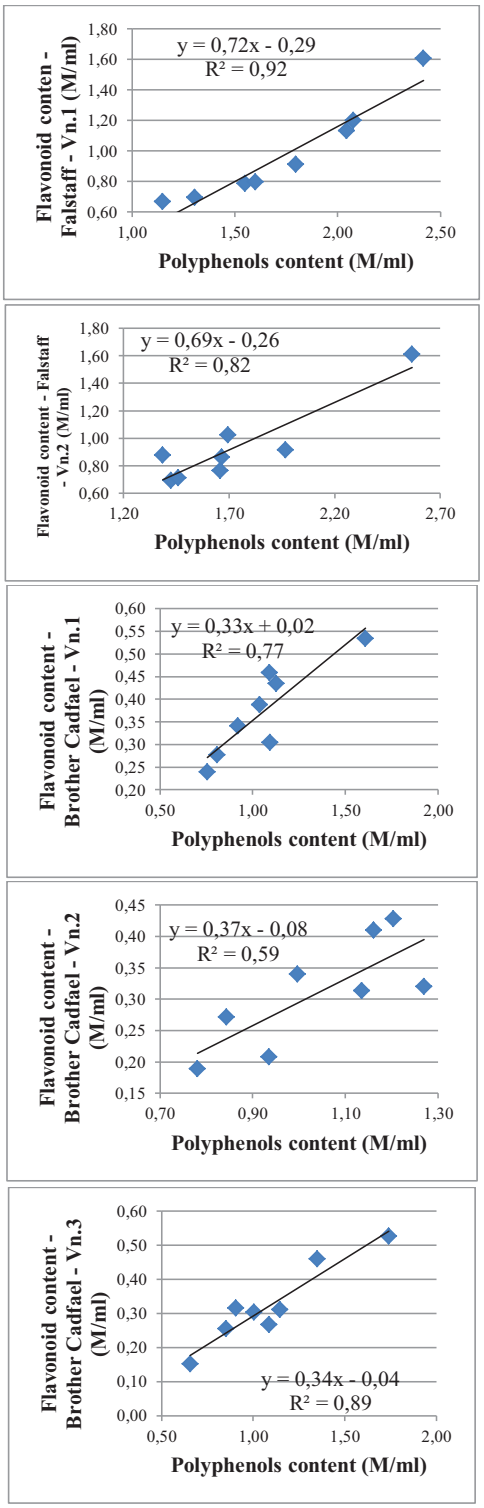


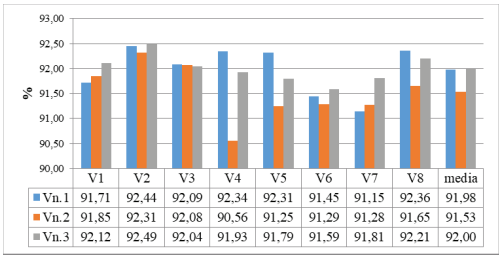
Figure 10. Correlation between flavonoid and polyphenols content în petale

Antioxidant capacity.

In 'Crown Princess Margareta' variety, the lowest values are present in variant V7.1 (91.15%) and the highest values are at V2.1 (92.44%). On the wool mulched rows, the lowest values are in V4.2 (90.56%) and the highest values are in V2.2 (92.31%) and V3.2 (92.08%). On the unmulched raws, the values range from 91.59% (V6.3) to 92.49% (V2.3) (Figure 11).

Comparing variants V1-V8, there are significant differences, with the lowest values being found in V7 variants and the highest in V2.

The values obtained are comparable to those determined by Sengul M. et al. (2017), who determined at *Rosa damascena* Mill. a high value of antioxidant activity, highlighting the importance of rose and its use alone or in combination with other herbal teas.

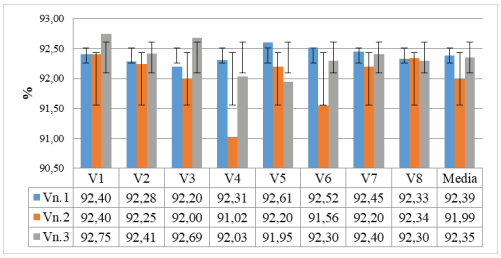


	V1	V2	V3	V4	V5	V6	V7	V8	media
Vn.1	91,71	92,44	92,09	92,34	92,31	91,45	91,15	92,36	91,98
Vn.2	91,85	92,31	92,08	90,56	91,25	91,29	91,28	91,65	91,53
Vn.3	92,12	92,49	92,04	91,93	91,79	91,59	91,81	92,21	92,00

Figure 11. Antioxidant capacity of fresh petals at 'Crown Princess Margareta'

At the 'Falstaff' variety there are significant differences between variants. On wood chips mulched rows, the values range from 92.20% (V3.1) to 92.61% (V5.1). In the wool mulched raws, the lowest values are present in variant V4.2 (91.02%) and the highest values are at V1.2 (92.40%). In the unmulched raws, the lowest value is present in variant V5.3 (91.95%) and the highest values at V1.3 (92.75%) (Figure 12).

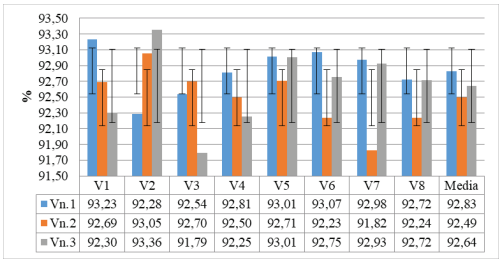
By comparing the variants, the smallest values are found in V4 and the highest in V1.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	92,40	92,28	92,20	92,31	92,61	92,52	92,45	92,33	92,39
Vn.2	92,40	92,25	92,00	91,02	92,20	91,56	92,20	92,34	91,99
Vn.3	92,75	92,41	92,69	92,03	91,95	92,30	92,40	92,30	92,35

Figure 12. Antioxidant capacity of fresh petals at 'Falstaff' variety

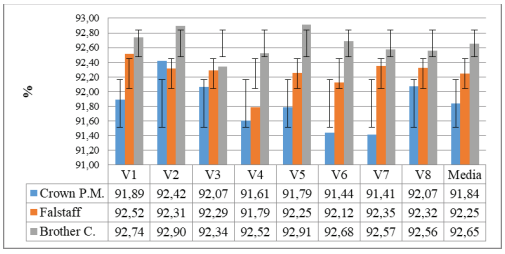
For the 'Brother Cadfael' variety, the values from the wood chips mulch raws ranged from 92.28 (V2.1) to 93.23% (V1.1). In the wool mulched variants, the smallest values are present in variant V7.2 (91.82%) and the highest values in V2.2 (93.05%). In the unmulched variants, the lowest value is present in variant V3.3 (91.79%) and the highest values are at V2.3 (93.36%) (Figure 13). By comparing the variants, the smallest values are in V3 and the highest in V5.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	93,23	92,28	92,54	92,81	93,01	93,07	92,98	92,72	92,83
Vn.2	92,69	93,05	92,70	92,50	92,71	92,23	91,82	92,24	92,49
Vn.3	92,30	93,36	91,79	92,25	93,01	92,75	92,93	92,72	92,64

Figure 13. Antioxidant capacity of fresh petals at 'Brother Cadfael' variety

By comparing the antioxidant capacity of the three varieties of roses, there are significant differences between them (Figure 14).

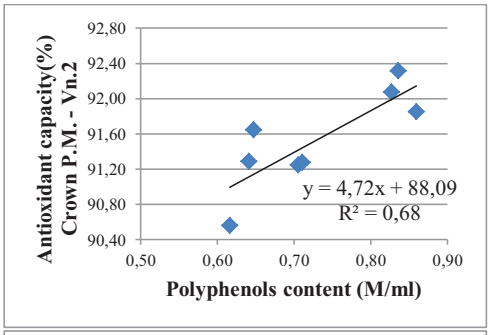


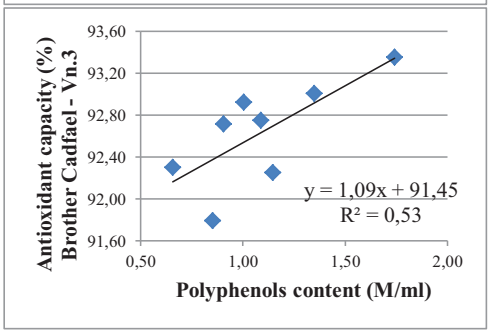
	V1	V2	V3	V4	V5	V6	V7	V8	Media
Crown P.M.	91,89	92,42	92,07	91,61	91,79	91,44	91,41	92,07	91,84
Falstaff	92,52	92,31	92,29	91,79	92,25	92,12	92,35	92,32	92,25
Brother C.	92,74	92,90	92,34	92,52	92,91	92,68	92,57	92,56	92,65

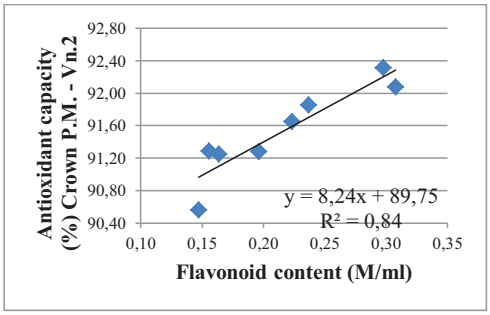
Figure 14. Antioxidant capacity of fresh petals to the three rose varieties

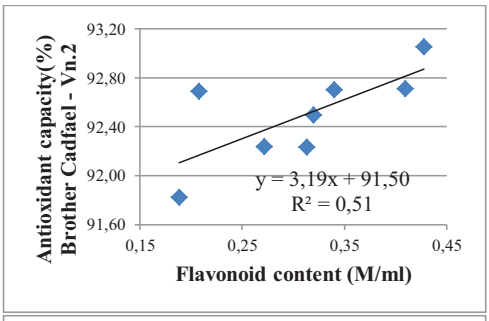
Significant positive correlations are noted between the antioxidant capability of petals and

the polyphenol content at the 'Crown Princess Margareta' variety in wool mulched variants and at the 'Brother Cadfael' variety in the unmulched variants. Also, significant positive correlations between the antioxidant capability of petals and the flavonoid content of the 'Crown Princess Margareta' and 'Brother Cadfael' varieties in wool mulched variants and the 'Brother Cadfael' variety in the unmulched variants (Figure 15).









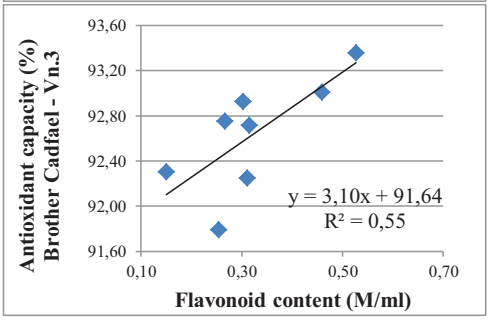


Figure 15. Correlation between antioxidant capacity and total polyphenols and flavonoids in fresh petals

Total polyphenols and flavonoid content, antioxidant capacity of rose petal jam

In Table 2 are presented the total content in polyphenols, flavonoids and the antioxidant capacity in the 9 rose petal jam variants.

Table 2. Total polyphenol, flavonoids and antioxidant capacity in rose petal jam

Variant	Polyphenol content (M/ml)	Flavonoid content (M/ml)	Antioxidant capacity (%)
D1	0.08±0.01	0	88.58±0.00
D2	0.17±0.01	0.25±0.01	93.15±0.00
D3	0.10±0.02	0	92.36±0.00
D4	0.07±0.00	0	87.82±0.00
D5	0.13±0.00	0.23±0.02	92.95±0.00
D6	0.08±0.00	0.02±0.00	92.64±0.00
D7	0.07±0.00	0.02±0.01	88.31±0.00
D8	0.15±0.00	0.29±0.01	92.91±0.00
D9	0.08±0.00	0.04±0.01	92.76±0.00

The values of total polyphenol content differ significantly between variants, those with 'Falstaff' petals (D2, D5 and D8, respectively) having almost double values compared to variants in the other two varieties. For all three varieties, the polyphenol content of rose petal jam is much lower than in fresh petals.

Variants of the 'Falstaff' variety have similar flavonoid values, significantly higher than those of the other variants, where the values are low or 0.

The values for antioxidant capacity are high in all variants. 'Crown Princess Margareta' jam has an average value of 88.24%, less than in fresh petals. The 'Falstaff' variety averages 93.00% similar to those of fresh petals and the 'Brother Cadfael' variety averages 92.59%, similar to those of fresh petals.

Total polyphenols and flavonoid content, antioxidant capacity of dry petals

In Table 3 are presented total content in polyphenols, flavonoids and antioxidant capacity in the dehydrated petals of the three varieties.

Table 3. Polyphenol, flavonoids and antioxidant capacity in the dehydrated petals

Variant	Polyphenol content (M/ml)	Flavonoid content (M/ml)	Antioxidant capacity (%)
'Crown Princess Margareta'	7.81±0.15	2.09±0.09	90.30
'Falstaff'	15.74±0.90	6.88±0.11	86.79
'Brother Cadfael'	8.91±0.07	2.41±0.11	90.20

Polyphenol content values differ significantly between variants, those of the 'Falstaff' variety having almost double values than those of the other two varieties. For all three varieties, the polyphenol content in petals is much higher than in fresh petals. Values of flavonoids in dehydrated petals also exceed the values found in fresh petals, the 'Falstaff' variety having more than double values than the other two varieties.

Antioxidant capacity values are high in all variants, for 'Crown Princess Margareta' and 'Brother Cadfael', similar to those in fresh petals. The 'Falstaff' variety has a smaller value than in fresh and jam.

CONCLUSIONS

Antioxidant capacity was similar in all the three variants. Total phenolic and flavonoid content varied according to the experimental variants, 'Falstaff' registering the highest amount. Dried petals registered similar biochemical parameters or higher than the fresh or processed ones, drying being recommended as an efficient preserving method.

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EFFECTIVENESS OF TREATMENTS ON THE ATTACK OF *POLYSTIGMA RUBRUM* PATHOGENS AND *STIGMINA CARPOPHILA* ON PLUM IN ŞOIMARI LOCATION, PRAHOVA COUNTY

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Abstract

The work studies the occurrence of Polystigma rubrum and Stigmina carpophila pathogens which are frequent yearly in plum orchards with varying degrees of attack intensity. It was followed by the attack of the two pathogens in the period 2017-2018 in the 'Stanley', 'Anna Spath' and 'Gras românesc' varieties. It was found that the attack of the Polystigma rubrum pathogen had values in 2018 ranging from 31% to the 'Stanley' variety and 19.5% to the' Gras românesc' variety. In the case of the Stigmina carpophila fungus attack, the most severe attack was 38% in the year 2017. The effectiveness of prophylactic and therapeutic treatments ranged between 65% and 71% during the analyzed period.

Key words*: cultivar, degree of attack, effectiveness, fungus, plum.*

INTRODUCTION

Polystigma rubrum and *Stigmina carpophila* are micromicetes found yearly in plum orchards in Romania.

Plum diseases produced by *Polystigma rubrum*, *Stigmina carpophila* and *Monilia laxa* are common in conditions of natural infection in the climatic conditions in north-west of Romania and cause significant losses (Mitre et al., 2015).

Polystigma rubrum are responsible for the disease, called plum-leaf blister, and *Stigmina carpophila* causes shot-hole disease of plum (Gheorghies & Geaman, 2003; Bubici et al., 2010). Iliev and Stoev (2011) show that *Polystigma rubrum* is one of the most common diseases of the plum. Stoev et al. (2017) shows, that plum-leaf blister manifested itself in all 7 varieties investigated.

Under the conditions of Bulgaria from 1996-2003, the Stanley and Anna Spath varieties were tolerant of diseases such as red patches of leaves (Vitanova et al., 2004).

The study of the biological parameters of the *Stigmina carpophila* fungus revealed the development of fungal colonies under certain *in vitro* conditions (Văcaroiu et al., 2009).

MATERIALS AND METHODS

The research has followed the evolution of the *Polystigma rubrum* and *Stigmina carpophila* pathogen attacks on plum leaves 'Stanley', 'Anna Spath' and 'Gras românesc' cultivars in a classical orchard in Şoimari, Prahova County. The frequency of the attack (F %), the intensity (I %) and the attack rate (GA %) on the leaves for the two years studied were determined. The following formulas were used: F = n x 100/N, in which n = the number of attacked plants/ organs, N = total plant/organ analysis, attack intensity was calculated using formula I = Σ (i x f) where: i = the percentage of the attack, f = the number of organs/plants with the respective attack percentage, n = the total number of attacked organs/plants analyzed, GA = F x I/100, where F = attack frequency, I = attack intensity. The fungal attack was monitored in control and variants: 2% bordeleza, 2% Confidor oil 1.5%, 0.2% Topsin WDG, and Luna experience 400 SC 0.05%, Signum FG 0.5%. Efficacy of treatment (E%) was calculated according to the formula E (%) = [(GAc-GAv)/GAm] x 100, where GAc = control; Gav = degree of attack in the treated alternative.

RESULTS AND DISCUSSIONS

The data in Table 1 show that in plum varieties monitored as control, the attack of plum-leaf blister and shot-hole disease of plum had a maximum incidence (F = 100%) during the analyzed period. In 2017 in the 'Stanley' cultivar, the attack of the *Polystigma rubrum* recorded an intensity of 29% and in 2018 31%. In the same variety, the *Stigmina carpophila* fungus attack on the leaves had an intensity of 38% and in the year 2018 36%. In the 'Anna Spath' variety, it was found that the intensity of the *Polystigma rubrum* attack was higher in 2018 (I = 28%) than in 2017 (I = 22.5%). The attack of leaf mycotic attack on the same variety recorded a lower degree of attack in 2018 (GA = 31.5%) compared to 2017, when the attack rate was 34%. The 'Gras românesc' cultivar recorded the smallest values of the disease attack monitored between 2017 and 2018. Thus, the attack of the *Polystigma rubrum* fungus was 19.5% in 2017 and 21.5% in 2018. Regarding the *Stigmina carpophila* fungus attack, it had values of about 30% during this period.

Table 1. Fungus attack *Polystigma rubrum* and *Stigmina carpophila* on plum (2017-2018) in Şoimari location, Prahova County

Variety	Year	Pathogen/disease					
		Polystigma rubrum/ plum-leaf blister			*Stigmina carpophila*/ shot-hole disease of plum		
		F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)
'Stanley'	2017	100	29	29	100	38	38
	2018	100	31	31	100	36	36
'Anna Spath'	2017	100	22.5	22.5	100	34	34
	2018	100	28	28	100	31.5	31.5
'Gras românesc'	2017	100	19.5	19.5	100	30.5	30.5
	2018	100	21.5	21.5	100	30	30

The prophylaxis and therapy of these diseases was provided by treatments with the products presented in Table 2. Their application was done in the recommended concentrations (Henegar & Andru, 2013) the data and the phenophase presented in Table 2. The products selected for application, applied against these diseases had an effect on other pathogens (e.g., *Monilinia laxa*) and were also accompanied by insecticide treatments against plum pests. Thus, treatment with Topsin WDG (0.2%) was associated with the insecticide Calypso 480SC (0.02%), and the fungicid Luna Experience 400CS (0.05%) and Signum FG (0.5%) with the insecticides Mospilan 20SG (0.045%) and Novadim Progress EC (0.075%) and insecticides Calypso 480SC (0.02%) and Mospilan 20SG (0.045%). Including copper-containing preparations can prevent diseases such as brown rot and red leaf spots (Stoev et al., 2017). The fungicide treatments were effective in controlling brown rot in different tree species (Popa et al., 2013; Chiţulescu & Cristea, 2017).

Table 2. Treatment scheme applied to control *Polystigma rubrum* and *Stigmina carpophila* pathogens on plum 2017-2018 in Şoimari location, Prahova County

The product	Concentration (%); dose (l, kg/ha)	Phenophase	Date of administration
Zeama Bordeleza	2%	Vegetative retention	15.03
Confidor oil	1.5%	Vegetative retention	05.03
Topsin WDG (+ Calypso 480SC)	02% (+0.02%)	Green button	05.04
Luna experience 400 SC (+ Mospilan 20SG)	0.05% (+0.045%)	White button	20.04
Signum FG (+ Calypso 480SC)	0.5% (+0.02%)	Flowering corolla10-15%	04.05
Luna experience 400 SC (+Novadim Progress EC)	0.05% (+0.075%)	Shake of the petals 10-15%	17.05
Signum FG (+ Mospilan 20SG)	0.5% (+0.045%)	Fruit development	01.06

The treatments applied significantly reduced the attack of fungi *Polystigma rubrum* and *Stigmina carpophila* against the control variant (Table 3).

In the 'Stanley' cultivar, in 2017 plum-leaf blister fell to 9.5%, compared with 29% in the control variant, and the attack of shot-hole disease reached 13%, compared to the untreated variant at which GA = 38%. In 2018 the attack of pathogens monitored in this variety was significantly reduced to the treated version.

In the 'Anna Spath' cultivar, application of fungicides reduced the attack that reached 7.5% and 8.5% in the case of the *Polystigma rubrum* pathogen, and 11% and 9.5% in the attack of *Stigmina carpophila* in the years 2017 and 2018 relative to the control variant (Table 3).

As for the 'Gras românesc' cultivar, it was found that the intensity of the attack had the lowest values for both pathogens analyzed following the application of the treatment scheme.

The effect on the plum-leaf blister was noted in 2017 when the attack reached 6% in the variant treated against the control where GA = 19.5%.

Table 3. The influence of the *Polystigma rubrum* and *Stigmina carpophila* on plum (2017/2018) in Şoimari location, Prahova County

Cultivar	Year	Variant Untreated (control) / treatment	Pathogen / disease					
			Polystigma rubrum/ plum-leaf blister			*Stigmina carpophila*/ shot-hole disease of plum		
			F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)
'Stanley'	2017	Control	100	29	29	100	38	38
		Treatment	100	9.5	9.5	100	13	13
	2018	Control	100	31	31	100	36	36
		Treatment	100	9	9	100	10.5	10.5
'Anna Spath'	2017	Control	100	22.5	22.5	100	34	34
		Treatment	100	7.5	7.5	100	11	11
	2018	Control	100	28	28	100	31.5	31.5
		Treatment	100	8.5	8.5	100	9.5	9.5
'Gras românesc'	2017	Control	100	19.5	19.5	100	30.5	30.5
		Treatment	100	6	6	100	9.0	9.0
	2018	Control	100	21.5	21.5	100	30	30
		Treatment	100	6.5	6.5	100	8.5	8.5

The effectiveness of the treatments applied against the pathogens monitored was calculated and it was found to have varied between 66.6% for 'Anna Spath' cultivar on the control of the *Polystigma rubrum* pathogen and 69.2% for the 'Gras românesc' cultivar (2017), 69% for the 'Anna Spath' and 'Gras românesc', 71% at Stanley cultivars (2018).

Regarding the efficacy of the treatments in controlling the *Stigmina carpophila* pathogen, the highest values were determined for the 'Stanley' cultivar (2018), and over 70% for the 'Gras românesc' cultivar during the analyzed period (Table 4).

Table 4. Effectiveness of the treatments applied in the control of the pathogens *Polystigma rubrum* and *Stigmina carpophila* (2017-2018) in Şoimari location, Prahova County

Cultivar	Year	Pathogen/disease				
		The variant untreated (control) / treatment	*Polystigma rubrum*/ plum-leaf blister		*Stigmina carpophila*/shot-hole disease of plum	
			GA (%)	E (%)	GA (%)	E (%)
'Stanley'	2017	Control	29	67.2	37	64.8
		Treatment	9.5		13	
	2018	Control	31	71	36	71
		Treatment	9		10.5	
'Anna Spath'	2017	Control	22.5	66.6	34	66.2

		Treatment	7.5		11.5	
	2018	Control	28	69.6	31.5	69.8
		Treatment	8.5		9.5	
'Gras românesc'	2017	Control	19.5	69.2	30.5	70.5
		Treatment	6		9	
	2018	Control	21.5	6.8	30	71.6
		Treatment	6.5		8.5	

CONCLUSIONS

The frequency of the attack was maximum during the analysis period in all the varieties monitored. Micromicete of *Polystigma rubrum* was more virulent in 2018, and the pathogen *Stigmina carpophila* recorded a lower attack in the same year. The treatments applied significantly reduced the attack of the pathogens monitored. The effectiveness of fungicides intervention ranged between 65% and 71%.

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STUDY OF MORPHOLOGIC CHARACTHERISTICS OF SOME FRUITS LESS KNOW IN ROMANIAN POMICULTURE

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Abstract

The researches were carried at the Frasinu Society in Buzău County, located on the outskirts of Buzău. The orchard was established in the spring of 2010, with the 'Caroa de Rei', 'Sharon', 'Ogosho' and 'Rojo Brillante' varieties, of nine years old. The trees were planted at a distance of 4 x 4 m. Four variants (represented by the variety), cultivated in 4 repetitions, were studied. Observations on the fruits were made from 2014 until 2018. Determinations were made on the average fruit weight, the height of the fruit and the diameter of the fruit and the shape index was calculated. From the chemical point of view, determinations have been made regarding the content in soluble solids determined by refractometry. The largest fruits were obtained in the 'Sharon' variety (190 g on average), followed by the 'Rojo Brillante' (180 g) and 'Ogosho' (174 g). The fruits of the 'Caroa de Rei' variety recorded a shape index of 1.14, and the 'Ogosho' variety had the lowest value of the shape index (0.64), the fruits being of the flattened form. The kaki fruit is sweet, due to its high content in soluble solids. The varieties studied had values higher than 10%, the sweetest variety being 'Caroa de Rei' (17.12%). Considering the quality of the fruits and the behavior of the trees during the experiments we consider that this species can be cultivated successfully in Romania.

Key words*: fruits, Japanese persimmon, shape index.*

INTRODUCTION

The kaki species (*Diospiros kaki*), experimentally cultivated in Romania, is a tree species that can be grown in warm areas. Known by its fruit present on the shelves of the big stores, it has been appealing to consumers in Romania in a short time, due to its special taste.

Diospiros kaki is known from the earliest times, fruits are considered to be the gods' food. It forms a globular crown and reaches heights of 6-8 m (Cepoiu N., 2001). The foliage is rich and leaves are is very large in size compared to other species. The relatively small greenish yellow flowers are solitary or clustered. They are formed from small buds and functional masks, females or hermaphrodites. The fruits are large, spherical, elongated, or may have a collar at the top (Păun C, 2017).

They have orange or red color, resemble mature tomatoes, are astringent when not ripened, they are consumed after a storage period (Cepoiu et al., 2005). They have a specific taste of ripe pumpkin or pumpkin pie. They have a very complex chemical composition (Table 1):

Table 1. Nutritional value per 100 g fruit pulp

Calories	70 Kcal	Total fats acid saturated	0.02 g
Protein	0.58 g	Total fats acid monosaturated	0.037 g
Lipid	0.19 g	Cholesterol	0 mg
Carbohidrates	18.59 g	Phytosterol	4 mg
Fiber	1.7 g		
AMINOACIDS (g)			
Tryptophan	0.01	Threonine	0.03
Alanine	0.029	Isoleucine	0.025
Leucine	0.042	Lysine	0.033
Fenil-alanine	0.05	Methionine	0.026
Cisteine	0.013	Thyrosine	0.016
Valeine	0.03	Arginine	0.025
Histidine	0.012	Serine	0.022
acid-aspartic	0.057	acid-glutamic	0.076
Glycine	0.025	Proline	0.022
VITAMINS (mg)			
Vitamin B_1	0.03	Vitamin C	7.5
Vitamin B_2	0.02	Vitamin A	217
Vitamin B_3	0.1	Vitamin E	0.59
Vitamin B_6	0.1		
MINERALS			
Ca	8 mg	Na	1 mg
P	17 mg	K	161 mg
Zn	0.11 mg	Cu	0.113 mg
Mn	0.335 mg	Se	0.6 µg

Source: Fichier canadien sur les éléments nutritifs, 2001b

MATERIALS AND METHODS

The observations were made during 5 years (2014-2018) at Frasinu S.A. from Buzău County. Four varieties with large fruit from Portugal were studied.
At first, the experience was aimed at studying the adaptability of this species to the conditions in our country, knowing that it preferred tropical and subtropical areas.
The 'Caroa de Rei', 'Sharon', 'Ogosho', 'Rojo Brillante' varieties have been studied under various aspects.
The trees were planted according to the scheme of a normal planting with a distance of 4 m between rows and 4 m between plants per row.
Observations have been made on the growth and development of varieties and the evolution of the foliar surface until the fructification has been established, and after that, the characteristics of the fruit have been studied.
The fructification was installed three years after planting, and production increased visibly from one year to the next.
During the 5 years of study, the temperatures during the winter were very low. In the winter of 2014, temperatures dropped to -25°C, and in 2016 to -30°C. As a result, in 2014 a large part of the fruit branches was affected and very few fruits were obtained.
The fruits were analyzed annually in the Horticultural Products Laboratory, determining their weight, fruit height, fruit diameter in the two-way median area, USS content and storage capacity.
On the crowns were not made the specific interventions, the plants being allowed to evolve freely, having the ability to adjust the size of the shoots.
Observations have been made regarding the evolution of the foliar surface of each variety. The large foliar surface can explain the high productive potential of this species.
Fruits were analyzed concerning the external appearance and chemical analyses were performed with respect to USS content.
The average weight was determined by weighing 30 fruits obtained as a result of the average analytical sample technique.

RESULTS AND DISCUSSIONS

The fruits of the kaki species (Japanese persimmon) developed very well every year of experimentation (Table 2). The data presented in Table 2 represents the average fruit weight of the 4 varieties of kaki.

Table 2. Average weight of fruits (g)

Variety	Year of experimentation					Average
	'14	'15	'16	'17	'18	
'Caroa de Rei'	140	110	135	115	125	125
'Sharon'	290	160	150	170	180	190
'Ogosho'	150	160	170	200	190	174
'Rojo Brillante'	220	180	170	180	150	180

It can be noticed that there are small differences between the fruit size from one year to the next. The largest fruits were obtained in the 'Sharon' variety (190 g on average), followed by the 'Rojo Brillante' and 'Ogosho' varieties (Figure 1).

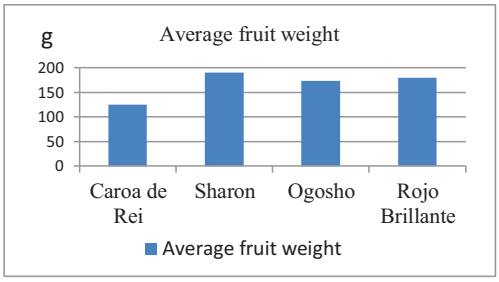

Figure 1. Average fruit weight of kaki varieties

All the studied varieties produced large fruit.
The fruit of the kaki species reaches a height of 40 to 71 mm (Table 3). The highest fruits formed 'Caroa de Rei' variety, in the 4 years of experimentation, the height of the fruit was between 62 and 71 mm.

Table 3. The height of the kaki fruit (mm)

Variety	Year of experimentation					Average
	'14	'15	'16	'17	'18	
'Caroa de Rei'	70	60	71	62	63	65.2
'Sharon'	65	51	53	52	52	54.6
'Ogosho'	46	45	47	53	51	48.4
'Rojo Brillante'	50	50	50	50	40	48.0

Because the fruits are not round, two measurements were made perpendicular on the middle of the fruit. Thus, the differences between the two measurements show the degree of asymmetry of the fruit.
The analysis of the data presented in Table 4 shows that the fruits are slightly asymmetrical in the four varieties.

Table 4. Determination of kaki fruit diameter (mm)

Variety	Year of experimentation					Ave rage
	’14	’15	’16	’17	’18	
‘Caroa de Rei’	60.0	55.6	58,4	54.2	58.4	57.3
‘Sharon’	86.0	73.0	68.0	71.0	74.3	74.4
‘Ogosho’	72.8	76.1	74.4	76.9	76.5	75.4
‘Rojo Brillante’	70.4	70.0	65.4	70.2	60.7	67.4

In order to determine the shape index, the relationship between the fruit height and the average of the two diameters was made (Figure 2).

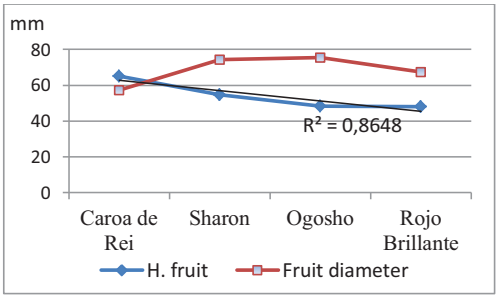

Figure 2. The correlation between high fruit and fruit diameter

The data presented in Table 5 shows that the fruits of the ‘Caroa de Rei’ variety had the value of a form greater than 1 each year of experience, the fruits being elongated. The other varieties had lower values of the shape index, the fruits being spherical.

Table 5. Shape index determination

Variety	Year of experimentation					Ave-rage
	’14	’15	’16	’17	’18	
‘Caroa de Rei’	1.16	1.07	1.21	1.19	1.08	1.14
‘Sharon’	0.75	0.69	0.77	0.73	0.70	0.72
‘Ogosho’	0.63	0.60	0.64	0.69	0.66	0.64
‘Rojo Brillante’	0.71	0.72	0.66	0.71	0.66	0.69

The kaki fruits are astringent and cannot be eaten immediately after harvesting. Content in USS grows as the fruit matures in the warehouse to a maximum, at which point the fruit pulp becomes soft. The soluble dry matter content is between 14.0 and 17.5%, depending on the variety and the year of experimentation (Table 6, Figure 3).

Table 6. Determination the fruit content in U.S.S (%)

Variety	Year of experimentation					Ave-rage
	’14	’15	’16	’17	’18	
‘Caroa de Rei’	17.4	17.0	16.8	17.4	17.0	17.12
‘Sharon’	14.0	15.4	16.0	14.8	15.0	15.04
‘Ogosho’	14.5	15.0	14.8	14.4	14.7	14.68
‘Rojo Brillante’	17.5	17.3	17.5	17.2	17.5	17.4

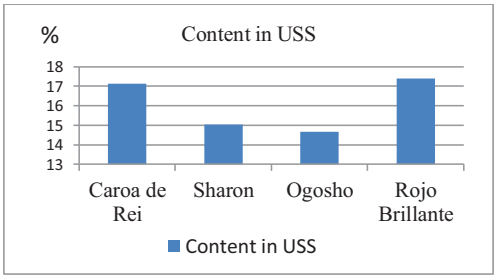

Figure 3. The content of pulp in USS

CONCLUSIONS

The ‘Sharon’ variety has formed the biggest fruit - 190 g. The highest height of the fruit was observed in the ‘Caroa de Rei’ variety - 65.2 mm. The ‘Sharon’ and ‘Ogosho’ varieties had fruit with similar diameter - 74.4 mm, respective 75.4 mm. Only the ‘Caroa de Rei’ variety had elongated fruit (1.14 shape index), the other varieties having spherical shaped fruit. The ‘Caroa de Rei’ and ‘Rojo Brillante’ varieties had fruit with the highest content in USS - 17.12%, respective 17.4%.
The four varieties can be grown successfully, because the fruits are tasteful and attractive (shape and color) being appreciated by consumers. The trees have resisted the most difficult environmental conditions in the winter and summer.

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BEHAVIOUR OF SOME NEW ROMANIAN PEAR DISEASE RESISTANT GENOTYPES CULTIVATED IN BUCHAREST AREA

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Abstract

In the last 30 years, pears orchards area diminished dramatically in Romania due to several factors, the most important being the sensitivity to some pests (pear psylla - Cacopsylla pyricola Förster) and diseases (fire blight - Erwinia amylovora Burrill). In the same time, the market demand for pears is increasing and the prices are continuously high. The aim of this study is to present the behaviour of some new Romanian pear disease resistant genotypes, produced at the Voineşti Research Station for Fruit Growing and planted in the Experimental Orchard of the Faculty of Horticulture within USAMV Bucharest. Six varieties and two new hybrid selections grafted on quince (CTS 212), on pear (Farold 40) and on own roots, in vitro propagated, were analysed. The planting distances varied from 3.0 x 0.8 m, for Parallel U to 3.0 x 1.6 m, for Trident canopy. The study presents the comparative biometrical data for each genotype respectively: average tree height, type, number and average length of annual fruiting shoots, trunk cross sectional area. Estimation of yield and fruits characteristics at the harvest moment are detailed.

Key words*: Pyrus communis, varieties, rootstock, canopy, Trident, Parallel U, fruits parameters.*

INTRODUCTION

In the last 30 years, pears orchards area diminished dramatically in Romania due to several factors, the most important being the sensitivity to some pests (pear psylla - *Cacopsylla pyricola* Förster) and diseases (fire blight - *Erwinia amylovora* Burrill). In the same time, the market demand for pears is increasing and the prices are continuously high. The aim of this study is to present the behavior of some new Romanian pear disease resistant genotypes, produced at the Voineşti Research Station for Fruit Growing and planted in the Experimental Orchard of the Faculty of Horticulture within USAMV Bucharest.

Pear breeding program had as objectives new varieties with disease resistance (*Venturia pirina*, *Erwinia amylovora*), tolerance to black and white spot (*Fabraea maculate*, *Mycosphaerella sentina*), fumagine (*Capnodium salicinum*) and *Psylla* sp.; extended period for winter season, good fruit appearance, high biological potential and adaptability (Andreieş, 2002; Andreieş, 2017). Few studies were presented with the new varieties from Voinesti pear breeding program, most of them with the pomological characterization of the cultivars.

MATERIALS AND METHODS

The trees were planted in 2014. Six varieties and three new hybrid selections 'Corina', 'Cristal', 'Euras', 'Orizont', 'Romcor', 'Tudor', H12/83/79, H5/5/84, R3/146 grafted on quince (CtS 212), on pear (Farold 40/Pear seedling) and on own roots, *in vitro* propagated, were analysed.

The planting distances varied from 3.0 m x 0.8 m, for Parallel U to 3.0 m x 1.6 m, for Trident canopy.

'Corina' is resistant to scab and tolerant to fire blight with medium vigour tree. Fruits have medium size, conical shape, yellowish - green skin colour, white flesh, fondant pulp. Ripening time is between October - November.

'Cristal' is resistant to scab and to fire blight with medium vigour tree. Fruits have medium-large size, conical shape, yellowish-green skin colour, white flesh, fondant pulp. Ripening time is between October-November.

'Euras' is resistant to scab, tolerant to fire blight and *Psylla* sp., medium tree vigour, fruits

with medium size, ovoid shape, semi-fondant pulp at full maturity. Ripening time is in October-April.
'Orizont' is resistant to scab, tolerant to *Psylla* sp., medium tree vigour, fruits with medium-large size, globular shape, yellow skin colour, semi-fondant pulp. Ripening time is between December-February.
'Romcor' is resistant to scab, no fire blight attack symptoms and tolerant to *Psylla* sp. The tree has middle to strong vigour. Fruits have medium to large size, conical shape, yellow-green skin colour, fondant pulp. Ripening time is between October-January.
'Tudor' is tolerant to scab, fire blight and *Psylla* sp. with medium to strong vigour of tree. Fruits have large size, pyriform, light yellow covered with bright red on about half of the fruit. Ripening time is between September-October (Andreieș, 2017; Branişte et al., 2007; Ghena et al., 2004; Asănică & Hoza, 2013; Grădinariu, 2002; Branişte et al., 2008).
Farold 40 (Daygon) is one of american OHF Selection (Old home x Farmingdale). The grafted cultivars have high vigour and good productivity (Hoza, 2000; Ghena & Branişte, 2003; Cimpoieș, 2018).
CTS 212 is a quince rootstock obtained in Italia (Pisa), with a good rooting system, high productivity to cultivars grafted on it and good fruit quality (Grădinariu, 2002; Cimpoieș, 2018).
The present study presents the comparative biometrical data, in 2018-2019 periods, for each genotype, respectively: average tree and trunk height; trunk cross sectional area; type, number and average length of annual flowering shoots. Estimation of yield and fruits characteristics at the harvest moment are detailed.

RESULTS AND DISCUSSIONS

Tree vigour

'Orizont' cultivar grafted on Farold 40 registered the biggest tree high (3.33 m). 'Tudor' on own roots had the smallest hight (1.38 m) (Figure 1). The cultivars self-rooted presented smallest heights than the cultivars on rootstocks.

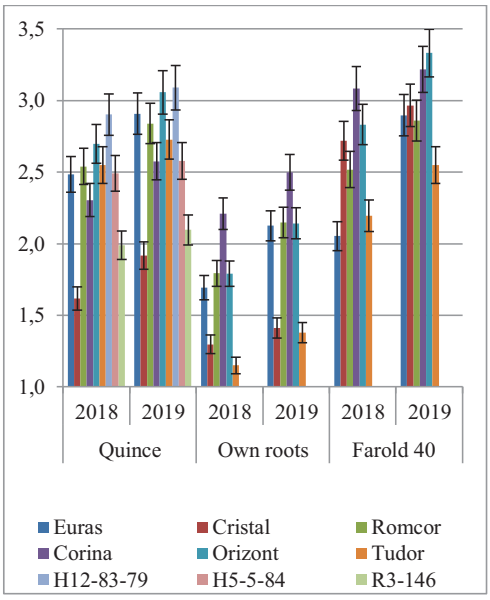

Figure 1. Comparison of tree height between varieties and rootstocks (m)

The cultivars self-rooted presented the smallest values also for trunk height comparing with the cultivars on rootstocks (Figure 2). Trunk height was bigger on Farold 40 rootstock for al cultivars and hybrids excepting 'Cristal', were quince rootstock registered for trunk height a bigger value.
The results are similar with the findings of Cimpoieș (2018), Ghena et al. (2004), Ghena and Branişte (2003), Hoza (2003).

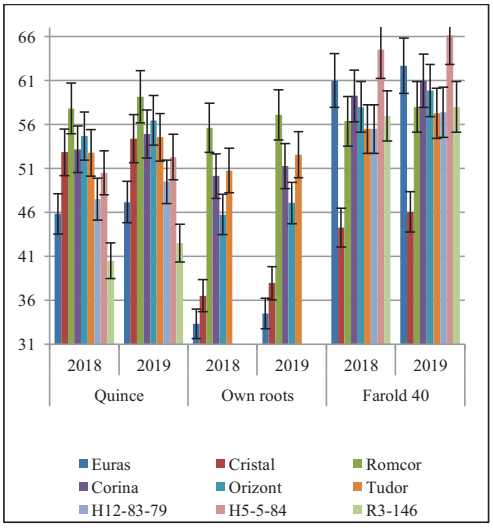

Figure 2. Trunk height influenced by cultivar and rootstock

Farold 40 rootstock led to more vigorous tree than the quince and self-rooted one, excepting 'Tudor'. The cultivars self-rooted presented the smallest values for trunk cross sectional area (TCS) comparing with the rootstocks used (Figure 3).

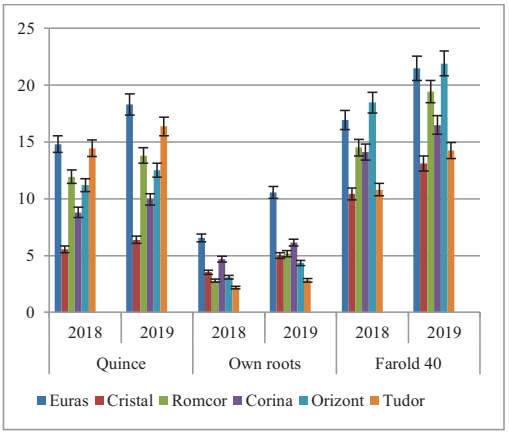

Figure 3. TCS comparison between varieties and rootstocks

The flower shoots number increased in 2019 comparing with 2018, influenced by cultivars, climatic factors and technology.

One of the main characteristic of these new cultivars is the production mainly on short flowering shoots, excepting 'Orizont' cultivar with long flowering shoots (Figures 4-8) (Andreieș, 2017; Asănică & Hoza, 2013; Grădinariu, 2002).

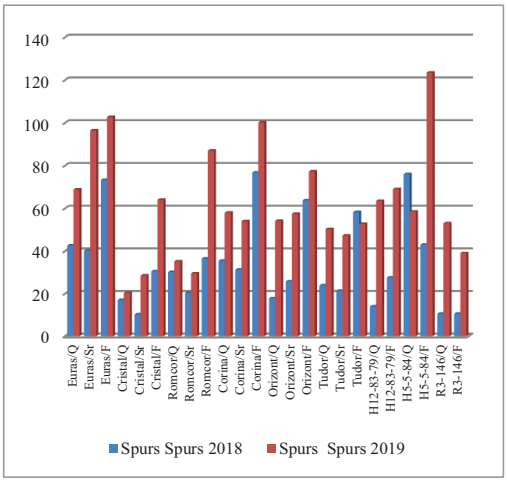

Figure 4. Spurs number between 2018-2019

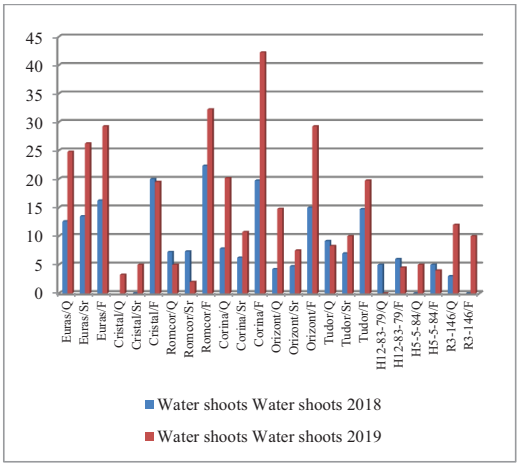

Figure 5. Water shoots number between 2018-2019

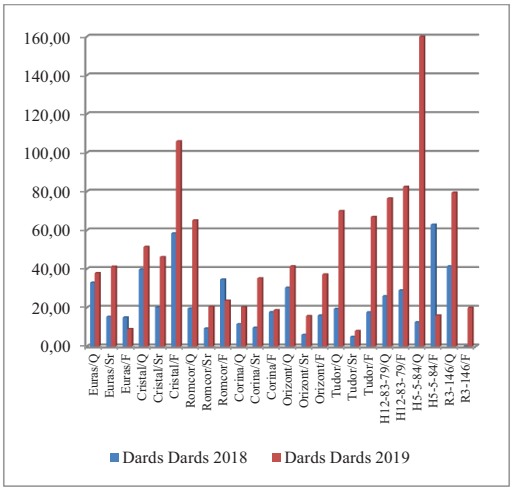

Figure 6. Dards number between 2018-2019

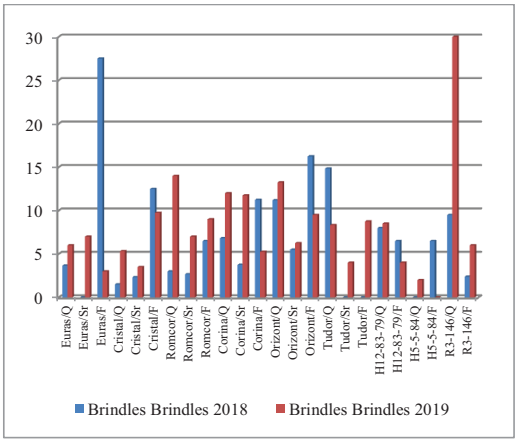

Figure 7. Brindles number between 2018-2019

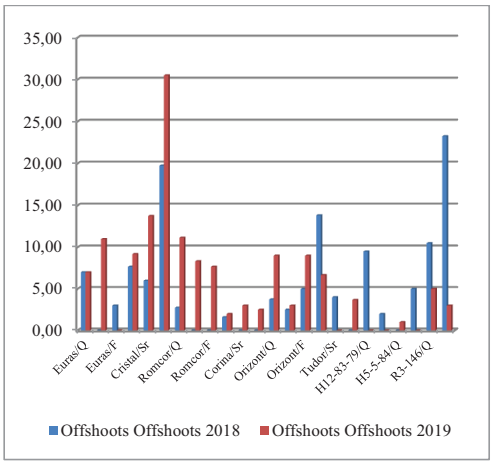

Figure 8. Offshoots number between 2018-2019

Total vegetative length of flowering shoots incresed also in 2019 comparing to 2018.
Farold 40 rootstock led to the highest vegetative growth to all cultivars. 'Corina' (2,496 cm), 'Cristal' (2,330 cm), 'Romcor' (2,221 cm) and 'Orizont' (2,220 cm) had the biggest values. Most of the quince grafted cultivars had bigger vegetative growth than the self-rooted ones (Figure 9).

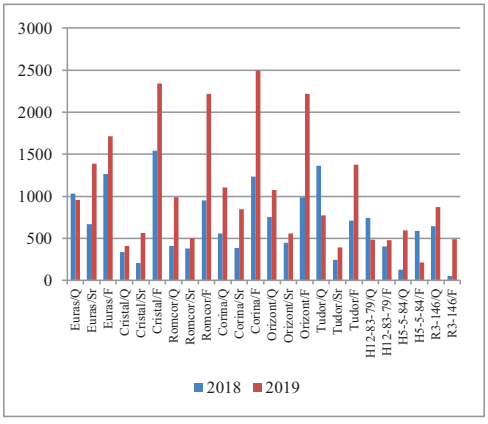

Figure 9. Total vegetative length between 2018-2019

'Euras' cultivar confirmed its characteristic to produce on short flowering shoots (Figure 10). Farold 40 grafted plants had the biggest values at spurs length followed by self-rooted once.

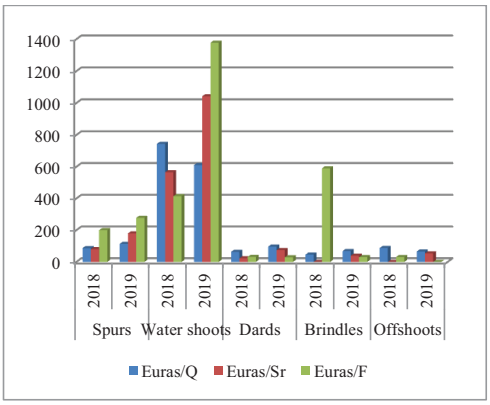

Figure 10. Total flowering shoots length to 'Euras' cultivar

'Cristal' presented significant vegetative growth on offshoots. Farold 40 led to the highest vegetative growths and quince to the smallest (Figure 11).

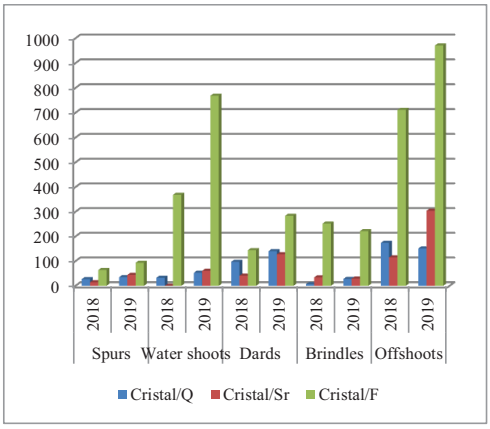

Figure 11. Total flowering shoots length to 'Cristal' cultivar

Spurs and brindles had significant growth at 'Corina' cultivar (Figure 12) similar to Andreieș (2017).

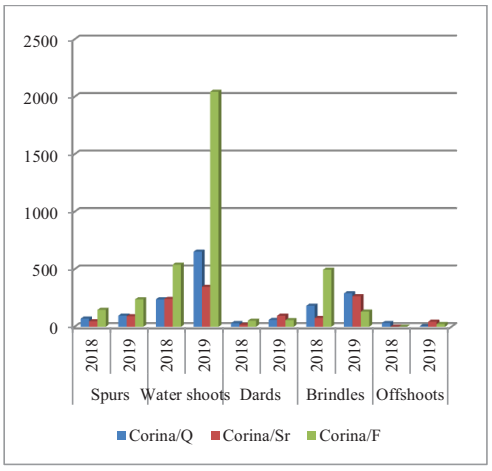

Figure 12. Total flowering shoots length to 'Corina' cultivar

'Romcor' presented significant values for brindles and offshoots (Figure 13). Quince grafted plants had bigger vegetative growth then the other two variants.

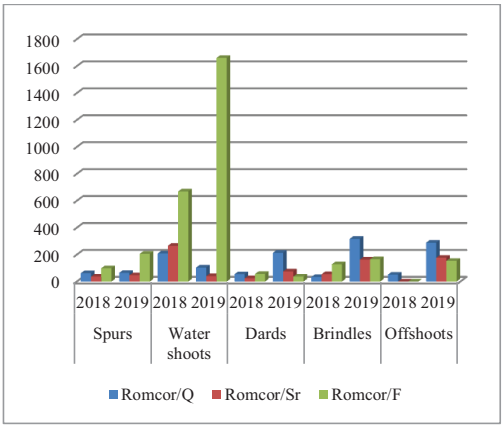

Figure 13. Total flowering shoots length to 'Romcor' cultivar

'Orizont' had brindles and spurs with the biggest growths (Figure 14).

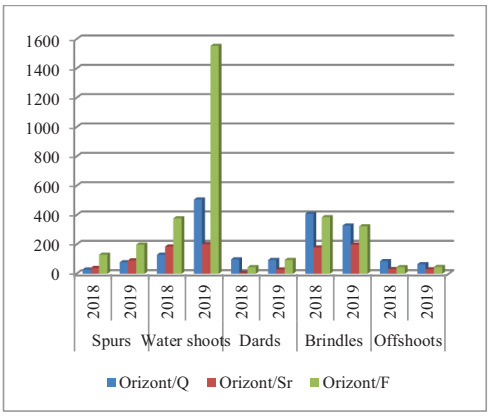

Figure 14. Total flowering shoots length to 'Orizont' cultivar

Spurs and brindles at 'Tudor' presented significant growths for the flowering shoots similar with Andreieș (2017) (Figure 15).

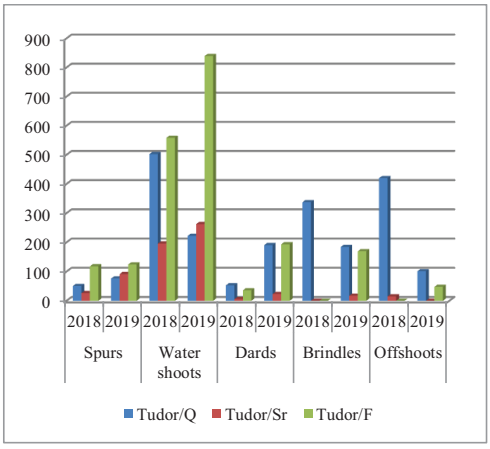

Figure 15. Total flowering shoots length to 'Tudor' cultivar

Production

Productivity was influenced by the rootstock. Farold 40 led to the highest production followed by quince and self-rooted trees excepting 'Euras' were quince led to bigger values. 'Cristal' presented the highest productivity on seedling rootstock, 'Euras', 'Romcor' and 'Orizont' on quince and 'Corina' on self-rooted rootstock. Seedling rootstock generally led to bigger production. Self-rooted trees registered the smallest values for production (kg/tree) (Figure 16).

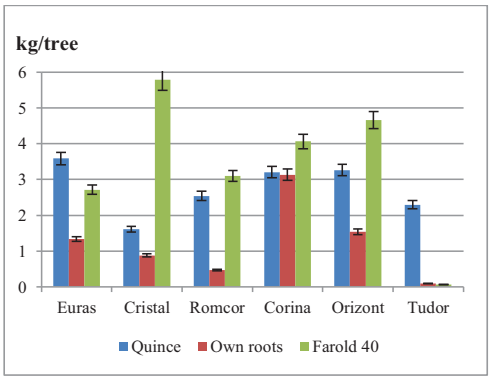

Figure 16. Estimation of yield/tree for each variety analysed

The highest productivity index was on 'Corina' and 'Orizont' self-rooted (0.67 kg/cm^2, respectively 0.50 kg/cm^2) and 'Cristal' on Farold 40 (0.56 kg/cm^2) (Figure 17)

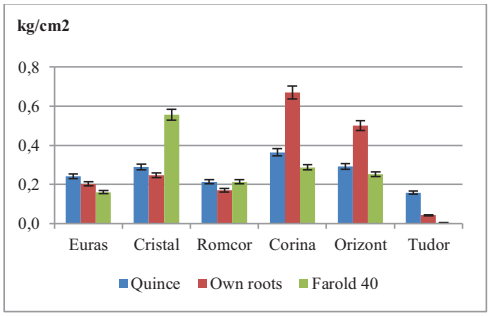

Figure 17. Productivity index influenced by cultivars and rootstock

'Romcor' cultivar self-rooted presented an average weight of 237 g/fruit, similar with the cultivar description (Andreieș, 2017); 'Corina' on Farold 40 (143.75 g/fruit) had also values according to the pontential of the cultivar (Figure 18). Similar with Wertheim (2002) it couldn't be registered a correlation between rootstock and fruit weight.

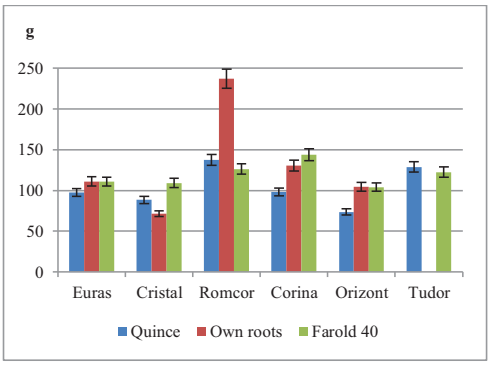

Figure 18. Fruit weight according to cultivars and rootstock

Fruit characteristics at the harvest moment

Rootstock influenced the fruit index form at 'Euras' and 'Tudor' cultivar (Table 1).

Table 1. The influence of cultivar and rootstock on fruit Index form

Cultivar	Form Index		
	Quince	Own roots	Farold 40
'Euras'	1.15	1.05	1.08
'Cristal'	1.08	1.08	1.07
'Romcor'	1.00	1.10	0.98
'Corina'	1.02	1.04	1.08
'Orizont'	0.98	0.98	0.96
'Tudor'	1.26	-	1.08
R3-146	-	-	0.96
H5-5-84	-	-	1.12
H12-83-79	1.04	-	1.08

The quince grafted cultivars presented the biggest values for firmness at the harvest moment comparing with the other two variants (Figure 19).

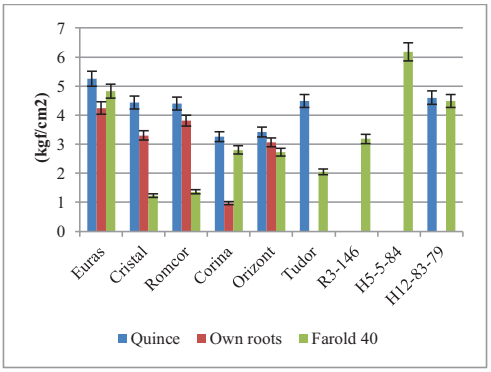

Figure 19. Fruit firmness at the harvest moment

Dry matter content varied between 17.28% ('Corina' self-rooted) and 27.06% ('Cristal' on Farold 40) (Figure 20). Quince grafted varieties presented similar values for dry matter.
The values were bigger than Ghena and Branişte (2003) and Andreieş (2017).

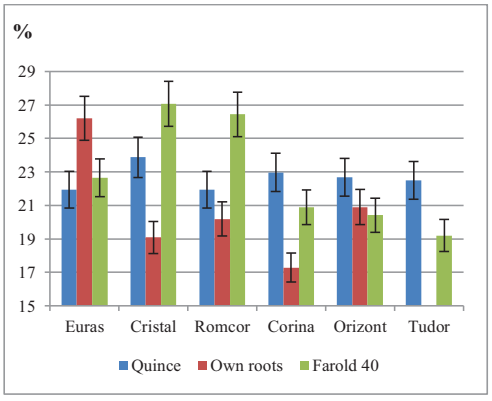

Figure 20. Dry matter influenced by cultivar and rootstock

Total soluble sugars at the harvest moment were between 12.66% ('Corina' on Farold 40) and 6.63% ('Orizont' on quince).
'Euras' registered the highest values comparing with other cultivars. Significant differences were on cultivars 'Corina' and 'Orizont' between quince and the other two experimental variants (Figure 21).
Quince grafted cultivars presented highest values for total soluble sugars, excepting 'Romcor'.

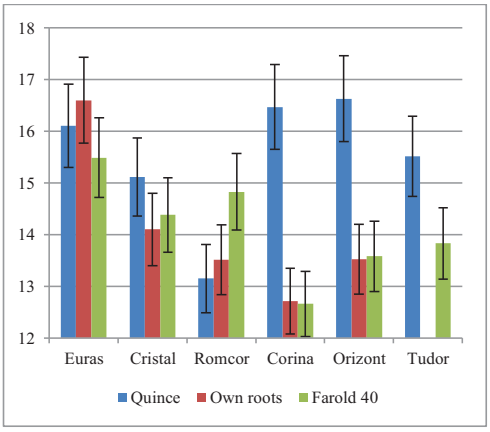

Figure 21. Total soluble sugars on pear cultivars

The results are similar with Ghena and Branişte (2003), Stănică and Branişte (2011), Hoza (2003), Grădinariu (2002), Cimpoieş (2018). 'Euras' presented higher values for total soluble sugars (more than 11%).

CONCLUSIONS

This research presents the first results of new Romanian pear disease resistant cultivars tested within the Experimental Orchard of USAMV Bucharest.
Results confirmed the biggest growth given by Farold 40 and also the characteristic of these new cultivars to produce mainly on short flowering shoots, excepting 'Orizont' cultivar.
Varieties grafted on Farold 40 presented the highest production followed by quince and self-rooted trees excepting 'Euras' were quince led to bigger values.
There wasn't noticed a correlation between rootstock and fruit weight.
Dry matter content varied between 17.28% and 27.06%, significantly higher than in other studies (14-16%). Quince grafted cultivars presented similar values for dry matter.
Total soluble sugars at the harvest moment were between 12.66% and 16.63%. Quince grafted cultivars presented highest values for total soluble sugars, excepting 'Romcor'.
The new Romanian pear resistant cultivars presented valuable qualities and can be promoted with success for intensive production.

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THE QUANTITATIVELY AND QUALITATIVELY POTENTIAL OF THE WINE GRAPE HARVEST IN RELATION WITH THE CLIMATE CHANGE

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Abstract

The style of wine produced in a region is a result of the baseline climate, while the climatic variability determines differences in the quality of the vintage. The production and quality of wines is likely to be affected by changes in meteorological and climatic conditions. Potential quantitatively evaluated on the basis of potential yield and qualitative potential has been defined by the weight and volume of grain, sugar, acidity, gluco-acidimetric index. In the present paper, we have investigated the relationships between climatic variability and the quantitatively and qualitatively potential of the grape production and wine quality in the Dealu Mare Valea Calugareasca. Regarding the yield potential of the grape production was found that the rate of increase was 0.28-0.74 kg/vine and the growth rate of gluco-acidimetric index was 0.5-1.0. Analysis of average air temperature ranges reveals changes in all seasons: significant warming of about 1.2°C during summer and 0.8°C in spring, and a slight cooling trend of 0.4°C was noted in autumn.

Key words*: climatic conditions, qualitative potential, sugar, acidity.*

INTRODUCTION

Climate change involves reducing greenhouse gas emissions and adapting ecological systems to the effects of climate variability. The Intergovernmental Panel on Climate Change V (IPCC-Climate Change 2013/AR-5, WG I-Physical Science Basis, SPM) published in 2013, mentions that global average air temperature has increased by about 0.85ºC in the last 100 years (1850-2012), the period 2001-2013 being one of the warmest of the data series since 1850.

Also, the number of hot days increased frequency of heat waves recorded a growth trend evident in most of Europe.

Annual rainfall amounts showed a variable distribution in time and space (increase/decrease) and dry periods followed by heavy rainfall brief generating floods and floods is growing fast.

The wine climate has an essential role in grape ripening. Over the last 20 years has experienced significant changes due to climate change.

The average global temperature could increase by up to 4.5°C, but the best estimate is 2.5°C, if the amount of CO_2 in the atmosphere doubled (Houghton et al., 1992). Global warming is 0.6°C in the past three decades and 0.8°C in the last century.

The reality of climate change is admitted by the vast majority of the scientific community (IPCC, 2014). The viticulture is highly dependent upon climatic conditions during the growing season. The climatic conditions vary from one year to the other. These variations induce the "vintage effect", year-to-year variations in yield, quality, and typicity (Cornelis van Leeuwen & Darriet, 2016). Sugar accumulation increases with temperature (Coombe, 1987), but certain secondary metabolites, like anthocyanins, are negatively affected by high temperature (Kliewer and Torres, 1972). Grape acidity, in particular the malic acid content, decreases in high temperature (Coombe, 1987).

MATERIALS AND METHODS

Climate data used in this analysis are for the Research Institute for Viticulture and Enology Valea Calugareasca station for 1936 to 2018. The data consist of daily observations of the

monthly average temperature, maximum temperature, minimum temperature and rainfall.

The simulation of computer climate scenarios allows the prognosis of future climate trends. Computer simulations of the climate system can be accomplished using general circulation patterns that show the global climate response to changes in the composition of the atmosphere.

Climate changes occurred gradually until now it seems that it will be produced all graduated modifications in the future. So, there were used programs RCP 4.5 and RCP 8.5, in order to predict global warming by the year 2100, with a view of adaptation and to counter their impact.

RESULTS AND DISCUSSIONS

The analysis of the thermal values for the period 1936-2018 indicates that the average annual air temperature increased over the last 38 years by 1.0°C (1981-2018/11.8°C) over the whole analyzed period (1936-1980/10.8°C), which exceeds the average global warming of 0.85°C in the last 100 years (1850-2012), according to AR 5 (IPCC, 2013). In the last 38 years, in our country the warmest year was 2007 (13.7°C), and the coldest 1942 (9.0°C) (Figure 1).

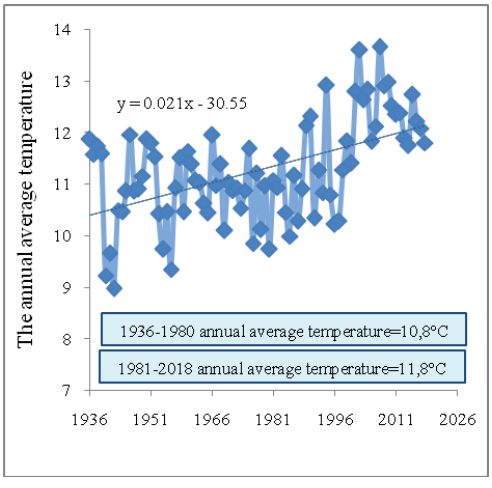

Figure 1. The trend of the average annual air temperature during the period 1936-2018

Jones and Alves (2012) mentioned that a region with a mean temperature during the vegetation season of 15.0°C, which is heated by 1°C, will be able to experiment with several varieties suitable for a faster rippening.

In the coldest month of the year, respectively January, the monthly average temperature increased over the last 38 years (1981-2018) by 1.9°C, compared to the period 1936-1980, and the warmest July, by 1.3°C (Figures 2 and 3).

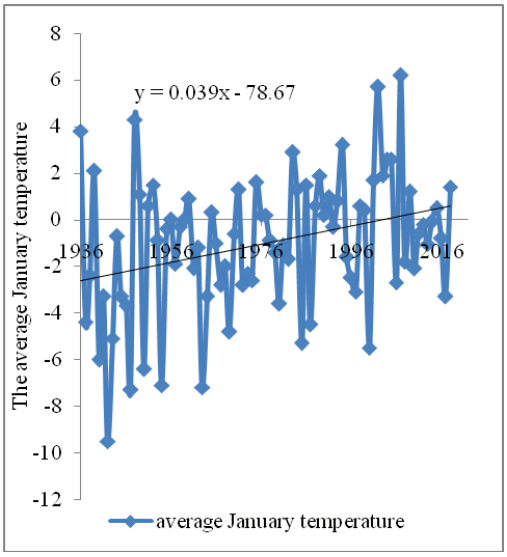

Figure 2. The trend of the average annual air temperature in January between 1936 and 2018

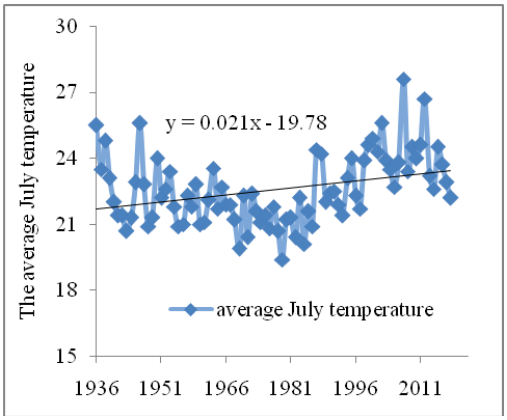

Figure 3. The trend of the average annual air temperature in July between 1936 and 2018

Table 1 shows in ascending order the warmest years recorded during the period 1936-2018 in Valea Calugareasca, 18 of them being reported during the period 2000-2018, when the positive thermal deviations were 2.7°C to 0.1°C compared to the climatic multi-annual average of the analyzed period of 11.3°C.

Table 1. The warmest years between 1936 and 2018

Year	The average annual temperature (°C)	Deviation (°C)
1961, 1968, **2000**	11.4	0.1
1952, 1958, 1983	11.5	0.2
1937, 1939, 1960	11.6	0.3
1938, 1975, **2014**	11.7	0.4
1951, 1999, **2005**, **2018**	11.8	0.5
1936, 1950, **2013**	11.9	0.6
1946, 1966	12.0	0.7
1989, **2006**, **2017**	12.1	0.8
2016	12.2	0.9
1990	12.3	1.0
2011, **2012**	12.4	1.1
2010	12.5	1.2
2003	12.6	1.3
2015	12.7	1.4
2001, **2004**	12.8	1.5
1994, **2008**	12.9	1.6
2009	13.0	1.7
2002	13.6	2.3
2007	13.7	2.4

The 2007 year marks the warmest year with a positive thermal deviation of 2.4°C compared to 1936-2018 and the second place with 5 years ago from 2001-2009 (2001, 2002, 2004, 2008 and 2009) with an annual average of 1.4°C above the average of 11.3°C.

Rainfall is the main source of water for the growth and development of vineyards, and the most significant elements of this meteorological parameter are the quantitative variability, the distribution and the spatio-temporal distribution.

The analysis of rainfall data reveals the fact that, from a pluviometric point of view, there was a trend of decreasing annual rainfall in the period 1981-2018 compared to the period 1936-1980.

The average multiannual rainfall (January-December) calculated for the period 1936-1980 is 624 l/mp. Between 1981 and 2018, annual rainfall decreased by 10 mm. The driest year is 1945 (294.6 l/mp), and the most rainy year 2005 (1079.2 l/mp) (Figure 4).

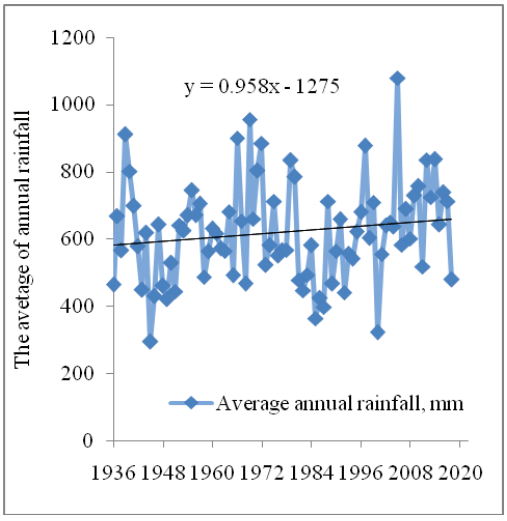

Figure 4. The trend of the average annual rainfall between 1936 and 2018

For vineyards, the quantities of rainfall that fall during the vegetation period and their distribution by phenological phases are particularly important.

During the vegetation period, rainfall may also present quantitative fluctuations and distribution (Figure 5).

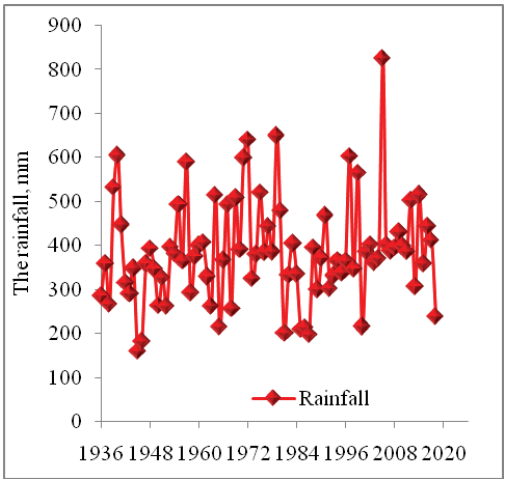

Figure 5. The trend of the rainfall during vegetative period between 1936 and 2018

The growing season (Apr.-Sept.) average rainfall was 48.5 mm in April, 63.2 mm (May),

79.9 mm (June), 76.3 mm (July), 61.6 mm (August) and 50 mm (September) in the period 1981-2018, comparative with 46.4 mm in April, 76.0 mm (May), 94.6 mm (June), 75.2 mm (July), 56.9 mm (August) and 41.3 mm (September) in the period 1936-1980.

The potential of grape harvest is a result of the interaction of natural, biological and agronomic factors and its level is the criterion for determining the destination for capitalizing the production of grape for wine.

Cabernet Sauvignon variety recorded an increase in the production of grapes from 2.30 kg/vine in the period 1936-1980 to 3.05 kg/vine in the period 1981-2018 (Figure 6).

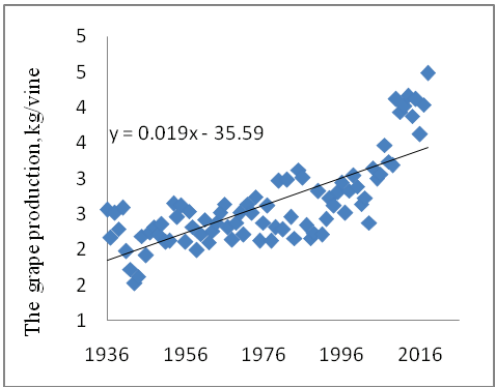

Figure 6. The trend of the grape production between 1936 and 2018

Potential quantitatively evaluated on the basis of potential yield has been defined by the sugar and acidity.

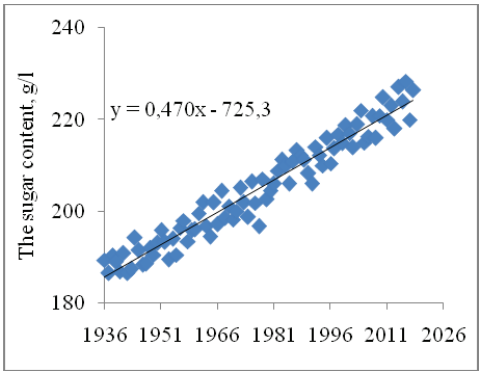

Figure 7. The sugar content of the grape between 1936 and 2018

Increasing the average air temperature by 1.3°C caused an increase in sugar accumulation by 20 g/l (196 g/l between 1936 and 1980; 216 g/l between 1981 and 2018), as well as a decrease in acidity by 0.92 g/l (5.54 g/l between 1936 and 1980; 4.62 g/l between 1981 and 2018) (Figures 7 and 8).

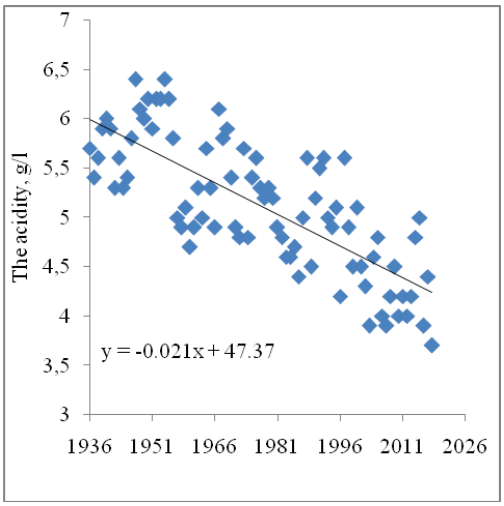

Figure 8. The acidity content between 1936 and 2018

Predictable Effects in Climate Change Scenarios

Climate change is a major challenge for viticulture in the coming decades.

In the recent past, wine quality has increased in most wine-growing regions because of higher temperatures and more frequent water deficits, while yields have decreased.

If the tendency continues, quality might be negatively affected in the near future.

Growers need to implement adaptive strategies to continue the production of high-quality wines at economically acceptable yields in a warmer and dryer climate.

The impact of projected global warming on the Valea Calugareasca wine industry was investigated using spatial modelling techniques.

Expected shifts in annual average temperature between present day and the year 2050 will be in the order 0.2 to 1.5°C.

By 2100, the projected increase in annual average temperature in viticultural areas is 0.4 to 3.5°C (Figure 9).

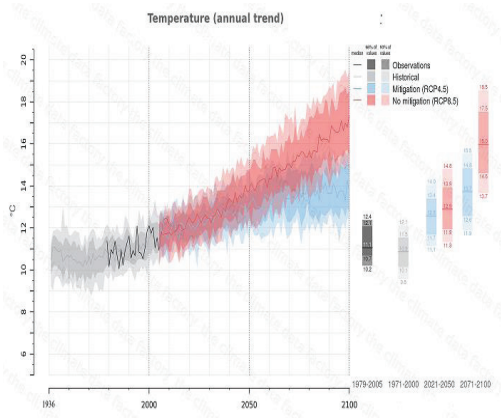

Figure 9. Results from one climate model projection showing average temperature

Regarding the maximum air temperature between present day and the year 2050 will be 1.2 to 1.5°C, and by the year 2100 it will be 2.5 to 3.0ºC (Figure 10).

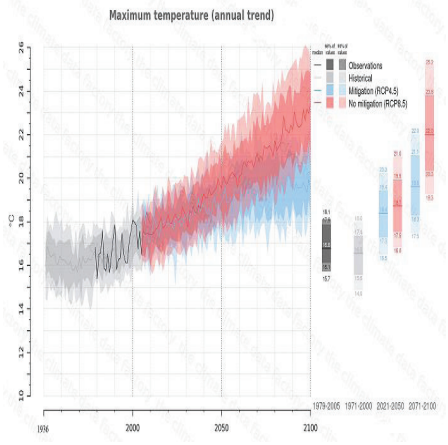

Figure 10. Results from one climate model projection showing maximum temperature

Higher temperatures will increase evapotranspiration. Modifications in rainfall patterns are difficult to predict. It is likely that rainfall will be subject to great regional and temporal variations. Of particular importance are the annual precipitations, the distribution of which will be very irregular, long periods of drought (Figure 11).

An increase in radiation can cause sunburn on grapes, particularly in the prevéraison phase. An increase in UV-B radiation might be favorable in red wine production because of increased skin phenolics but can impair white wine quality and induce atypical aging.

The solar radiation will increase by 30-60 w/m^2 until 2100 (Figure 12).

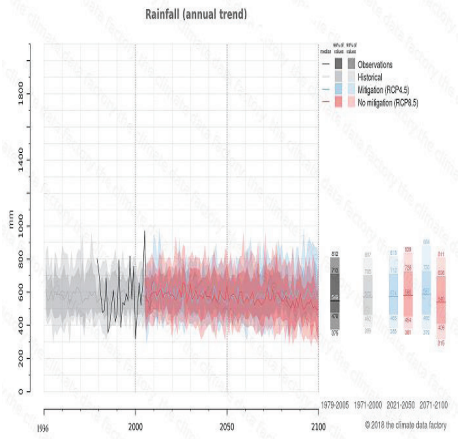

Figure 11. Results from one climate model projection showing average rainfall

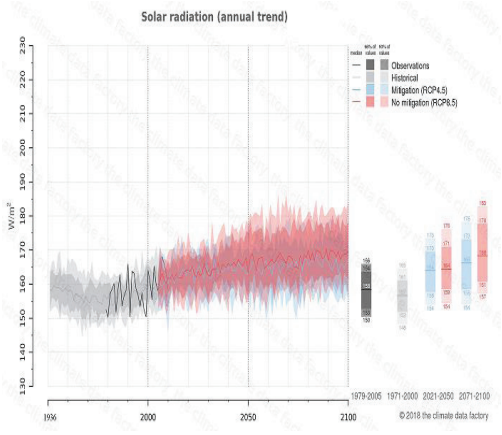

Figure 12. Results from one climate model projection showing solar radiation

CONCLUSIONS

Climate change is a major challenge for viticulture in the coming decades. In the recent past, wine quality has increased in most wine-growing regions because of higher temperatures and more frequent water deficitswhile yields have decreased. If the tendency continues, quality might be negatively affected in the near future.

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THE GRAPE MARC AS POTENTIAL SOURCE OF BIOLOGICALLY ACTIVE COMPOUNDS

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Abstract

Grape culture is one of the most widespread in the world, with about 77 million tonnes of grapes harvested only in 2016. After the winemaking process, the main waste is grape marc. Traditionally, grape marc was thrown into the field, thus losing an important source of valuable compounds. The main purpose of this paper is to present some aspects of the grape marc valorisation obtained from vinification of Romanian autochthonous grapes varieties (Fetească Neagră, Busuioacă de Bohotin, Băbească Neagră etc.). This information will be analysed in terms of the compounds present in widely used grape varieties. Regarding Romanian grape varieties, the bibliographic sources are limited and it is necessary to carry out some analyses and characterizations of the grape marc obtained from native grape varieties as a potential source of alternative products (use in cosmetics, animal feeds, composting etc.).

Key words*: grape marc, Romanian grapes.*

INTRODUCTION

Biomass is a residual material resulting from food production processes, from agriculture, horticulture or human waste. Its issued in various industrial processes, such as energy production, supplement in animal feed, or as raw materials for chemical products.

As the need for food is higher day by day, agriculture generate large amounts of biomass residues. Some of the residues are left in the field to improve the quality of the soil by return of the nutrients. Others, can provide different added-value products by various processing techniques with applications in cosmetics industry or as alternative sources of feed or as compost.

With around 63 million tons produced worldwide, grapes are one of the most cultivated crops around the world (FAOSTAT, 2013). From the total weight of the grapes, approx. 20% constitute the main winemaking by-product, the grape or wine marc (Laufenberg et al., 2003). Grape (or wine) marc is a residue of pressed grapes, pieces of stalks and yeast from the wine fermentation process.

The wine production process generates huge amounts of by-products consisting mainly in organic wastes (Musee et al., 2007; Rondeau et al., 2013). For a long time, due to the lack of knowledge, wine marc was an under-valued product. Traditionally, wine marc was subjected to distillation, producing alcohol (Silva et al., 2000) or used as fertilizer (Arvanitoyannis et al., 2006). Around the world, grape marc is processed in different manners: recovery of grape seed for oil extraction (Vera, 2016), in the soap manufacture, as animal feed, fertilizer or as part of varnish and paints. Usually, grape marc consists in seeds, skins, stalks and residual moisture, residual sugars, organic acids and some quantities of alcohol. Residual alcohol and sugars concentration in marc varies according to the processes and practices used in winemaking (Devesa-Rey et al., 2011; Hixson et al., 2014; Zheng et al., 2012).

One of the operations that can influence the quality and content of the marc is pressing. Depending on the type of press used (traditional presses, vertical presses, horizontal presses, pneumatic horizontal presses, horizontal presses with membrane or presses

with continuous action), the yield of the marc is inversely proportional to the must. The higher the yield of the presses, the resulting marc is less rich in nutrients (Baltes, 2016).

Sustainable aspects of winemaking

Wine making is a process that produce a large amount of organic and inorganic waste. Bibliographic sources specify that during cultivation and harvesting about 5 tons of solid waste is generated per hectare per year (Zacharof, 2017). In this respect, the adoption of sustainable cultivation practices for grape and wine crops has been encouraged. (Rugani et al., 2013; Cuccia, 2015; Da Ros et al., 2016).

A sustainable winemaking process consists in maximizing resources and reducing emissions from the production process (Castillo-Vergara et al., 2015; Cuccia, 2015). At global level, the wine sector produces about 0.3% of annual greenhouse gas emissions (Amienyo et al., 2014).

European legislation explicitly uses the term by-products only for grape marc and lees.

Based on the by-product definition given by the Waste Framework Directive (Directive 2008/98/EC on waste), grape marc are substances resulting from a production process, the primary aim of which was not the production of such residues and, furthermore, they are able to be used afterward (Spigno, 2017).

The added- value conversion of the bio-products from winemaking can help in reducing the negative costs and demonstrating sustainability in winemaking. It is important to maximize product recovery and minimize the secondary wastes. Is necessary to understanding the potential benefits of the reuse of winery wastes and by-products (Nerantzis, 2006).

In order to reduce the negative environmental impact, companies have to take into account technologies proposed in the industry to add value to the residues produced in the wine-making industry (Rosa et al., 2011).

Depending on the winemaking process, the marc characteristics may vary. For the red wine production process, the must and the marc are fermented together. This allows the release of pigments (such as anthocyanins) that give the specific colour to red wine. Also, this process is the only one that brings changes to the marc, because fermentation reduces its sugar content. In the case of white wine, the must is fermented separately (Dwyer et al., 2014) and the resulting marc has a higher content in pulp and sugars (Mendes et al., 2013). Figure 1 presents the process of white and red wine production and the marc resulting.

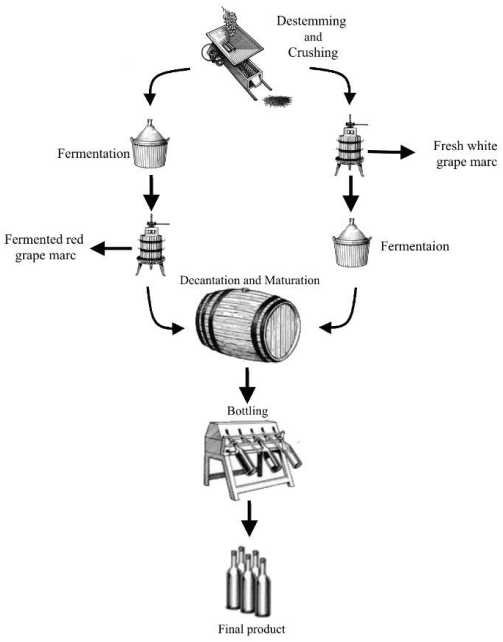

Figure 1. White and red grape marc resulted from the winemaking process (adapted after Muhlack et al., 2018)

GRAPE MARC APPLICATIONS

Grape marc is a rich source of high-value products such as ethanol, tartrate and maltase, citric acid, grape seed oil, hydrocolloids and dietary fibres. In addition, the grape marc is characterized by a high content of phenol, due to poor extraction during winemaking, which makes their use useful and supports sustainable agricultural production (Kammerer et al., 2004).

The products resulting from the wine making process are cheap raw materials with a high amount of antioxidant substances that can be used in human or animal nutrition or in the production of substances with phytochemical activity (Alonso et al., 2002; Negro et al., 2003; Gonzalez-Paramas et al., 2004).

In Romania, a few studies have been focused

on the use of grape marc. An important study was carried out by Olteanu et al. (2014), in which the marc from South Eastern Romanian winery was studied for the feeding value and antioxidant capacity. In their study were used three marc varieties consisted of: (1) fresh grape peals, pulp and seeds of red grapes, with no stems; (2) dry, seed-less red grape peals and pulp, with no fermentation smell, and (3) the seed cakes, by-product of oil extraction, with no smell of fermentation or rancidity.

A close correlation between the antioxidant capacity and the polyphenols concentration shows a higher antioxidant character for the dry grape marc sample.

Usually, when studying the feed properties of winemaking by-products some standardized analysis methods are recommended:

- the dry matter (DM) to be determined by gravimetric method according to Regulation (CE) nr. 152/2009 and standard SR ISO 6496:2001;
- crude protein (CP) to be determined by the Kjeldahl method;
- the amino acids to be determined by High Pressure Liquid Chromatography (HPLC);
- the crude fat to be determined by extraction in organic solvents;
- the fatty acids to be determined by gas chromatography;
- the crude fibre to be determined by the method with intermediary filtration;
- the ash to be determined with the gravimetric method;
- the calcium, copper, iron, manganese and zinc to be determined by atomic absorption spectrometry;
- the phosphorus to be determined by spectrophotometry;
- the gross energy to be determined by calculation, using the dry matter, protein, fibre, fat, nitrogen-free extractives and ash, using the equations developed by Burlacu et al. (2002).
- the polyphenols concentration (mg equivalent gallic acid/g sample) to be determined according to the modified method described by Mihailovic et al. (2013);
- antioxidant capacity (mM equivalent Trolox/g sample) to be determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method proposed by Marxen et al. (2007).

The use of marc as a component of compost is the most readily available. In his book, Madjar et al. (2007), concludes that compost with marc helps to improve soil with nutrients needed for plants. Also, due to the high-level content of organic matter in the composted marc, it helps to increase the level of life in the soil layers by increasing the level of respiration in the soil, supporting the catalase activity, the urease and the phosphatase activity.

Marc compost can also help sandy soils. When applying a rotation of appropriate crops, the application of marc compost can lead to the improvement of trophic and biological soil characteristics (Ion et al., 1996).

Another approach was taken by Bărbulescu et al. in 2017 with fermented Merlot grape marc, taken from the Oprișor area. The fermentation was carried out under natural light and in darkness; in the study were used in the fermentation yeast isolates also from the Oprișor area. The dried grape marc was grounded and added in the fermentation mix after 13 hours of fermentative process. The authors have noticed that in the presence of natural light, both unfermented and fermented grape residues, compared to similar samples processed at darkness, have lower values (content in polyphenols, protein and antioxidant activity). Also, the biomass obtained through fermentation shows higher antioxidant activity in both light and darkness in comparison with similar samples to the initial grape residue.

Baltes et al. (2016) have analysed quantitatively and qualitatively the polyphenols from the varieties of Cabernet Sauvignon, Merlot, Pinot Noir and Fetească Neagră harvested in 2014 and 2015. For the study, the marc was dried and crushed and then subjected to solvent extraction consisting of ethyl alcohol and water (1: 1 ratio) and then quantitative analysed using the modified Folin-Ciocâlteu method. Following the analyses, the studied samples showed values ranging from 558.6 mg to 608.2 mg/100 g grape marc.

Higher values showed the samples of Pinot Noir grape marc (approx. 600 mg/100 g), and the lowest were the marc of Cabernet Sauvignon (558.6 mg/100 g). Merlot and Fetească Neagră varieties showed similar values: from 566.3 mg to 587.5 mg/100g grape marc.

The values obtained for total phenolic content place grape marc on an equal footing with:

- black currant - 758 mg/100 g;
- capers - 654 mg/100 g;
- black olives - 569 mg/100 g;
- high bush - 560 mg/100 g (J Pérez-Jiménez et al., 2010).

Some Romanian studies have covered also health aspects related to the marc extracts. Studies conducted by Balea et al. (2018) on Fetească Neagră and Pinot Noir marc harvested in Romania in 2015 showed that the marc extracts (fresh and fermented) have improved cardiac parameters and oxidative stress when were administrated to rats. They assumed this result are due to the higher values of phenols and antioxidant activity in the fermented marc compared to fresh marc extracts. Regarding the cardio-protective effect, the fresh Fetească Neagră marc had a higher cardio-protective effect than fermented marc. Pinot Noir extract presented equal cardiovascular effects.

CONCLUSIONS

In the current global context, when the tendency is to use the most of the resources (even some by-products like grape marc), efficient valorisation is a win-win situation. Grape marc brings, besides a significant amount of protein, fats and fibres, a large amount of bio-compounds with a strong antioxidant effect.

The high concentration of polyphenols recommends grape marc utilization particularly in diets enriched in polyunsaturated fatty acids like diets used for the production of animal feed high in omega 3 fatty acids. The use of winery by-products, in animal feeding, in composting or in different extractions also solves the problems of their storage, transformation or disposal, thus maintaining the environmental balance.

The identification of beneficial compounds present in grape marc is important because the identified elements can be a natural source of beneficial compounds. As it is demonstrated, grape marc is an important source of polyphenols.

In Romania, the bibliographic sources are limited and it is necessary to carry out some analyses and characterizations of the grape marc obtained from native grape varieties as a potential source of alternative products.

ACKNOWLEDGEMENTS

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INSECT SPECIES DIVERSITY IN STEFANESTI VITICULTURAL CENTRE FROM ROMANIA

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Abstract

The grapevine, being a multiannual crop, favors the installation, growing and spreading of insect populations whose attack increases proportionally with the number of years of cultivation, unless rigorous plant protection measures are applied. In this work were studied the beneficial and harmful fauna from the Stefanesti vineyards, identifying 43 species of insects, of which 14 species belong to the beneficial fauna and 29 species are pests. The data were interpreted using the diversity indices: K - Dominance, Rarefaction, SHE analysis and Bray - Curtis analysis both for the insect of the beneficial fauna but also for the other species identified in the Stefanesti - Arges vineyard, Romania.

Key words*: biodiversity, fauna, precipitation, temperature, vineyards.*

INTRODUCTION

Grapevine, one of the most spread horticultural crop in the temperate climate, is exposed, as all plants, to the influence of diseases (fungal and bacterial diseases, phytoplasmas, virus and virus-like diseases), pests (insects, mites, nematodes), and climatic changes.

Among the pests, the insects can lead to serious economic losses in the quality and quantity of grapes (Goussard, 2013). The range and the behavior of harmful insects is influenced by the environmental temperatures (Boudon-Padieu & Maixner, 2007; Reineke & Thiéry, 2016; Bois et al., 2017). Monitoring of beneficial and harmful insects in vineyards has been done in different viticultural countries and conditions (Altieri et al., 2005; Retallack, 2011; Soares et al., 2016).

The paper deals with the evolution of the insect diversity in Stefanesti Arges vineyard, located in the South of Romania.

MATERIALS AND METHODS

The study was carried out on the territory of the National Research and Development Institute for Biotechnology in Horticulture Stefanesti - Arges, located in the Stefanesti vineyard, in the southern central part of the Subcarpathian Mountains.

During the study, the temperature, relative air humidity and precipitation were monitored using the iMETOS 1 weather station, positioned near to the vineyards, at 44°85' latitude, 24°95' longitude and 278 m altitude.

The Stefanesti microclimate is relatively wet due to the proximity of the forests to the north, the fragmentary platform relief of deep valleys located almost perpendicular to the corridor formed by the Arges river on the one hand and the human settlements with woody vegetation on the other hand, which shuts down the airflows to the south.

The wind influences by its nature and intensity. The winds of NW, E and NE have the highest intensity and the NW, E and NE - the smallest.

The cutting system in the vineyard is Guyot. The distance between the rows is 2.5 m and between the plants 1 m in a row. The rows are NS oriented.

In the studied plots, the soil is the Eutric Regosol type in association with the Eutricambosol type (Toti et al., 2017). The upper horizons are mixed at the depth of 0-40 cm as a result of the soil works and the Bv horizon presents "in situ" clay. Soil texture being loamy-clayey on the whole soil profile, its content is moderately good in humus, good in nitrogen but decreasing on the soil profile, and weak in mobile phosphorus. The soil

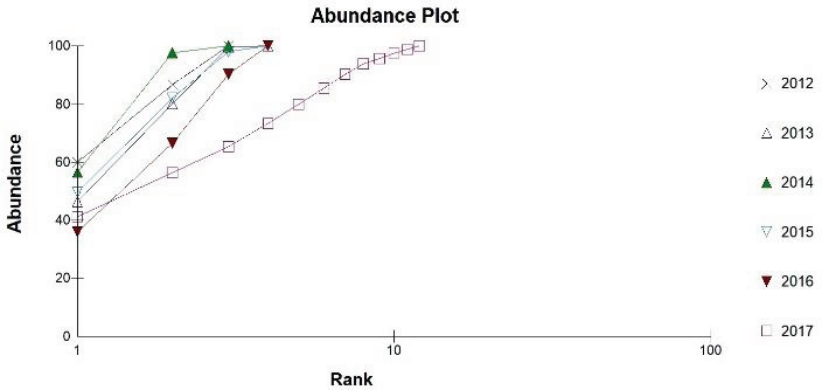
response ranges from weak acid to neutral, between 6.2 and 7, and increases with depth.
The insects have been captured using sticky traps and butterfly net and monitoring have been done for a period of 6 years, between 2012 and 2017.
The results were interpreted with the BioDiversity Professional 2 program. With this program were calculated: K - Dominance, Rarefaction, SHE analysis and Bray - Curtis analysis.
SHE analysis examines the relationship between S (species richness), H (information - the Shannon-Wiener diversity index) and E (evenness as measured using the Shannon-Wiener evenness index). This approach highlights the impact of the number of species and the balance between them on biodiversity changes.
The Bray-Curtis similarity is based on abundance data and was measured to show the difference between years both in the case of beneficial insects and in the case of pests.

RESULTS AND DISCUSSIONS

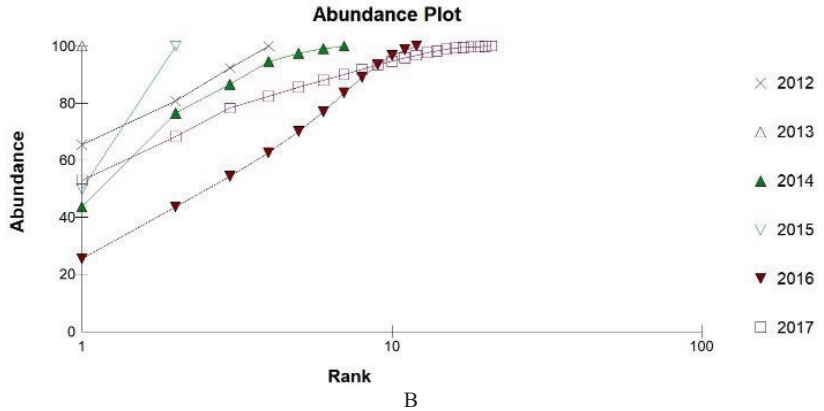
In a vineyard, where the grapevine is the dominant species, it is important to have a spontaneous flora to encourage the insect species that can provide beneficial services to the grapevine plants.
In the studied vineyard plantations, the following insect species were captured and identified with the aid of yellow sticky traps: *Harmonia axyridis, Psyllobora 22-punctata, Coccinula 14-pustulata, Adalia 10-punctata, Vibidia duodecimguttata, Scymnus frontalis, Cantharis rustica, Panorpa* sp., *Mordella sp., Andrena* sp., *Coccinella 7 punctata, Adalia bipunctata, Pirates hybridus, Apis melifera, Empoasca vitis, Lygus spinolai, Musca domestica, Culex* sp., *Poecilimon schmidti, Diplolepis rosae, Lytta versicatoria, Sialis* sp., *Centrotus cornutus, Anthaxia nitidula signaticollis, Anthaxia nitidula, Agrilus sp., Cercopis vulnerata, Forficula auricularia, Philaenus spumarius, Cortodera diferens, Polistes dominulus, Anthomyia procellaris, Paederus fuscipes, Sarcophaga* sp., *Vespula vulgaris, Trichaetipyga juniperina, Tettigonia viridissima, Neomyia cornicina, Cicadella viridis, Meiosimyza* sp., *Stenolophus teutonus, Helophilus* sp., *Graphosoma lineatum*.
None of the species mentioned above caused damage in the studied vineyard plantations. The first 14 listed species are part of the beneficial fauna, as in Bairrada region where losses due to insects were almost negligible in all vineyards (Nereu et al., 2018).
At the same time, with the help of the butterfly net, were also identified the species: *Colias croceus, Eucarta amethystina, Diacrisia sannio, Thymelicus sylvestris, Pieris sp., Apatura ilia f. clytie, Argynnis paphia*.
Other insect species have been identified, but not quantified, as well: *Lucanus cervus* (has not been captured because it is a protected species in Romania), *Oryctes nasicornis, Oulema obscura, Dermestes murinus, Harpalus pubescens*.
It is important to understand the biodiversity (existing and potential) from a vineyard due to the complex range that taking place between flora (grapevine, cover crops, shelterbelts etc), fauna (insects, birds, soil and aquatic organisms) and the natural balance of this environment (Altieri et al., 2005; Retallack, 2011).
The insect populations growing and spreading in a vineyard is influenced by the soil maintenance system. For example, the grassing experimental variants favoured diversity and abundance of insect species as compared with the black field variant (Vizitiu et al., 2018).
The following weeds species have been frequently encountered in the studied vineyards: *Convolvulus arvensis, Polygonum convolvulus, Amaranthus retroflexus, Setaria* sp., *Galium aparine, Chenopodium album, Sonchus arvensis, Stenactis annua, Polygonum aviculare, Agrostis alba, Erigeron canadensis, Echinochloa crus-galli, Rumex obtusifolius, Agropyron repens, Calamagrostis epigejos, Cirsium arvense, Matricaria inodora, Xanthium strumarium, Taraxacum officinale, Veronica hederifolia, Avena fatua, Poa pratensis, Cardaria draba, Agropyron repens, Digitaria sanguinalis, Senecio vulgaris, Stachys annua, Matricaria chamomilla, Daucus carota, Dactylis glomerata*.
The forest vegetation near the vineyard is represented mainly of: *Quercus robur, Prunus*

padus, Fagus sylvatica şi *Carpinus betulus, Salix* sp., *Alnus glutinosa.*

In order to compare diversity between years was used the K - Dominance graphical method. So, the dominance curves of beneficial fauna during the 2012-2017 period show that 2017 had the higher diversity than the other years (Figure 1A). On the other hand, the beneficial fauna was smaller as compared with the other identified insects (Figure 1B).



A



B

Figure 1. K - Dominance curves of insect diversity during the 201-2017 period (A - beneficial insects and B - harmful insects)

No community had the same biodiversity, even in homogeneous landscapes, due to site-specific characteristics of vegetation, soil, topography, climatic factors, and other environmental factors that may govern each species settlement (Mokam et al., 2014).

The assessment of beneficial species richness from 2012-2017 period shows their increasing number in 2016 both for beneficial fauna as well as for pest insects (Figure 2 A, B).

SHE analysis is useful for identifying ecotones (regions where different ecological communities intersect) (Hayek & Buzas, 1997).

Through the SHE analysis it has been examined the relationship between S (Species Richness), H (Information) and E (Evenness as measured using Shannon-Wiener evenness index) in the 2012-2017 period.

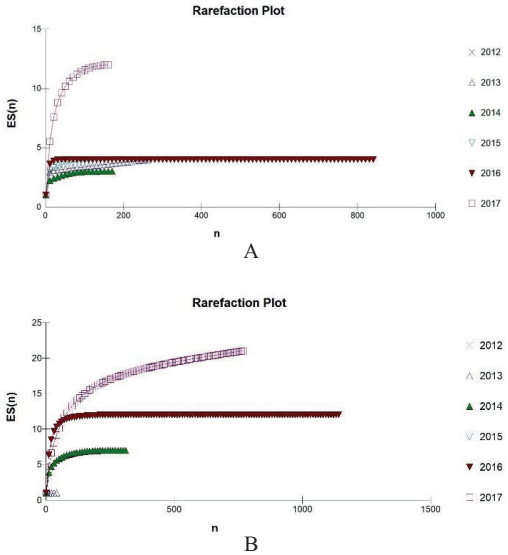


Figure 2. The assessment of species richness in 2012-2017 period (A - beneficial insects and B - harmful insects)

SHE analysis of diversity for the species identified from the grapevine plantations in 2012-2017 period indicates a mixture of comunities because H increase while ln(E) and ln(E)/ln(S) decrease (Figure 3).

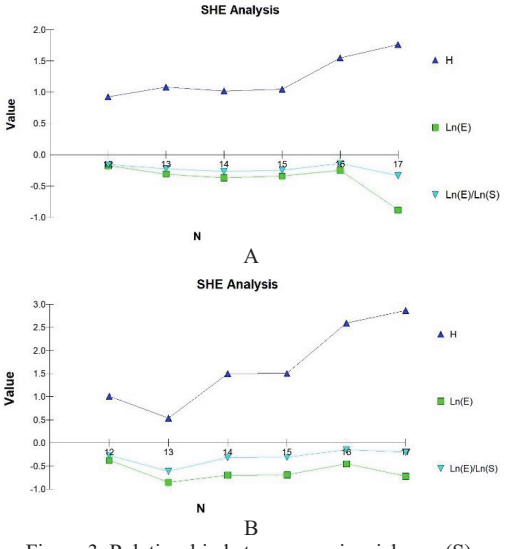


Figure 3. Relationship between species richness (S), Shannon-Wiener diversity index (H) and evenness (E) during 2012-2017 period (A - beneficial insects and B - harmful insects)

For each data set a multivariate cluster analysis using Bray-Curtis similarity measure was applied to ascertain the similarity between the years.

The Figure 4 demonstrates a high degree of dissimilarity between group A and B, recording a larger number of species and individuals in the case of harmful fauna in 2015, 2016 and 2017 years (Figure 4B), as compared to the same period but corresponding to the beneficial fauna (Figure 4A).

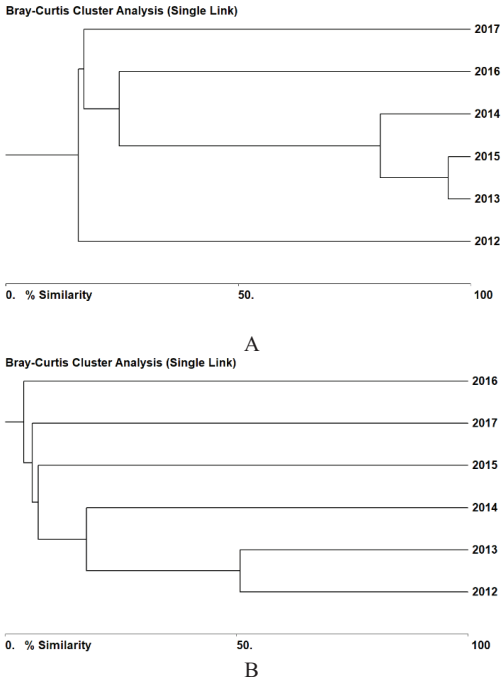


Figure 4. Bray-Curtis analysis for the insect identified in 2012-2017 period from the vineyard plantations (A - beneficial insects and B - harmful insects)

The medium temperature evolution and the level of precipitation registered along the 2012-2017 period showed differences from one year to another.

In 2012-2017 period have been registered 8 months/year with temperature ranged in 5-45˚C interval, favourable to the biological activity development of the insects (Figure 5).

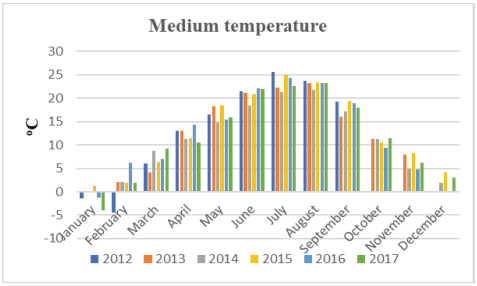


Figure 5. Medium temperature evolution in 2012-2017 period

Also, the precipitations level influence the insects evolution and development.

The lack of precipitation in the winter months were improper for biological stages of the insect which are wintering at the soil surface or to a small depth of the soil.

The precipitation abundance (Figure 6) in the summer period had a negative influence in 2012 and 2013 on the number of individuals (Figure 2) as a results of their eggs and larvae deposited on the vegetative organs lavation.

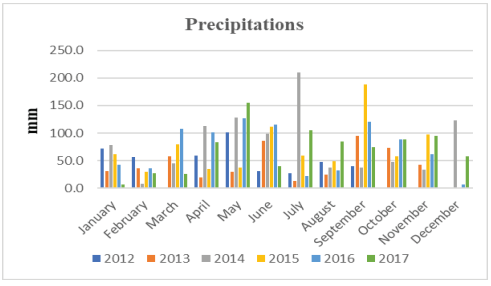


Figure 6. Level of precipitation in the 2012-2017 period

However, was not correlation between the annual sum of precipitations and number of individuals in the vineyard plantation along the entire studied period (Figure 7).

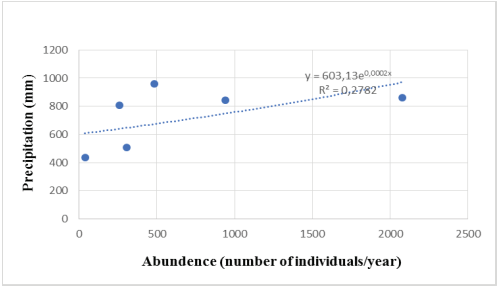


Figure 7. Relationship between the total number of insects and annual sum of precipitation from 2012-2017 period

CONCLUSIONS

In 2012-2017 period, the number of insect species identified and quantified in the grapevine plantation located in the Stefanesti vineyard was 43, of which 14 insects are part of the useful fauna and the remaining 29 were other insects species.

The K - Dominance graphical method showed that 2017 year had the higher diversity than the other years; however, the beneficial fauna was smaller as compared with the pest insects

The assessment of beneficial species richness from 2012-2017 period shows that how richness increases in 2016 both for beneficial fauna and for pest insects.

In the 2012-2017 period in vineyard plantations the SHE analysis indicated a mixture of insects comunities.

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VEGETABLE GROWING


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EFFECTS OF ORGANIC FERTILIZERS ON THE GROWTH SEEDLINGS OF ASPARAGUS

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Abstract

The study was conducted within the Horticulture Faculty in Bucharest using asparagus (Asparagus oficinalis L.) the Argenteuil cultivar. We used three types of substrate, peat, perlite and mixture of pearlite and peat. After 15 days of emergence they were transplanted in 10 cm diameter pots. The fertilization variants were as follows: V1 - unfertilized control; V2 - Amalgerol; V3 - Formulex; V4 - Vermiplant; V5 - Poco and V6 - Iguana. Care work has been aimed at observing the technology of culture, maintaining temperature and light, applying watering, proper fertilization. We watched the dynamics of seed germination, the dynamics of transplant growth in the height.
The paper reports preliminary results of a research in order to identify the best option for obtaining quality asparagus seedlings.

Key words*: asparagus, transplans, fertilizers, quality.*

INTRODUCTION

Asparagus (*Asparagus officinalis* var. *altilis* L.) In Romania, asparagus (*Asparagus oficinalis* L.) is grown on very small areas compared to other countries from Europe, such as England, Italy, Spain, France. The shoots are mainly consumed for their high mineral content. Usually asparagus is grown in temperate climates, so harvesting is restricted to the spring and early summer period. In recent years there has been a growing interest (particularly by supermarkets) in developed and affluent countries to have fresh asparagus available year round (Nicola & Basoccu, 2000; Soare & Duta, 2011; Drăghici et al., 2015). Dinu et al. (2012) conducted a study to accelerate germination of seeds using humic acids and seed extract of *Vitis vinifera*.
The optimum germination temperature of the asparagus seeds is between 20-25^{o}C (Drăghici, 2014). The initial seed quality has a marked effect on the success of the priming (Nascimento, 1998; 2003). Evans and Pill (1989) did not obtain a priming benefit for asparagus seeds with high physiological quality (Mário Lúcio de Carvalho Bittencourt et al., 2005) They show that the asparagus seeds have germinated in a higher percentage compared to the non-moistened seeds, also the crop has emerged in a shoter time. The asparagus variety react differently to the temperature, some growing faster at high temperature, while others prefer low temperatures (Chen et al., 2018).
Another aspect is the quality of the seedlings, they depend on the percentaje of rooting after planting (Elmer, 2018).
It is very important that the transplant is of a very good quality because the next culture depends on this.

MATERIALS AND METHODS

The experiment was made in the Research greenhouse condition and in the Research Center for Studies of Food Quality and Agricultural Products of University of Agronomic Sciences and Veterinary Medicine of Bucharest.
In this study we used Argenteuil cultivar. The seeds were sown in peat substrates and at ten days after emergence they were transplanted in pots filled with growing substrates. We used two types of substrates, peat and a mix of peat and perlite with 4 mm granulation and five types of fertilizers . The fertilized variant was: V1 - control; V2 - Amalgerol; V3 - Formulex;

V4 - Vermiplant; V5 - Poco and V6 - Iguana. Amalgerol - a product with a unique combination of natural oils, plant extracts and organic carbohydrates with effect on the plant and the soil. Effects on the plant: Increases the root mass of plants and the absorption of nutrients: N, P, K and microelements; It leads to better water retention in the soil and better development of the root system due to good aeration; Vermiplant. The liquid is a totally natural product, it is obtained from earthworms. It contains a wide range of microelements such as barium, zinc, iron, manganese, as well as a number of amino acids, all of which contribute to better growth and development of plants. POCO contains - Calcium 0.04-0.05%; Iodine: 6.30-12.70 mg/l; Magnesium 0,50-0,80 mg/l; Nitrogen: 0.025-0.038 mg/l; Potassium: 0.50-0.64%; Sodium: 0.088-0.120%; Sulfur 0.028-0.050%; Orange oil: 0.10-0.12%, rape oil: 0.04-0.06%; Organic acids: 0.20-0.25%; pH: 8.5-9.5. Iguana is a 100% organic product. Contains 4% nitrogen, 3% phosphorus, 6% potassium.

Formulex contains nitric nitrogen 2.19%; ammoniacal nitrogen 0.21%; phosphorus (P_2O_5) 0.85%; Potassium (K_2O) 3.36 %; Calcium (CaO) 1.85 %; Bor 0.0108 %; Cobalt 0.0006%; Copper EDTA 0.0025 ; Iron Chelate EDTA 0.0526%; Manganese Chelate EDTA 0.0131%; Molybdenum 0.0012%; and Zinc chelated EDTA 0.0036%.

The care work consisted of watering, fertilization, temperature and light monitoring.

I followed the dynamics of seed germination, seedling growth dynamics in height. We have correlated the plant mass, fertilizer type and substrate type. The purpose of the study was to identify the best option for obtaining quality asparagus seedlings.

The moisture and dry matter content of the asparagus roots was determined by an oven-drying method at 105°C for 24 h until the samples reached a constant mass, according with European Pharmacopoeia 7th and Commission Regulation (EC) No 152/2009.

RESULTS AND DISCUSSIONS

Analysing the data regarding the germination of asparagus seeds we have found that it varied according to the crop substrate and the fertilizer used for wetting. We have found that the asparagus seeds in the perlite substrate have germinated in a lower percentage of 90.22% (Table 2), in comparison with the other variants, 93.33% for the substrate with 50% peat and 50% perlite (Table 3), respectively 92% for the peat substrate (Table 5). The lowest percentage was recorded to all variants in which we watered the substrate only with water. In the variants that have been watered with Iguana product, the percentage of emerged seeds was the biggest.

Another observation is that at three day after sowing, the seeds have emerged in a higher percentage than any other cases in which we used organic fertilizers, exception being variants 5 and 6 (Table 3).

Table 1. The percentage of seeds of Asparagus emerged - in peat substrate

Variants	after 4 days	after 5 days	after 6 days
	(%)	(%)	(%)
V1 - control	0	20.67	69.55
V2	17.66	55.67	21.75
V3	21.33	70.67	4.00
V4	22.25	62.67	9.67
V5	17.00	50.67	27.67
V6	23.25	71.33	2.00

Table 2. Influence of fertilizers on the emergence of asparagus seeds - the variant in perlite

Variants	Percentage of seeds sprung (%)	Differences (%)	(%)	Signifcation
V(0) average	94.67	4.45	104.93	*
V(1)	90.22	0.00	100.00	Ct
V(2)	95.08	4.86	105.39	**
V(3)	96.22	6.00	106.65	**
V(4)	94.59	4.37	104.84	*
V(5)	95.34	5.12	105.68	**
V(6)	96.58	6.36	107.05	**

LSD 5% = 3.370 LSD5% in % = 3.7353; LSD1% = 4.800
LSD 1% in % =5.3203 LSD1% =6.940 LSD1% in %=7.6923

Table 3. The percentage of seeds of asparagus emerged - in perlite substrate

Variants	after 4 days	after 5 days	after 6 days
	(%)	(%)	(%)
V1 – control	0	21.33	72.00
V2	15.75	57.00	20.33
V3	28.55	66.55	-
V4	24.25	64.33	5.25
V5	8.25	60	27.67
V6	25.25	72.33	0

Table 4. The influence of fertilizers on the emergence of asparagus seed - perlite variant

Variants	Percentage of seeds sprung (%)	Differences (%)	(%)	Significance
V(0)	94.81	1.48	101.58	N
V(1)	93.33	0.00	100.00	Ct
V(2)	93.08	-0.25	99.73	N
V(3)	95.10	1.77	101.90	N
V(4)	93.83	0.50	100.54	N
V(5)	95.92	2.59	102.78	*
V(6)	97.58	4.25	104.55	**

LSD 5% = 2.280 LSD 5% in % = 2.4429 LSD 1% = 3.250 LSD 1% in % = 3.4823 LSD 01% = 4.710 LSD 01% in %= 5.0466

Table 5. The percentage of seeds of asparagus emerged - in mixed substrate 50% perlite + 50% peat

Variants	after 4 days	after 5 days	after 6 days	total
	(%)	(%)	(%)	(%)
V1 - control	0	41.00	51.00	92.00
V2	6.00	80.25	5.00	91.25
V3	21.25	75.55	-	96.80
V4	21.25	73.00	1.00	95.25
V5	2.00	64	29.25	95.25
V6	27.00	69.85	0.00	96.85

Table 6. The influence of asparagus seeds emergence on the variant - substrate 50% perlite + 50% peat

Variants	Percentage of seeds sprung (%)	Differences (%)	(%)	Significance
V(0) Average	92.90	0.90	100.98	N
V(1)	92.00	0.00	100.00	Ct
V(2)	81.25	-10.75	88.32	N
V(3)	96.80	4.80	105.22	N
V(4)	95.25	3.25	103.53	N
V(5)	95.25	3.25	103.53	N
V(6)	96.85	4.85	105.27	N

LSD 5% = 15.290 LSD 5% in % = 16.6196 LSD 1% = 21.760 LSD 1% in % = 23.6522 LSD 01% = 31.470 LSD 01% in %= 4.2065

In the case of asparagus seedling that were obtained in peat substrate, in the first year, at 90 days after sowing, the greatest height was obtained in variant 3 - 38.33 cm, and the lowest height in variant 1 (Figure. 1.).

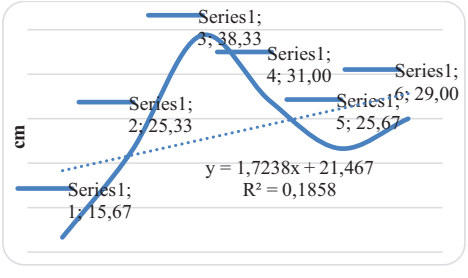

Figure 1. Influence of the fertilizer on the height of seedlings of asparagus - in peat substrate

In the case of the perlite substrate, the seedlings have obtained a height of 34.55 cm in variant 3, and the lowest in variant 1 (Figure 2).

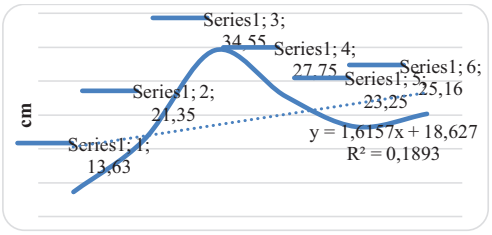

Figure 2. Influence of the fertilizer on the height of seedlings of asparagus - in perlite substrate

In the case of the variant grown in 50% perlite susbtrate and 50% peat substrate, the lowest height was obtained in variant 1 and the greates in variant 3 (Figure 3).

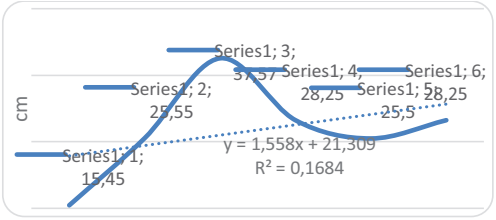

Figure 3. Influence of the fertilizer on the height of seedlings of asparagus - in peat substrate

We have found that on perlite substrate the height of the asparagus seedling was bigger in all the variants in which organic fertilizers were applied. There have been statistical differences that varied in every variant. The biggest difference was in variant 3 (Table 7).

Table 7. The height of the asparagus seedling grown on peat susbtrate.

Variants	Hight (cm)	Diferences (cm)	(%)	Significance
V(0) average	27.50	11.83	175.49	***
V(1)	15.67	0.00	100.00	Ct
V(2)	25.33	9.66	161.65	***
V(3)	38.33	22.66	244.61	***
V(4)	31.00	15.33	197.83	***
V(5)	25.67	10.00	163.82	***
V(6)	29.00	13.33	185.07	***

LSD 5% =1.970 LSD 5% in % = 12.5718 LSD 1% = 2.800 LSD 1% in % =17.8685 LSD 01% = 4.050 LSD 01% in %= 25.8456

In the case of the seedling grown on perlite substrate we have found that the statistic differences were very significant results

concerning the height of the seedling. The greatest height was recorded in variant 3 with 253.48 cm and the lowest with 156.64 cm (Table 8).

Table 8. The height of the Asparagus seedling grown on perlite substrate

VARIANT	Hight (cm)	DIFERENCES (cm)	(%)	SIGNIFICNCE
V(0) average	24.28	10.65	178.15	***
V(1)	**13.63**	**0.00**	**100.00**	**Ct**
V(2)	21.35	7.72	156.64	***
V(3)	34.55	20.92	253.48	***
V(4)	27.75	14.12	203.60	***
V(5)	23.25	9.62	170.58	***
V(6)	25.16	11.53	184.59	***

DL5% = 0.390 DL5% in % = 2.8613 DL1% = 0.550 DL1% in % = 4.0352 DL01% = 0.800 DL01% in % = 5.8694

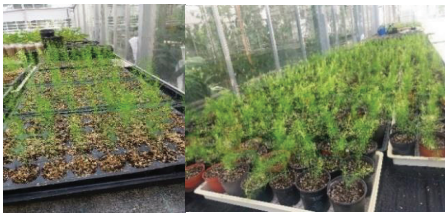

Figure 4. Asparagus seedlings

In the case of the seedling grown on substrate made of 50% peat and 50% perlite we have discovered that the height varied between 165.05 cm in V5 and 243.17 cm in V3. Also in this variant the statistic differences were very significant, in a positive way (Table 9).

Table 9. The height of the asparagus seedling grown on peat substrate

VARIANT	Hight (cm)	DIFERENCES (cm)	(%)	SIGNIFICNCE
V(0) Average	26.76	11.31	173.21	***
V(1)	15.45	0.00	100.00	Mt
V(2)	25.55	10.10	165.37	***
V(3)	37.57	22.12	243.17	***
V(4)	28.25	12.80	182.85	***
V(5)	25.50	10.05	165.05	***
V(6)	28.25	12.80	182.85	***

DL5% = 0.260 DL5% in % = 1.6828 DL1%= 0.370 DL1% in % = 2.3948 DL01% = 0.530 DL01% in %= 3.4304

The content of dry basis was bigger in V6 in the case of using the peat substrate and the both peat and perlite substrate, of 0.16980%, respectively 0.63850%. The lowest content of dry basis was recorded in V1, in the case of all types of substrate (Table 10).

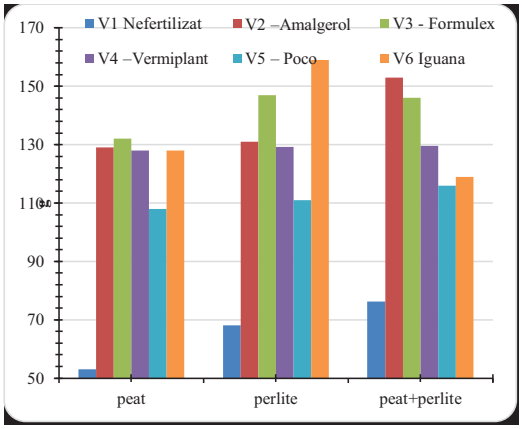

Figure 5. Average weight of asparagus roots

Figures 5-11 show how was influenced the average weight of the roots by the type of substrate and fertilizers.

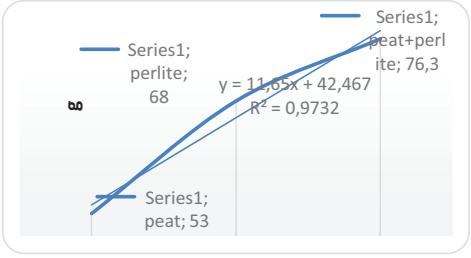

Figure 6. The influence of the substrate on the average weight of the root - Control

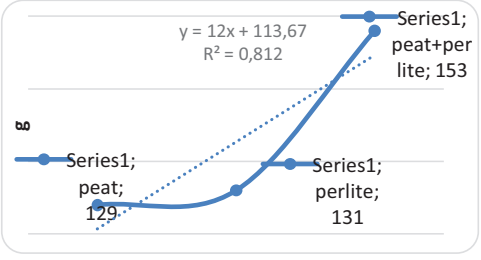

Figure 7. The influence of the substrate on the average weight of the root - variant V2 with Amalgerol

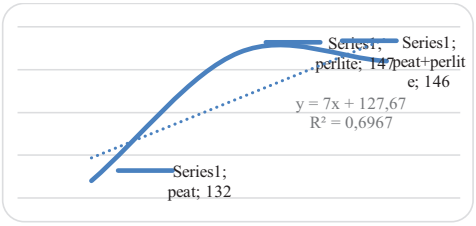

Figure 8. The influence of the substrate on the average weight of the root – variant V3 with Formulex

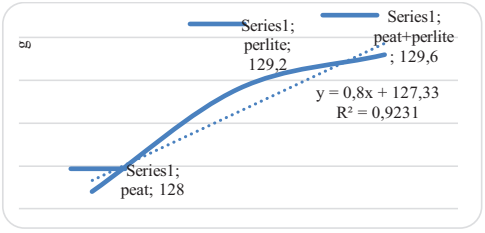

Figure 9. The influence of the substrate on the average weight of the root – Variant V4 with Vermiplant

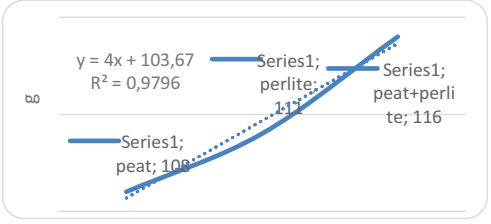

Figure 10. The influence of the substrate on the average weight of the root - Variant V5 with Poco

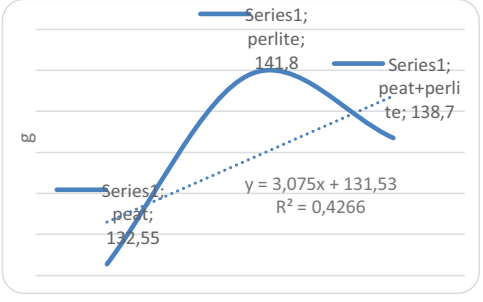

Figure 11. The influence of the substrate on the average weight of the root - Variant V6 with Iguana

Asparagus dry matter content it is influenced both by the substrat and fertilizer variants (Figure 12). Between V1-V3 and V1-V4 fertilizer variants it can be noteced a strong positive correlation (r = 0.999). A negative strong correlation it was found between V1-V5 and V2-V6 (Table 10).

Table 10. Correlation between fertilization variants depending on the substrate

Variants	V1	V2	V3	V4	V5	V6
V1	1.000					
V2	-0.774	1.000				
V3	0.999	-0.745	1.000			
V4	0.999	-0.745	1.000	1.000		
V5	-0.999	0.745	-1.000	-1.000	1.000	
V6	0.421	-0.900	0.380	0.380	-0.380	1.000

V1 - Control ;V2 -Amalgerol; V3 - Formulex; V4 - Vermiplant; V5 – Poco; V6 - Iguana

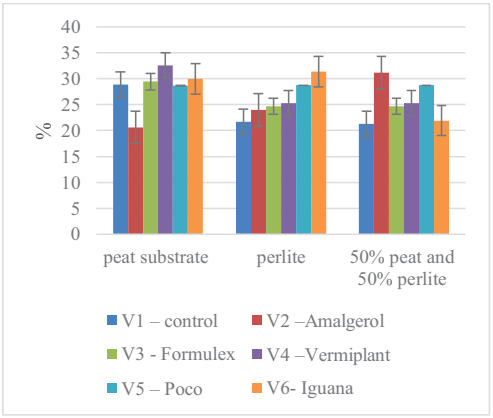

Figure 12. Asparagus dry matter content, in percent

CONCLUSIONS

In the variant in which we used peat as a substrate, the asparagus seeds have emerged after four days in the lowest percentage of 17.00% in V5 and 23.25% in V6. In the case of the unfertilized variant, there aren't any emerged seeds. At 5 days after sowing, the highest percentage of emergence of 71.33% was recorded in V6. Statistically, the differences were very significant, in a positive way, in the variants V2, V3, V5 and V6.

In the case of perlite substrate, the germination percentage was between 93.08% in V2 and 97.58% in V6. A high percentage of emerged seeds was recorded at 5 days after sowing, of 21.33% and 72.33% in V6. In V6 the highest percentage of emerged seeds was recorded, of 72%. In the case of the fertilized variants, insignificant differences were recorded in V2, V3 and V4, but very significant in V6.

In the case of the mixed substrate (50% peat and 50% perlite), at 5 days after sowing the highest percentage of germinated seeds was recorded, of 41% in V1 and 80.25% in V2.

Statistically, there weren't any differences regarding the final percentage of germination. The greatest height of asparagus seedling was recorded in V3 (38.33 cm), obtained in the case of peat substrate, and the lowest in V3 (34.55 cm), in the case of the perlite substrate variants. In the case if all organic fertilized variants we have recorded a biggest content of organic substance, in comparison with the unfertilized variant.

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SELECTIVITY OF SOIL HERBICIDES AT PUMPKINS

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Abstract

In the growing season of 2018, a field pot experiment with the pumpkin (Cucurbita pepo L.) hybrid 'Prince' F1 was conducted. In the trial, the selectivity of soil herbicides was evaluated. The variants of the trial were: 1. Untreated control; 2. Dual Gold (960 g/l S-metolachlor) - 1200 ml/ha; 3. Dual Gold - 1200 ml/ha; 4. Stomp New (330 g/l pendimethalin) - 4000 ml/ha; 5. Stomp New - 5000 ml/ha; 6. Spectrum (720 g/l dimethenamid-P) - 800 ml/ha and 7. Spectrum - 1400 ml/ha. The selectivity of the herbicides was reported by 9-score phytotoxicity scale of EWRS on the 7th and on the 14th day after germination (0 - no damage, and 9 - complete crop destruction). The earliest germination was found for the untreated control – 6 days after sowing, and for the plants of treatment 5 germinated last - 12 days after sowing. For variant 2 the phytotoxicity was score 0 and on both evaluation dates. For variants 4 to 9 the phytotoxicity was from score 2 to score 4 on the first reporting date, but on the second date, the phytotoxicity reached lethal scores of 8 to 9. The earliest flowering time was reported for the control, while the latest flowering time was found to be for treatment 3-10 days after the control. At the end of the vegetation, the control plants were the longest - 3.09 m, and the shortest was the pumpkins from treatment 3-2.46 m. The control had the highest number of fruits - 2.40 per plant.

Key words*: pumpkins, herbicides, selectivity.*

INTRODUCTION

Pumpkins are grown all around the world for agricultural purposes (animal feed), commercial and ornamental sales (Wolford & Banks, 2008).

The importance of pumpkins is determined by the nutrients contained in their fruits and their valuable dietary and taste qualities. They are a source of carotene, proteolytic enzymes, and sugars, and their seeds contain large amounts of oil (Genkova, 2008).

Between 2011 and 2015 there was an increase in production areas planted with pumpkins in Bulgaria - from 619 da to 27,420 da, respectively (Agrostatistical Reference Book, 2016).

The yields of pumpkin fruits can reach 50-60 t/ha (Shaban et al., 2014).

The weed infestation is one of the main factors leading to a reduction in yields, a deterioration in the quality of the production and an increase in its cost. In the pumpkins, mainly late-spring weeds occur. From the broadleaf weeds the most common are *Amaranthus retroflexus* L., *Capsella bursa-pastoris* Medik., *Chenopodium album* L., *Datura stramonium* L., *Xanthium strumarium* L. The most often observed grass weeds are *Sorghum halepense* (L.) Pers., *Cynodon dactylon* (L.) Pers., *Setaria* spp., etc. (Tonev et al., 2007).

The herbicides used in this crop must meet a large number of requirements. They should be highly efficacious against weeds and safe for pumpkin plants, because Pumpkins are sensitive to herbicides, therefore the chemical control of weeds is limited (Tonev et al., 2007).There are at least three major species differences among the pumpkins. Their response to herbicides differ, so it is important to know which types are sensitive to a particular herbicide to avoid crop injury.

Most herbicides labeled for pumpkins are applied pre-emergence and only provide limited control of broadleaf weeds (Brown & Masiunas, 2002; Grey et al., 2000).

The aim of the study is to evaluate the selectivity of soil herbicides applied at pumpkins in low and higher rates.

MATERIALS AND METHODS

The experiment was carried out in the experimental base of the Department of Agriculture and Herbology of the Agricultural University of Plovdiv, Bulgaria. The studied pumpkin (*Cucurbita pepo* L.) hybrid was 'Prince' F1. The pumpkins were grown in square steel pots without bottom with the size of 625 cm^2. The pots were dug into the ground. The content of available nutrients before the beginning of the experiment was N_{min}- 35.66 mg/kg soil, P_2O_5 - 32.75 mg/100 g soil and K_2O - 31.80 mg/100 g soil. The soil organic matter content is low - 0.96% and the $pH_{(H2O)}$ is 7.1. The sowing was done on the 30th of May 2018.

The experiment was designed to evaluate pumpkin responsiveness to different rates of 3 herbicide products in low and high rates. The variants of the trial were: 1. Untreated control (weed free); 2. Dual Gold 960 EC (960 g/l *S-metolachlor*) - 1200 ml/ha; 3. Dual Gold 960 EC - 1500 ml/ha; 4. Stomp New (330 g/l *pendimethalin*)- 4000 ml/ha; 5. Stomp New - 5000 ml/ha; 6. Spectrum (720 g/l *dimethenamid-P*) - 800 ml/ha and 7. Spectrum - 1400 ml/ha. All treatments were replicated 8 times. The spraying solution was 30 l/ha.

The herbicides were applied at BBCH 00 - just after seeding. The selectivity was evaluated by the 9 score scale of EWRS on the 7th and on the 14th day after the germination of the plants (at score 0 there are no damages on the crop, and at score 9 the crop is completely destroyed).

Statistical analysis of collected data was performed by using Duncan's multiple range test of SPSS 17 program. Statistical differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Germination time after seeding is shown in Figure 1. The earliest germination was found to be for the plants from the untreated control - 6 days after sowing. The plants from treatments 2 and 3 (Dual Gold - 1200 ml/ha and Dual Gold - 1500 ml/ha) germinated 8 days after sowing. The difference for the germination time was proved according to Duncan's multiple range test ($p < 0.05$).

After the application of Stomp New (5000 ml/ha) at treatment 5, the latest germination of the plants in the study was reported. The pumpkins from the studied variety germinated 12 days after sowing. The differences of this result were with a proven difference according to all other treatments in the experiment. Song et al. (2006) also found that the application of herbicides at pumpkins can affect the germination.

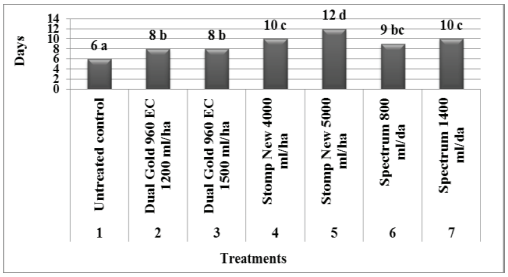

Figure 1. Germination time after seeding - days. Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

The visual evaluation of the phytotoxicity caused by the herbicide application is shown in Figure 2. On the 7th day after the plants' germination the phytotoxicity levels varied from very weak symptoms (score 1) for treatment 2 and 3 (Dual Gold 960 EC- 1200 ml/hand Dual Gold 960 EC – 1500 ml/ha) to better-expressed symptoms - thinning of the crop, strong chlorosis (score 5) for treatment 5 (Stomp New - 5000 ml/ha). On the 14th day after pumpkins germination, the phytotoxicity caused by some of the treatments increased and reached lethal values for some of the treatments.

The lowest herbicide toxicity was found for treatment 2. For this variant, the phytotoxicity score was 1 -very weak symptoms (slight stunt effect).

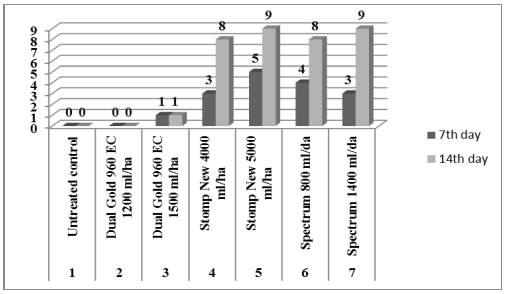

Figure 2. Score of phytotoxicity 7 and 14 days after germination

After treatment with Stomp New 4000 ml/ha (treatment 4) and Stomp New - 5000 ml/ha (treatment 5) the phytotoxicity reached score 9 - Heavy damage (perishing of the plants). The same was observed for after the application of the herbicide product Spectrum. For both application rates, the phytotoxicity score reached the lethal scores - 8 for the treatment at the rate of 800 ml/ha and 9 for the treatment at the rate of 1400 ml/ha.

The observed phytotoxicity levels and the death of the plants from the variants treated with the herbicide products Stomp New and Spectrum is the reason that there is no data shown in the next figures presenting the results of the pot experiment.

The stem length 14 days after germination is shown on Figure 3. The longest stems were found to be for the untreated control - 122.24 mm. This result was not with proved differences according to the statistical analyses used with treatment 2 (Dual Gold - 1200 ml/ha), but it proved with treatment 3 (Dual Gold - 1500 ml/ha).

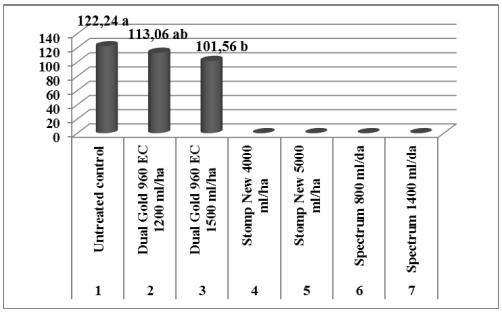

Figure 3. Stem length14 days after germination, mm.
Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

The width of the stems at the plants of the untreated control was the highest (9.88 mm) and was with a proved difference by Duncan's multiple range test ($p < 0.05$) only with treatment 3 (Dual Gold - 1500 ml/ha) (8.01 mm) (Figure 4).

After the treatment with Dual Gold - 1200 ml/ha the stem width (9.47 mm) was found to be only with 0.41 mm lower than those of the untreated control.

The obtained data for these two biometrical parameters showed that the high rate of Dual Gold (1500 ml/ha) led to decrease of the length and width of the stem.

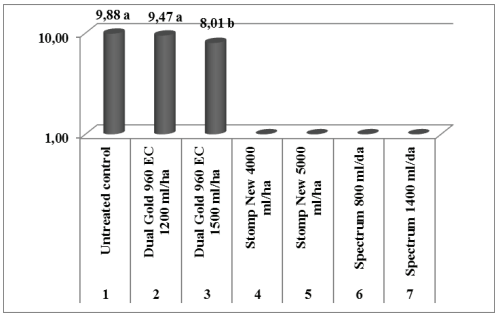

Figure 4. Stem width14 days after germination, mm.
Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

On Figure 5 are shown the results for the number of true leaves 14 days after germination.

It was observed that the untreated control had formed 5.20 true leaves per plant. The statistical test showed proved differences in comparison with the treatments with Dual Gold independently the used application rate. The plants from treatments 2 and 3 had formed one leave less than the untreated control.

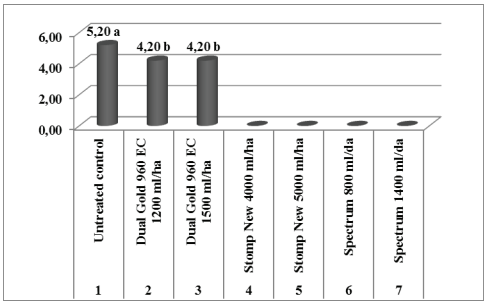

Figure 5. Number of true leaves14 days after germination.
Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

On Figures 6 and 7 are presented the obtained results considering the petiole length and petiole width.

These two parameters were also influenced by the herbicide application.

The petiole length as well as petiole width were with highest values for the untreated control. The petiole length for the control plants was 85.39 mm and the width was 5.60 mm.

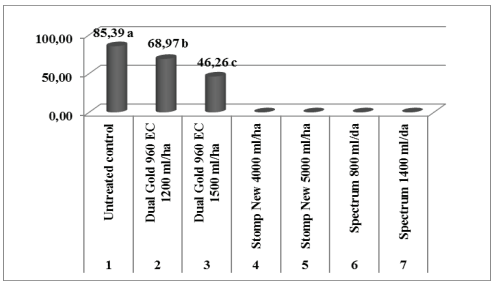

Figure 6. Petiole length 14 days after germination. Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

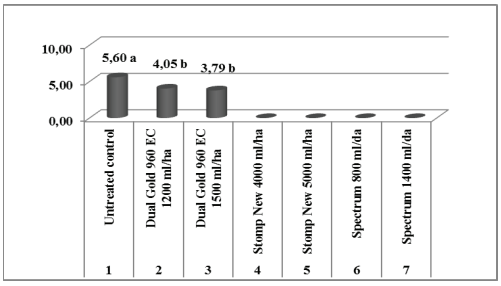

Figure 7. Petiole width 14 days after germination. Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

The earliest flowering time was reported for the control 37 days after the sowing – on the 6th of June 2018 (Figure 8).

The latest flowering time was found to be for treatment 3 - 10 days after the control (47 days after the sowing). The flowering time for treatment 2 (Dual Gold 960 EC - 1200 ml/ha) was recorded to be 4 days after the untreated control (Figure 8).

It was found that injury from herbicides can also stress and lead to the death of flower buds at pumpkins (Maynard & Egel, 2016).

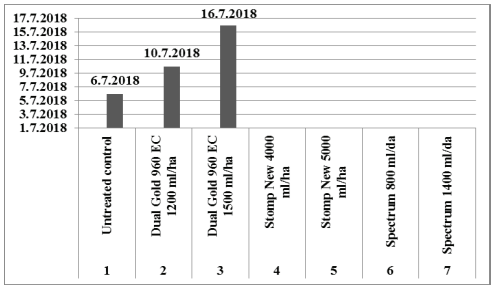

Figure 8. Beginning of flowering. Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

On figure 9 is shown the length of the plants at the end of the vegetation. At the end of the vegetation, the control plants were the longest – 3.09 m. The difference for Length of the plants at the end of the vegetation, was proved according to Duncan's multiple range test ($p < 0.05$) only with treatment 3 (Dual Gold 960 EC - 1500 ml/ha). Varhney et al. (2015) also found that herbicide-induced toxicity affects the growth of crop plants.

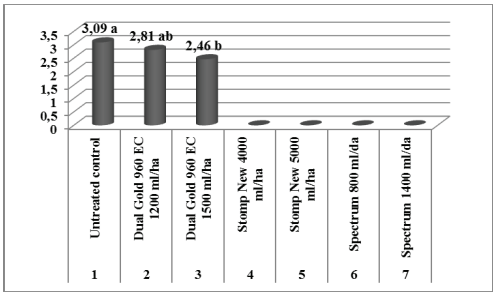

Figure 9. Length of the plants at the end of the vegetation, m. Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

In a study conducted by El-Nahhaland Hamdona (2017) the application of some soil herbicides led to growth retardation of the plants grown in the experiment.

In the trial conducted by Nosratti et al. (2017) pumpkin species have exhibited pronounced differences in tolerance to the different herbicides that were studied. Despite early-season phytotoxicity caused by some of the herbicide applications, the pumpkin plants were able to recover and produce yield, but it was lower than those of the untreated controls.

These results correspond to our trial data. Despite the phytotoxicity caused by Dual Gold 960 EC applied at the rate of 1500 ml/ha (treatment 3) were overcome, the yield was lower in comparison to the untreated control (Figure 10). The control had the highest number of fruits - 2.40 per plant. The difference for the productivity of the control according to treatment 3 was with a proved difference by Duncan's multiple range test ($p < 0.05$) and was not proved with treatment 2 where Dual Gold 960 EC was applied at a rate of 1200 ml/ha.

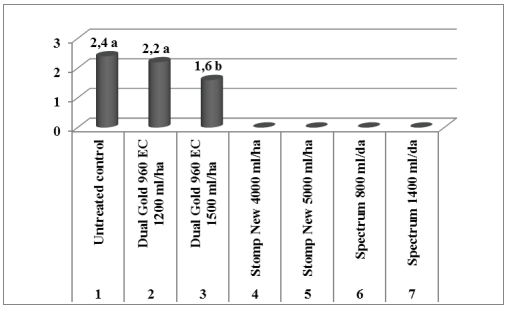

Figure 10. Number of fruits per plant.
Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

The crops are exposed to different biotic and abiotic stress in the production conditions.
The herbicides are very often a stress factor to the crop.
The interaction with other biotic (insects, diseases, nematodes, etc.) and abiotic (temperature, moisture, etc.) stress factors can lead to yield loss (Bagavathiannan et al., 2017).
It is also very important to remember that the yield decrease from not controlling the weed infestation is greater than the application of herbicides that can cause different damage symptoms to the crops (Hartzler, 2013).
In our trial the highest weight of one pumpkin fruit for the untreated control was reported – 3.89 kg (Figure 11).
The weight of one fruit from treatment 2 (Dual Gold 960 EC - 1200 ml/ha) was lower - 3.65 kg. The differences in the values were with not proved difference according to the control.
The fruits from the pumpkins treated with the higher rate of Dual Gold 960 EC had fruits weighting 3.16 kg that was 73 grams lower than the control.

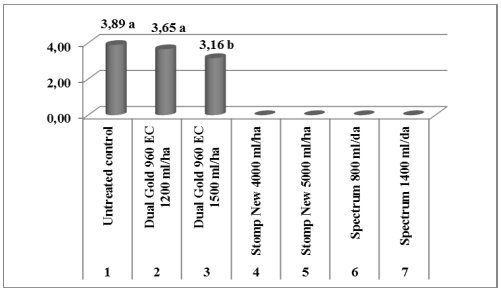

Figure 11. Weight of 1 pumpkin fruit, kg.
Columns with different letters are with a proved difference by Duncan's multiple range test ($p < 0.05$)

CONCLUSIONS

The herbicide application delayed pumpkins germination independently the herbicide product and studied application rate.
The application of Stomp New (330 g/l pendimethalin) and Spectrum (720 g/l dimethenamid-P) lead to high phytotoxicity score causing perishing of the plants.
The studied biometrical parameters of the pumpkins like stem length and width, number of true leaves, petiole length and width 14 days after germination were also influenced by the herbicide application. The highest values were obtained for the untreated (weed free) control.
The flowering time, as well as the length of the pumpkins, was with lowest values after the application of Dual Gold 960 EC at rate of 1500 ml/ha.
The highest productivity was recorded for the untreated (weed free) control and treatment 2 (Dual Gold 960 EC - 1200 ml/ha).
The highest weight of one pumpkin fruit for the untreated (weed free) control was reported, but the difference with treatment two were not statistically proved.

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THE EFFECT OF DIFFERENT SOWING DATES ON YIELD AND SOME AGRONOMIC CHARACTERISTICS OF CARROT (*DAUCUS CAROTA* L.)

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Abstract

The objective of this study was to determine the effect of different sowing dates on yield and some agronomic characteristics of different carrot varieties ('Romance' F1, 'Nansun' F1, 'Maestro' F1, 'Soprano' F1 and 'Vac-64' F1). The results of the study showed that the effect of sowing dates on the agronomic characteristics was significant. While the highest yield was obtained from 'Soprano' F1 at 4th sowing date, the highest plant height, the highest leaf length, maximum number of leaves, the highest carrot weight, the highest carrot length and the highest carrot diameter was obtained from 'Romance' F1 at 2nd sowing date, 5th sowing date, 4th sowing date, 3rd sowing date, 4th sowing date and 2nd sowing date, respectively.

Key words*: carrot, sowing date, yield.*

INTRODUCTION

Carrot (*Daucus carota* L.), a vegetable species of *Apiaceae* (*Umbelliferae*) family, is widely cultivated in the world (Yamaguchi, 1983; Peirce, 1987; Simon et al., 2008).

In recent years, with the increasing awareness of the nutritional value of carrot, especially in the content of vitamin A (α and β carotene), B1, B2 and C vitamins, has increased its worldwide importance (Leclerc et al., 1991; Warmanand Harvard, 1996; Yawalker, 1985; Arscoot & Tanumihardjo, 2010; Khan, 2011; Carvalho et al., 2014). In addition, carrot contains bioactive compounds such as polyacetylenes and isocumins and fiber that promote human health (Kidmose et al., 2004).

For this reason, carrot is a vegetable that people should consume daily of all ages (Guerra et al., 2001).

Carrot is widely used in canned, juice and cezerye industries as well as in fresh consumption (Chauhan, 1989).

The largest production amount of carrot was China with 20.274.393 tons in 2018, followed by Uzbekistan with 2.249.733 tons and Russia with 1.805.787 tons in the world.

Turkey ranks 10th in world carrot production (Anonymous, 2019a).

Turkey has a satisfactory level of production and consumption of carrot. In 2018, 642.837 tons of carrot were produced in 12347.8 ha area. The proportion of carrot production in total vegetable production is 2.14%, and carrot ranks 9th among all vegetables produced. The provinces have the highest carrot production are Konya, Ankara and Hatay in Turkey. Carrot production in Hatay province is increasing day by day due to climate conditions, export opportunities and contribution to employment. Not only carrots grown in the region are exported to countries such as Syria, Saudi Arabia and Romania, but also are marketed within the different countries of Turkey. Total 58.190 tons of carrots were produced in 2121.9 ha area in Hatay province in 2018 (Anonymous, 2019b). In this region, carrot production starts with the seed sowing from the beginning of August. The harvest starts at December and lasts until the end of April.

The most important climate factor affecting on carrot cultivation is temperature. In addition to plant growth, temperature is also effective in the root shape, root color and growth of the green parts of shoots. Carrot is mainly a temperate crop grown during spring through autumn in temperate countries and during winter in tropical and subtropical countries of

the world especially because it is resistant to frost at germination stage and early period of growing (Bose & Som, 1986).The optimum growth temperature for carrots is between 15.6 and 21.1°C. Higher and lower temperatures adversely affect growth and decrease the quality of roots (Barnes, 1936; Khan, 2011). Sowing date has important effects on the growth, development and yield of carrot due to environmental factors such as temperature and light intensity (Mack, 1979). Therefore, factors affecting the yield of carrot also include sowing date and it may play a critical role to extend availability of carrots in the market in the early and late season (Rashid & Shakur, 1986; Khan, 2011).

In this study, it is aimed to determine the effect of different sowing dates on plant growth, yield and some agronomical characteristics of carrot has great economic importance in Kırıkhan-Hatay, province of Turkey.

MATERIALS AND METHODS

The research was carried out in Kırıkhan district of Hatay province and the laboratories of Kilis 7 Aralik University Advanced Technology Application and Research Center between the years of 2016-2017. 'Nansun' F1, 'Maestro' F1, 'Romance' F1, 'Soprano' F1 and 'Vac-64 ' F1 carrot varieties were used as plant material. In order to determine the effect of different sowing dates, five sowing dates have been tried; 20th July 2016, 10th August 2016, 1st September 2016, 20th September 2016 and 10th October 2016.The experiment was established as randomized blocks design with three replications. Sprinkler irrigation system was used for irrigation of plants. Yield, carrot weight, carrot length, carrot diameter, plant height, number of leaves and leaf length values were determined. Tukey test was used to determine different groups after variance analysis.

RESULTS AND DISCUSSIONS

Yield

The carrot yield varied depending on the sowing dates and the varieties. According to the variance analysis, the effect of different sowing dates on the carrot yield was significant ($p \leq 0.05$). The highest carrot yield was obtained from the 'Soprano' F1 with 469.8 kg ha^{-1}at 4th sowing date (20th September), while the lowest carrot yield was obtained from 'Nansun' F1 with 168.5 kg ha^{-1} at 5th sowing date (Table 1). There have been significant differences in yields between varieties and sowing dates. Similar results were obtained by Sarı and Paksoy (2004). They reported that the yield varied depending on the varieties and sowing dates and the highest total carrot yield. Similarly, Elgin Karabacak (2010) used 24 different sowing dates on a monthly period between 15th March, 2006 and 15th February, 2008 in order to determine the most suitable sowing date for Parmex mini carrot variety. In the study, it was reported that the effect of sowing dates on total yield was significant and the highest yield (251.6 kg ha^{-1}) was obtained from mini carrots sown in February.

Nilsson (1987) determined that delaying of sowing time for 1 or 2 months after the beginning of May resulted in a reduction in the growth of both roots and foliage. Khan (2011) reported that the effect of different sowing date on plant growth of carrot and yield is important.

The highest yield value was reported as 22.63 t ha^{-1}, at 2nd sowing date (15th November). Mason and Tong (1971) used two different sowing dates (October and January) and 12 different carrot varieties.

They reported that the highest yield was obtained from varieties cultivated at the beginning of October. Pashine et al. (1993) investigated effects of sowing date on yield of different carrot cultivars and local carrot genotypes.

They used seven sowing dates at two-week intervals from 5th November to 5th February. The highest yield (155.18 q/ha) was obtained from the sowing on 5th November.

Carrot Weight

The carrot weight varied depending on the sowing dates and the varieties. The highest carrot weight was obtained from 'Romance' F1 (230.22 g/plant) at 3rd sowing date while the lowest carrot weight was obtained from 'Nansun' F1 with 66.58 g/plant at the 2nd sowing date (Table 2).

In a similar study, it was stated that the carrot weight changed depending on the sowing date and variety characteristics and the highest carrot weight (105.60 g/plant) was obtained from Asubeni F1 cultivar (Sarı & Paksoy, 2004).

Carrot Length and Diameter

In the study, the carrot length and carrot diameter varied depending on the sowing dates and the variety.

The highest carrot length was obtained from 'Romance' F1 with 29.33 cm at 4th sowing date (20th September) while the lowest carrot length (11.99 cm) was obtained from 'Maestro' F1 and 'Vac-64 'F1 varieties at 5th sowing date (10th October).

The highest carrot diameter (40.17 mm) was determined at 'Romance' F1 at the 2nd sowing date (10th August) and the lowest carrot diameter was 14.06 at 'Nansun' F1 at 5th sowing date (Table 3).

Different researchers reported that the length and diameter of the carrots obtained from the late sowing dates were higher than the carrots sown in the early period (Hussain et al., 2008). For example, Sarı and Paksoy (2004) reported that the effect of sowing dates and variety on carrot length was significant Similarly, Khan (2011) reported that the effect of sowing dates on plant growth was important, and the maximum root length (22.46 cm) was obtained from the carrots sown on 15th November.

Plant height, Leaf length and Number of leaves

The highest plant height, leaf length and number of leaves were obtained from 'Romance' F1.

These values were measured as 75.66 cm at the 2nd sowing date, 54.33 cm at the 5th sowing date and 8.21 numbers per plant at the 4th sowing date, respectively (Table 4).

The more number of leaves during plant development period of carrot is an important criterion for better growth of the roots.

For this reason, seed sowing date also has an effect on the number of leaves.

Jaiswal et al. (2003) reported that the highest plant height (156.25 cm) and the maximum number of leaves per plant in carrot were obtained from sowing on 20th July.

In another study, it was found that the highest plant height (46.83 cm) was obtained from the plants sowed on November 15 (Khan, 2011; Ali et al., 2014).

In contrast to these findings, Nilsson (1987) reported that delaying of sowing date for one or two months from the beginning of May led to a decrease in both root and leaf growth.

Table 1. Effect of sowing dates on yield of carrot (kg ha^{-1})

Cultivars	Sowing Dates				
	20th July	10th August	1st September	20th September	10th October
'Nansun' F1	318.8 fg	318.3fg	363.0ef	400.3de	168.5k
'Maestro' F1	358.9ef	339.1 f	438.6a-d	448.0a-c	236.3ij
'Romance' F1	194.3 jk	459.6ab	441.6a-d	405.0c-e	230.9ij
'Soprano' F1	421.7 bcd	433.6 a-d	458.6ab	469.8a	214.3ijk
'Vac-64 'F1	284.3 hi	337.0f	253.3hi	320.0fg	181.3k

Levels not connected by same letter are significantly different ($p \leq 0.05$)

Table 2. Effect of sowing dates on carrot weight (g/plant)

Cultivars	Sowing Dates				
	20th July	10th August	1st September	20th September	10th October
'Nansun' F1	76.95 jk	66.58 k	106.05 g-k	106.84 g-j	69.68 jk
'Maestro' F1	119.95 f-i	148.16 d-f	124.16 f-h	182.18 b-d	89.44 h-k
'Romance' F1	81.12 I-k	174.07 c-e	230.22 a	215.41 ab	89.10 h-k
'Soprano' F1	153.72 d-f	95.55 g-k	150.66 d-f	229.22 a	79.43 jk
'Vac-64 'F1	135.16 e-g	175.97 b-d	152.29 d-f	211.59 a-c	73.55 jk

Levels not connected by same letter are significantly different ($p \leq 0.05$)

Table 3. Effect of sowing dates on carrot length (cm) and carrot diameter (mm)

Cultivars	Sowing Dates				
	20th July	10th August	1st September	20th September	10th October
	Carrot length (cm)				
'Nansun' F1	16.25 hi	13.50 ij	15.33 h-j	16.88 g-i	17.33 f-h
'Maestro' F1	17.63 f-h	20.60 d-f	18.38 e-h	23.10 cd	11.99 j
'Romance' F1	15.58 hi	21.48 de	25.44 bc	29.33 a	17.00 g-i
'Soprano' F1	21.61 de	17.33 f-h	20.16 d-g	28.88 ab	16.55 hi
'Vac-64 'F1	16.44 hi	18.66 e-h	15.49 h-j	18.22 e-h	11.99 j
	Carrot diameter (mm)				
'Nansun' F1	29.87 de	18.08 f-i	22.12 f-h	22.28 f-h	14.06 i
'Maestro' F1	36.09 a-d	36.94 a-c	23.67 e-g	24.45 ef	16.33 hi
'Romance' F1	32.53 b-d	40.17 a	40.03 a	35.93 a-d	16.87 hi
'Soprano' F1	35.69 a-d	32.49 b-d	24.26 ef	35.80 a-d	17.41 g-i
'Vac-64 'F1	39.39 a	38.21 ab	29.94 de	31.17 c	18.17 f-i

Levels not connected by same letter are significantly different ($p \leq 0.05$)

Table 4. Effect of sowing dated on plant height (cm), leaf length (cm) and number of leaves (number/plant)

Cultivars	Sowing Dates				
	20th July	10th August	1st September	20th September	10th October
	Plant height (cm)				
'Nansun' F1	44.11 ij	43.49 j	43.77 j	57.88 c-h	64.99 a-f
'Maestro' F1	56.88 d-i	59.65 b-h	53.22 f-j	64.66 a-f	55.77 e-j
'Romance' F1	61.94 b-g	75.66 a	70.55 a-c	75.44 a	71.10 ab
'Soprano' F1	56.33 e-j	50.77 g-j	62.33 b-g	69.55 a-d	48.99 h-j
'Vac-64 'F1	47.99 h-j	65.33 a-f	50.10 g-j	66.99 a-e	53.66 f-j
	Leaf length (cm)				
'Nansun' F1	28.72 g	30.55 fg	27.55 g	43.10 b-d	48.33 a-c
'Maestro' F1	38.99 c-f	40.66 c-e	34.77 d-g	41.44 b-e	26.44 g
'Romance' F1	47.33 a-c	54.10 a	45.44 a-c	46.22 a-c	54.33 a
'Soprano' F1	35.66 d-g	34.77 d-g	40.55 c-e	39.99 c-f	32.66 e-g
'Vac-64 'F1	33.22 efg	45.44 a-c	34.33 d-g	50.55 ab	41.44 b-e
	Number of leaves (number/plant)				
'Nansun' F1	4.61 hi	5.22 f-i	5.11 f-i	6.00 c-h	6.99 a-e
'Maestro' F1	4.88 g-i	6.77 a-f	6.77 a-f	7.55 a-d	5.66 e-h
'Romance' F1	6.38 b-g	6.44 b-g	7.77 ab	8.21 a	7.44 a-d
'Soprano' F1	4.99 g-i	3.55 i	7.21 a-e	7.55 a-d	6.43 b-g
'Vac-64 'F1	7.64 a-c	6.32 b-h	6.77 a-f	6.77 a-f	5.88 d-h

Levels not connected by same letter are significantly different ($p \leq 0.05$)

CONCLUSIONS

In the study, it was determined that the effect of sowing dates on carrot yield and some plant properties was important because of the ecological conditions.

When the varieties and sowing dates interacted, it was determined that the highest yield was obtained from 'Soprano' F1 at 4th sowing date (20th September) with 4698 kg/da. However, the highest carrot length, carrot diameter, plant height, leaf length and number of leaves were obtained from 'Romance' F1. These values were measured as 29.33 cm at 4th sowing date, 40.17 cm at 2nd sowing date, 75.66 cm at the 2nd sowing date, 54.33 cm at the 5th sowing date and 8.21 number/plant at the 4th sowing date, respectively.

The results obtained from the study conducted in Kırıkhan district of Hatay province revealed that 'Soprano' F1 and 'Romance' F1 carrot varieties could be higher yields than the other varieties, provided that seeds sown between 1stSeptember and 20th September.

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WATER-YIELD RELATIONSHIP IN GRAFTED MELON

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Abstract

As in many plants, the water need of the plant cannot be met with the rainfall and irrigation of the plant to the root area causes significant increases in the yield in the cultivation of melon. There are many studies aiming to determine the amount of reduction that can be applied in irrigation water without causing any significant decrease in the quality and yield. However, the number of studies, if there is, in which a variety of melon is grafted on another kind of melon is extremely limited. In this article which is on irrigation, a grafted melon plant is discussed.

Key words*: deficit irrigation, grafted melon, water-yield relationship.*

INTRODUCTION

In grafting, two plant cultivars with similar organic structure are combined into one plant with the use of different methods and they grow as a single plant. This is what is called grafting. Whereas the parts of the plants on the soil surface are called as scion for grafted plants, the root parts are called as rootstock (Yarşı & Rad, 2004).

The cultivation of grafted garden plants started in late 1920 when watermelon plants were grafted on the pumpkin rootstock in Korea and Japan (Yamakawa, 1983). Following the first experiments, the cultivation of such grafted plants gradually increased and the cultivation of grafted watermelon, cucumber and various *Solanaceae* plants have become more and more common either in greenhouses or fields (Kurata, 1992).

In recent years, the production of grafted plants has increased in many countries. In parallel with this increase, the intended use of grafted plants has also been diversified; for example, to increase plant growth and development, to control plant growth, to increase resistance to diseases caused by pathogens, to reduce viral, fungal and bacterial infections, to provide tolerance to abiotic stresses such as temperature, salinity, drought stress, to strengthen the root system, to increase nutrient and mineral intake (Rivero et al., 2003).

The first grafted seedling production for commercial purpose started with the production of tomato in 1998 in Turkey. In the following years, the number of grafted seedlings produced and the rate of production has increased hay by hay.

In the first years of the production of grafted seedlings, tomato seedlings were produced commonly, but in recent years the production of grafted watermelon seedlings reached significant amounts (Balkaya, 2013).

The production of grafted seedlings reached about 50 million units annually in Turkey when the year 2000 was considered. About 60% of this production is watermelon, 35% is tomato and 5% is eggplant (Abak et al., 2010).

In 2013, the number of companies producing grafted seedling reached 28, and the amount of seedling production reached approximately 120 million (Balkaya et al., 2015).

There are studies in the literature examining the adaptation of rootstock-cultivar (Salehi-Mohammadi et al., 2009; Haegi et al., 2013; Yıldız & Balkaya, 2016), nutrient intake in melons and other vegetables (Yarşı & Sarı., 2006), resistance to salinity stress (Colla et al., 2006; Orsine et al., 2013; Kiran et al., 2017), fusarium endurance (Crino et al., 2007;) and resistance to nematodes (Haunay & Halmasso,

1985; Rahman et al., 2002), endurance to drought, water use (Agelea & Cohenb, 2009).
In this study, Kırkağaç type 'Ünlü' melon varieties grafted on Ferro rootstock was examined.

MATERIALS AND METHODS

The study was carried out on the land of West Mediterranean Agricultural Research Institute located 20 km east of Antalya province near the Antalya-Alanya highway.
The research site is located at 36^0 52′ north latitude and 30^0 50′ east longitude and the average elevation is 15 m.
The research area has the type of soils with clayey loam and clayey loam silty structure, and there is not any drainage problem.
The irrigation water used was provided by pumping from the deep well in the research area.
As the plant material, non-grafted 'Ünlü' variety (*Cucumis melo* L.) and Kırkağaç type 'Ünlü' melon varieties grafted on Ferro rootstock were used.
Drip irrigation method was used as the irrigation method in the research.
Experimental treatments consisted of 4 irrigation levels applied to non-grafted 'Ünlü' variety and 'Ünlü' variety grafted on Ferro rootstock.
In the study, eight treatments consisting of a combination of two varieties and four levels of irrigation were discussed.
In drip irrigation system, dripper laterals were placed at the nearest point to the plant in a way to be lateral to each plant line.
The parcels were arranged so that the distance between the plants was 1.5 m and the distance from the plant was 1 m and the row length was 15 m.
The area of a parcel was 15 m x 4.5 m = 67.5 m^2. In the drip irrigation system, laterals had a diameter of 16 mm and drippers had a flow rate of 4 l/hour.
The distance between the drippers was 0.5 m. The manifold pipeline was made of polyethene (PE) material, and it was resistant to 50 mm diameter and 8 atm pressure. The main pipeline was made of PE material with a pressure of 90 mm and a pressure of 10 atm. The control valve, pressure gauge and water meter were installed while passing from the main pipeline to the manifold pipeline. After the pump unit of the irrigation system, there are 2-inch hydrocyclones, 150 mesh sieve filter, ball valve and manometer. Butterfly valves were placed in the irrigation system at the beginning of laterals.
In the irrigation treatment was applied: (1) I100, when 30-40% of the water is consumed in the soil, the existing soil water content is completed to the field capacity; (2) I80, in each irrigation, 80% of the irrigation water applied to the I100 treatment was applied; (3) I60, in each irrigation, 60% of the irrigation water applied to the I100 treatment was applied; (4) I40, in each irrigation, 40% of the irrigation water applied to the I100 treatment was applied.
In the study, soil moisture content was observed in three layers in 0-30, 30-60 and 60-90 cm depths from the soil surface by gravimetric method. In the soil samples taken before the irrigation, the moisture content of the three layers was determined and the soil moisture content was determined for 0-90 cm depth (Yıldırım et al., 2009).
Irrigation treatments were planned as control treatment (I100) in which the moisture was completed to the field capacity and the treatments in which 80%, 60% and 40% of the amount of water applied to the control treatment were applied.
The soil moisture content before the irrigation was found out for each treatment, and the amount of irrigation water to be applied was calculated by using the following equation.

$$d = \left(\frac{TK_{0-90} - MN_{0-90}}{100}\right) D * P$$

In the equation, d = quantity of irrigation water applied, TK0-90 = 0-90 cm is the field capacity in the soil layer (in the form of volume percentage), MR0-90 = Moisture measured in the soil layer at the start of irrigation 0-90 cm (in the form of volume percentage, %), D = is the depth of soil layer, mm (90 mm) and P = wetted area ratio.
In the study, the wetted area ratio was calculated by measuring the wet strip width up to 30-40 cm of soil depth at the beginning, middle and end of the laterals, at the beginning

and near the end of the manifold measuring the average value to the lateral range.

The amount of irrigation water that should be given to each treatment was applied according to the irrigation time with the help of the equation below (Yıldırım et al., 2009).

$$Ta = \frac{1000\ d}{q\ N}$$

In the equation: Ta = watering time, hour; d = amount of irrigation water to be applied, mm; q = dripper flow rate, l hour^{-1}; N = number of unit area drippers (1333 pcs/ha).

RESULTS AND DISCUSSIONS

The total amount of irrigation water applied to the treatments discussed in the study ranged from 67 mm to 117 mm in the grafted melon and from 79 mm to 145 mm in the 'Ünlü' variety.

In the study, yield values obtained according to irrigation levels ranged from 15.7 to 27.0 t ha^{-1} in grafted 'Ünlü' variety and between 29.6 and 40.3 t ha^{-1} in non-grafted 'Ünlü' (Figure 1).

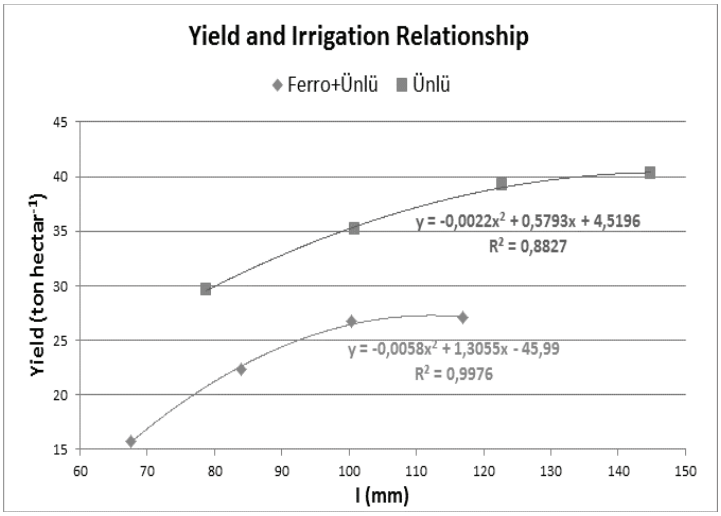

Figure 1. Change in yield due to the amount of irrigation water

Seasonal plant water consumption (ET) values in the melon varieties discussed in the study ranged from 191 mm to 225 mm in the grafted (Ferro + 'Ünlü') plants and ranged from 246 mm to 284 mm in the 'Ünlü' variety.

In general, as the ET value increased, the yield also increased in the plants and a positive correlation was found between ET and yield. However, although an equal amount of irrigation water was applied to grafted and non-grafted plants, ET values were found to be lower in the grafted plants than in the non-grafted plants. This may be due to the fact that the development of the grafted plants due to the rootstock adaptation is slower than the non-grafted plants.

Adaptation between rootstock and species is very important. The adaptation between the rootstock and the plant cultivar directly affects the intake of water and mineral elements necessary for optimal growth of the grafted plant. In other words, vascular discontinuity of the grafting joint may cause plant growth to slow down, and the plant may also cause physiological disorders, and consequently, the continuity of communication between plant cultivar and rootstock may be adversely affected.

The adverse effects of non-adaptation situation can be observed from the seedling stage and in later development periods, when the nutrient and water requirements of the plant increased and during the fruitage period (Martinez-Ballesta et al., 2010).

In the study, the coefficient of determination of the mathematical equation used for the

estimation of yield based on ET was found to be (= 'Ünlü') R^2 = 0.990 for non-grafted plants, and R^2 = 0.977 for grafted plants (Ferro + 'Ünlü') (Figure 2). Kirnakand Dogan (2009) stated that the watermelon plant yields decreased as the ET value decreased. The determination coefficient for the mathematical equation which they used to calculate ET and yield relationship was R^2 = 0.99. Castellanos et al. (2016) reported that the levels of ET in the water vary between 356 and 472 mm in irrigations with different nitrogen levels and the yields obtained varied between 32.4 and 52.0 t ha^{-1}.

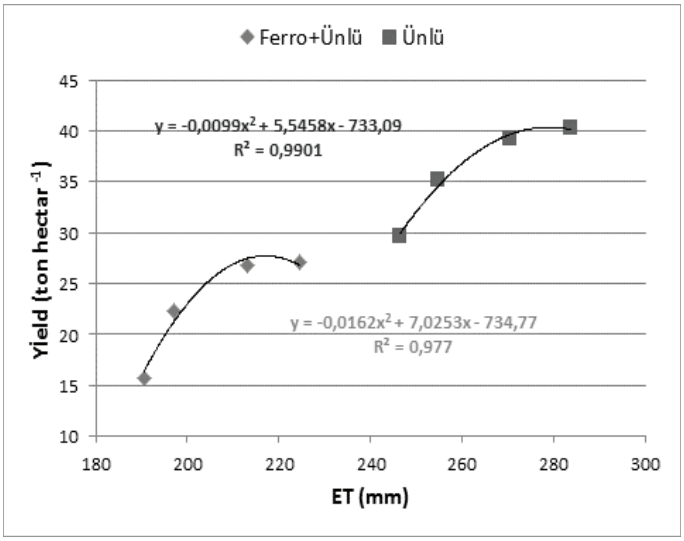

Figure 2. Plant water consumption (X = ET, mm) and yield relationship

In our study, the yield values of the grafted plants were found to be lower than the non-grafted plants. As a result of variance analysis conducted for the yield values, the effect of species effect on the fruit yield and the level of the irrigation level were found to be significant while the interaction of the species irrigation level was not significant. There were two statistics between the yield values of the species. The average yield obtained from the grafted 'Ünlü' species (36.0 t ha^{-1}) were found to be higher than the yield obtained from Ferro+'Ünlü' grafted species (22.9 t ha^{-1}). No statistically significant difference was found between the levels of I100 and I80 in terms of irrigation levels, and both treatments had higher yields than I60 and I40. The I60 treatment was found to be in group b with the second highest yield. The yield values of the I40 treatments were found to be the lowest in group c.

The effect of species effect on irrigation water use (IWUE), irrigation level effect and species level interaction were found statistically significant.

In the study, the efficacy of irrigation water use in non-grafted plants ('Ünlü') was higher than that of grafted (Fero + 'Ünlü') plants.

Whereas the irrigation level which has the lowest water use efficiency among the irrigation levels was I100, no significant difference was found among the other levels (I40, I60, I80).

There was no significant difference between the other levels (I40, I60, I80) and I100 with the lowest water usage efficiency average among the irrigation levels.

Out of the averages of species irrigation interaction, all irrigation levels of the grafted plants (Ferro+'Ünlü') (I100, I80, I60, I40) and non-grafted plants ('Ünlü'), I100 level IWUE was found to be in the lowest statistical group.

There was no statistically significant difference between I40 and I60 irrigation levels in the non-grafted plants with respect to IWUE, while

irrigation water use efficiency was higher than I80 and I100 levels at I40 level. In the case of non-grafted plants, in the I60 treatment, the IWUE value was found to be higher than I100.
In the study, the water use efficiencyin non-grafted plants was higher than that of grafted plants. In contrast to the results obtained in our study, San Bautista et al. (2011) reported that water use efficiency increased by 35% compared to non-grafted plants in double-grafted plants. Among the irrigation levels, the water use efficiency was the lowest in I40. The WUE averages of other irrigation levels were not statistically significant.

CONCLUSIONS

The total amount of irrigation water applied ranged from 67 mm to 117 mm in the grafted melon and from 79 mm to 145 mm in the 'Ünlü' series.
Seasonal plant water consumption (ET) values in grafted and non-grafted plants ranged from 191 mm to 225 mm in the grafted (Ferro + 'Ünlü') plants and ranged from 246 mm to 284 mm in the 'Ünlü' variety.
The average yield obtained from the grafted 'Ünlü' was found to be higher in the non-grafted plants compared to Ferro + 'Ünlü' grafted plant yield.
When the efficiency values f irrigation water are taken into consideration together with the yield; in cases where water is scarce and expensive, 40% irrigation water deficit is recommended in the cultivation of 'Ünlü' variety.
The recommended water deficit for 'Ünlü' variety grafted on the Ferro rootstock is 20%.

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ESTIMATION OF DISTINCTION IN SEED FORMATION AND PRODUCTIVITY IN DIFFERENT GENOTYPES OF TOMATILLO (*PHYSALIS IXOCARPA* BROT.)

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Abstract

The main aim of the present study was to establish the responses in seed formation and seed production in several genotypes of tomatillo (Physalis ixocarpa Brot.) with a target to predict the seed yield and its realization. The experiments were carried out in Agricultural University of Plovdiv, Bulgaria with five genotypes with a different origin. The number of seed per fruit, the percentage of fully developed seeds, the linear seed sizes, the weight of 1000 seeds, germination energy, germination, main germination time, uniformity of germination, length of embryo root and hypocotyls, fresh weight and deviation in seedlings were investigated. The yields of fruit and seed also have been determinate. Significant variation in the numbers of seed per fruit as well as in the in weight of seeds per fruit was observed. High germination was an account of each genotype. The differences in seed production and in some sowing qualities between genotypes were registered. These results can be applied for the prediction of seed productivity and also in the determination of price during the realization of seed according to the genotype behaviors of the seed yield.

Key words*: germination, seed formation, seed production, sowing quality, tomatillo.*

INTRODUCTION

The genus *Physalis* is characterized by big diversity of annual and perennial species, according to various authors between 70 according to Christov (2010) and 110 (Skvorcova, 1997). Da Silva et al. (2016) also reported for the existence of a wide variety of species in Physalis and they claimed that in this genus comprising more than over one hundred species. The four main species as *Physalis peruviana* L., *Physalis pruinosa* L., *Physalis ixocarpa* Brot. and *Physalis pubescens* L. are the most popular (Moriconi et al., 1990; Crawford, 2004) and De Souza et al. (2017) emphasized that they cultivated more in the production of fruit used for several purposes.

The tomatillo (*Physalis ixocarpa* Brot.) is most popular in Latin America and it is one of the new vegetable crops for Europe and for Bulgaria also. The information about the development and productivity of tomatillo is very limited (Díaz et al., 2005). Freyre and Loy (2000), investigating five tomatillo genotypes in New Hampshire, USA region, established statistical significance influence of environmental conditions, related to fruit number and average fruit weight per genotype. In view of a higher seeds yield Peña-Lomelí et al. (2018) pointed out that the pollination is best to be accomplished on the second or third day of flowering.

Because the tomatillo reproduces primarily by seed, Barroso et al. (2017) pointed out that the investigations about seed maturation are very important to determine the most appropriate term of harvest, when the seeds are with a high physiological potential, even more so that the fruit until the end of the vegetation is completely enveloped by the sepals. According to them, the best time to harvest the seeds is 45 days after flowering. Similar studies have been carried out by Pérez et al. (2012), reporting that the influence of environmental conditions on germination and the physiological state of tomatillo seeds are poorly studied and with the highest quality are the tomatillo seeds, that have been harvested 55-65 days after flowering. In a previous publication, these authors (Pérez et al., 2008) also underline that the processes of seed development in the tomatillo have not been sufficiently studied, but here concluded that the highest viability has the seed at 42 days after flowering. De Souza et al. (2016), in

their studies, highlighted that the seeds of species of the genus Physalis are characterized by high storability. In view to establishing of the sowing qualities of tomatillo seeds, Martínez et al. (2006) indicates that it is appropriate to use a standard paper germination test and they have studied the germination of these seeds in the temperature range between 20^0C and 35^0C.

The main aim of the present study was to establish the responses in seed formation and seed production in several genotypes of tomatillo (*Physalis ixocarpa* Brot.) with a target to predict the seed yield and its realization, and at the same time to supplement the existing information about plant development throughout their life cycle.

MATERIALS AND METHODS

The experiments in this study were carried out in South Bulgaria conditions in 2016-2018 years at the Agricultural University-Plovdiv, Bulgaria, in his scientific field and also in scientific laboratories of the Department of Horticulture with the following genotypes and breeding lines of tomatillo (*Physalis ixocarpa* Brot.) with different origin:

018-2010 – origin from Colombia;
09-2012 – origin from Venezuela;
10-2012 – origin from Mexico;
14-2013 – origin from Mexico;
22-2015 – origin from China.

The plants were grown by unpricked seedlings. These seedlings were grown in a plastic unheated greenhouse and term of sowing was March 15th. The seed rate per one hectare was 80-100 g, but per square meter was sown at 1.5 g. The planting done in May 20-25, depends on the environmental conditions during different years. The scheme for planting was on furrows, on the scheme 70 x 50 cm, according to the previous investigation and recommendation (Panayotov & Tcorlianis, 2000). All necessary agro-technological practices were implemented in the optimum time and volume. The experiments carried out in four replicates with 25 plants, with an experimental plot from 9 m^2.

At the stage of full botanical maturity, all the ripe fruits were harvested and the fruit yield was established. After that, the seeds were extracted throughout fermentation. The seed yield was determined. The quantity of seed in one kilogram of fruits was established. The ratio between these fruits (one kilogram) and the seeds inside these fruits also was calculated. In other 5 fruits from each replicates all seeds were extracted and their number was counted.

In purpose to determine the seed development behaviours, the following indexes: the insemination by the percentage of fully developed seeds in one fruit to the quantity of a total number of seeds per fruit was registered. The linear seed sizes (length, width and thickness of seed) on 15 seeds with electronic calliper; the weight of one seed - set on 15 seeds, the weight of 1000 seeds (ISTA, 2013), in 4 replicates were determined. The sowing quality of the seeds were established by the following parameters: germinating energy (ISTA, 2013), in 4 replicates; germination (ISTA, 2013) in 4 replicates; uniformity of germination (according to Strona, 1966); mean germination time (MGT) - in four replicates, each of 100 seeds.

in addition, in order to gain a better understanding of the differences between the studied genotypes in the sowing qualities the fresh mass of a one seedling, by measuring of all developed seedlings; length of the embryo root and the hypocotyl - by measuring 10 seedlings from each replicates on the day of counting of germination in the four replicates were recorded. The deviations from the normal structure of the seedlings - short embryo root, lack of branches in embryo root, lack of hairs on the embryo root, undeveloped cotyledons, unopened cotyledons, lack of hypocotyls, by Welington (1970) were established. Data of the study were subjected to ANOVA test and correlation coefficients between some parameters also were established (Fowel & Cohen, 1992). The trend of the results of all vegetation are similar, therefore the presented data are average values from three years of study.

RESULTS AND DISCUSSIONS

Some characteristics of tomatillo seed formation are presented in Table 1. Genotypic differences were established about the number of seeds that setting up in one fruit, as the significant variation was established and it

ranging from 105.2 for genotype 09-2012 (Venezuela) to 154.7 for genotype 14-2013, originating from Mexico. Relatively smaller numbers of seed were developed in breeding line 22-2015 (112.6) numbers. The average numbers of seeds per fruit for all tested genotypes are 128.9. The variation to this among genotypes with the lowest and highest number of seeds is between -23.7 and +26.7. The difference between the highest and the lowest result, for seed number is even greater, almost double, 50.4 numbers. The differences between the variants are with statistical significance with exception of those between 10-2012 and 14-32013. Higher differences are observed in the weight of seeds in one fruit. Average in tomatillo fruit the content of seeds are approximately 83.4 mg. The highest weight of seed was established in genotype 14-2013 (102.7 mg), followed by 10-2012 with 89.0 mg, both originating from Mexico. The lowest seed weight per tomatillo seed established in № 09-2012, which is from Venezuela, only 59.0 mg. The strong positive correlation was calculated between the number and weight of seeds per fruit, with correlation coefficient r = +0.79.

Not only the number of seeds is important, but the most significant for a better understanding of seed formation is also the percentage of fully developed seeds. In almost all tested tomatillo genotypes, the most seeds are fully developed. These which originate from Mexico and Venezuela - 10-2012, 14-2013 and 09-2012 reached up to 100.0%. In 018-2010 the percentage of the fully developed seed is weaker (87.1%) as in this genotype the number of set seeds, found in the fruit was also relatively lower. The pollination and fertilization have a strong influence on the setup and development of the seed. Peña-Lomeli et al. (2018) found that the highest efficiency of tomatillo seed formation and production is obtained in the condition of good pollination and they also observed high genotype response.

Table 1. Tomatillo seed formation

№	Genotype	Seeds number/fruit	Weight of seed/fruit (mg)	% of normal developed seeds	Weight of 1000 seeds (g)
1	018-2010	116.3	87.7	87.1	0.927
2	09-2012	105.2	59.0	100.0	0.867
3	10-2012	155.6	89.0	99.1	1.005
4	14-2013	154.7	102.7	100.0	1.013
5	22-2015	112.6	78.8	98.8	0.940
	average	128.9	83.4	97.0	0.950
LSD p = 0.05%		6.4			0.098
r = +0.79					

r - correlation coefficient between number and weight of seeds per fruit.

The description of seed sowing quality is complemented by the weight of 1000 seeds. The variations in this behaviour are low. The highest weight of 1000 tomatillo seeds was measured in 14-2013 (1.013 g), followed by 10-2012 (1.005 g). The lowest, with difference the highest values from 0.146 g was the weight of 1000 seeds of line 09-2012 (0.867 g), even though the above mentioned the highest percentage of fully developed seeds - 100%. The low weight of 1000 seed was established also for 018-2010 (0.927 g). The average weight of 1000 tomatillo seeds is 0.950 g and variation between both the limit values established in the study is from -0.083 g to +0.063 g.

Statistical significances are established about the weight of one seed (Table 2). The average weight of one tomatillo seed is 0.71 mg. Between the investigated genotype and breeding lines it is within wide limits and changes from 0.35 mg in 22-2015 to 1.38 mg for 09-2012. Somewhat this tendency is also preserved in relation to the length of the seed. This sign varies between 1.49 mm to 2.22 mm in 14-2013 and in 10-2012, respectively, while the average value is 1.84 mm. More close are the results about seed width – from 1.24 mm to 1.81 mm in the above-mentioned genotypes and the deviation from the average wide is -0.26 mm and +0.31 mm. A similar tendency was also observed for the thickness of one seed,

that is from 0.16 mm (09-2012) to 0.44 mm (10-2012), which is with 48.0% less and with 57.14% more in comparison with the average value (0.28 mm). More of differences are statistically proved. About the importance of the seeds, morphology reported Zhang and Wen (1996) and point out that the seed behaviours can be used as an indicator for taxonomic identification between species and genotypes of the genus *Physalis*. Between the investigated lines in these conducted experiments, some differences in seed size were established. Inzunza et al. (1999) also observed some genotype differences in the tomatillo seed dimensions.

Table 2. Morphology behaviours of one tomatillo seed

№	Genotype	Weight (mg)	Length (mm)	Width (mm)	Thickness (mm)
1	018-2010	0.76	2.21	1.70	0.38
2	09-2012	1.38	1.68	1.36	0.16
3	10-2012	0.62	2.22	1.81	0.44
4	14-2013	0.42	1.49	1.24	0.22
5	22-2015	0.35	1.62	1.40	0.26
	Average	0.71	1.84	1.50	0.28
LSD p = 0.05%		0.06	0.15	0.21	0.04

Germination is the most important for characterization of the seed as a sowing material. From carried out experiments each genotype is with high germination energy (Table 3). The highest energy of germination was observed in genotype 09-2012 (96.1.%) followed by this of 10-2012 (90.0%). The lowest one was in line 22-2015 (65.5%). As Balck et al. (2008) and Copeland and McDonalds (2001) emphasized the seeds that have demonstrated higher germinating energy are with better vital performance because they have germinated for a shorter period.

Da Silva et al. (2016) reported that between each species in the genus *Physalis* the seeds from *Physalis ixocarpa* Brot. are with the lower percentage of emergence, while according to Thomson and Wit (1987) and Panayotov (2018) the seed from *Physalis peruviana* L. are characterized with high germination. Notwithstanding these statements in the carried out study good and high germination of tomatillo seeds was established. The highest one was also for Venezuelan genotype - 09-2012 (97.3%) which is with 12.1% more than average data. In the next place is the germination of Mexican genotype 10-2012 (92.0%). The lowest one was the germination of genotype 018-2010 (76.4%). The most intense, in the period between the two accounts, were the germination processes in 22-2015, where the difference between germinating energy and germination was 13.3%. In this genotype as mentioned above the germination energy was the lowest. The results are with statistical significance.

The indicators mean germination time and uniformity of germination (Table 4) promote for more prices study of the seed qualities. The highest mean germination time was registered in genotype 10-2012 (2.24 days) and the lowest one, the most slowly germinated seeds, was in 22-2015 (8.40 days). Average for germination of one tomatillo seeds are necessary 4.76 days. Between other genotypes about this sign the response did not establish. More significant are the differences in uniformity of germination. Black et al. (2008) and Panayotov (2015) maintains, that this characteristic of seeds is important because it is related to the possibilities the seed to overcome easier the resistance of soil during sprouting. This characteristic range from 5.18% to 11.25% in 22-2015 and in 10-2012, respectively. Considering the average values of 7.81%, it can indicate that the tomatillo seeds are characterized by low uniformity of germination. The low germination at 22-2015, mentioned above, may be due to the significantly lower values of these two indices. In the study of sixteen tomatillo genotypes, Sánchez et al. (2007) found strong differences in parameters that related to the seed vitality.

The morphological features of the seedling (Table 5) provide additional information about the seed vitality status. Statistically significant differences were registered about the fresh weight of the seeding. In the fresh weight of the

seedling of one seed, the differences are more significant. Copeland and Mc Donalds (2001) and also Panayotov (2015) reported that the weight of the seedling very often applied in several methods for determining the vigour as well as it demonstrates the opportunity for easier germination of the seeds. The average weight of tomatillo seedling is 10.86 mg and for the investigated genotype it is in big diapason from 5.9 mg to 11.9 mg in 22-2015 (from China) and 10-2012 (from Mexico). The correlation coefficient between mean germination time and seedling weight is middle but negative with r = -0.55. It can be assumed that as the germination time is shorter, the period of seedling development, to the end of germination, is longer, which eventually contributes to the accumulation of higher biomass and vice versa at a lower mean germination time. Therefore this correlation coefficient is negative but strong. Sánchez et al. (2007) and Martínez et al. (2006) also reported about the positive correlation with speed of germination and emergence, shoot and root length, and seedling dry weight. The highest length of the hypocotyls have developed the seeds from № 018-2010 (2.76 cm) and the lowest one was in 22-2015 (1.47 cm). The differences of these results to the average value are +0.69 cm and -0.6 cm. Long hypocotyls have developed also the seeds from 09-2012 and 10-2012. Genotype 22-2015 had developed also the lowest length of embryo root 2.81 cm, which are with 1.48 cm lower than the average values. The seedlings from Mexican genotype 14-2013 are with the highest embryo root (5.48 cm), followed by these of 10-2012, also from Mexico and 018-2010, which is from Colombia. These results unambiguously show that the variation between morphological behaviours of tomatillo seedlings for investigated genotypes is strong.

Table 3. Germination behaviours of tomatillo seeds

№	Genotype	Germination energy (%)	Germination (%)
1	018-2010	74.7	76.4
2	09-2012	96.1	97.3
3	10-2012	90.0	92.0
4	14-2013	80.7	81.3
5	22-2015	65.5	78.8
	average	81.6	85.2
LSD p = 0.05%		5.5	4.2

Table 4. Sowing parameters of tomatillo seeds

№	Genotype	MGT (days)	Uniformity (%)
1	018-2010	4.42	7.56
2	09-2012	4.04	6.95
3	10-2012	2.24	11.25
4	14-2013	4.70	8.12
5	22-2015	8.40	5.18
	average	4.76	7.81
LSD p = 0.05%		1.28	1.12

Table 5. Morphological characteristics of tomatillo seedling

№	Genotype	Fresh weight (mg)	Lenght of hypocotyl (cm)	Length of embryo root (cm)
1	018-2010	17.7	2.76	4.59
2	09-2012	9.2	2.39	3.77
3	10-2012	11.9	2.16	4.83
4	14-2013	9.6	1.59	5.48
5	22-2015	5.9	1.47	2.81
	average	10.86	2.07	4.29
LSD p = 0.05%		2.4	0.12	1.06
		r = -0.551		

r - correlation coefficient between fresh weight and MGT.

Not each seed developed normal seedlings. Some of them are characterized by different deviations from their normal morphology (Table 6). Average values of abnormally seedling grown between studied genotypes are 12.2%. This percentage of deviation was the highest in № 10-2012 followed by № 14-2013 with 13.5% and 12.8%, respectively. The lowest one, it was recorded in genotype 22-2015 (11.1%). The most common deviations of seedling developed, calculated towards all, not well-developed seedlings which were accepted for 100%, are the lack of branches of the embryo with 26.4% and also undeveloped cotyledons with 22.1%. The highest percentage of seedlings with lack of branches on embryo

root was observed in genotype 09-2012 (36.2%), followed by these of 10-2012 and 14-2013 with 27% approximately. The deviation undeveloped cotyledons ranged from 0% in 09-2012 to 35.3% in 22-2015. The lowest abnormal seedling are these with unopened cotyledons, average - 5.3% and it was presented only in genotype 09-2012 (26.4%), followed by these with lack of hairs on embryo root - 14.6%.

Table 6. Deviation from normal developed tomatillo seedlings (%)

Genotypes	%	Short embryo root	Lack of branches on embryo root	Lack of hairs on embryo root	Undeveloped cotyledons	Unopened cotyledons	Lack of hairs on hypocotyls
018-2010	12.2	32.8	18.3	22.2	26.7	0.0	0.0
09-2012	11.4	0.0	36.2	19.8	0.0	26.4	17.6
10-2012	13.5	10.2	27.4	18.2	26.9	0.0	17.3
14-2013	12.8	29.3	27.9	0.0	21.8	0.0	21.0
22-2015	11.1	13.8	22.2	11.6	35.3	0.0	17.1
Average	12.2	17.2	26.4	14.4	22.1	5.3	14.6

The main assessment of a given genotype or agrotechnological event is related to production, which determines its agronomic and economic significance. In order to predict the expected seed yield, the seed quantity in one kilogram of fruits in botanical maturity was determined, and it was an average of 7.39 g (Table 7) for the studied genotypes. The highest quantity of the seed in one kilogram of fruit was established for 10-2012 (8.10 g) and on the next place is 14-2013 with 7.72 g, both genotypes from Mexico. The values of this index are low about genotypes 22-2015 and 05-2012 (6.73 g and 6.85 g), respectively.

Through the ratio between the fruit and the extracted from them seeds are achieved in full assessment of the insemination. This also indicates what is the quantity of fruits from which can be obtained necessary seeds. The variation in this sign between investigated genotype is very high, the values are in diapason from 123.4 for 10-2012 to 148.6 for 22-2015, while the average ratio is 135.9.

The seed yield is in direct relation to the fruit yield. Therefore the quantity of harvested fruits is very important. The average fruits yield of the studied tomatillo population is 17411.0 $kg.ha^{-1}$. The highest one was obtained in the genotype 14-2013 (22422.8 $kg.ha^{-1}$), followed by 10-2013 (20265.2 $kg.ha^{-1}$). The lowest fruits yield was registered in 22-2015 and in 08-2010 (12012.2 $kg.ha^{-1}$ and 15651.2 $kg.ha^{-1}$), respectively. The differences are with mathematical significance. Statistical significance genotypic differences were established about tomatillo seed yield in South Bulgarian conditions. The highest quantity of seeds is obtained from Mexican genotype 14-2013 (173.3 $kg.ha^{-1}$), which is more with 45 $kg.ha^{-1}$ to the average yield from each genotype. On second place is the № 10-2012 with 154.2 $kg.ha^{-1}$. This may be due to the high yields of fruit and low ratio fruit: seeds and also to the higher weight of 1000 seeds, to the greater number and weight of seeds per fruit as well as the high percentage of fully developed seeds in these two genotypes. This is confirmed also from the established strong and positive correlations between seed yield on one hand and their number of seeds per fruit ($r = +0.87$), a weight of the seeds in one fruit ($r = +0.65$) as well as the weight of 1000 seeds ($r = +0.70$) on the other hand. In № 22-2015 was registered the least seed yield from 80.9 $kg.ha^{-1}$, this genotype is characterized with the lowest fruit yield, the highest ratio fruits: seeds and also with the relatively low number of seed in one fruit and weight of 1000 seeds. The average seed yield of the tomatillo genotype in this experiments carried out under environmental conditions of South Bulgaria is 128.3 $kg.ha^{-1}$. The differences in seed productivity are statistically proven, except for that between genotypes 018-2010 and 09-2012.

The established results can be used both to predict the expected seed yield and to more accurately determine the commercial price, depending on genotypic characteristics. The price must be higher for samples with a lower seed yield and a higher fruit : seeds ratio, since

the input costs for fruit production, are almost the same for each genotype, but for those with low seed yields there are additional incurred expenses about the extraction of higher quantities of fruits to be obtained the adequate amount of seed.

Table 7. Productivity behaviours of tomatillo genotypes

№	Genotype	Seed yield/ 1 kg fruits (g)	Ratio fruit: seeds	Yield of fruit ($kg.ha^{-1}$)	Yield of seeds ($kg.ha^{-1}$)
1	018-2010	7.57	132.1	15651.2	118.6
2	09-2012	6.85	145.9	16683.6	114.4
3	10-2012	8.10	123.4	20265.2	154.2
4	14-2013	7.72	129.5	22442.8	173.3
5	22-2015	6.73	148.6	12012.2	80.9
	Average	7.39	135.9	17411.0	128.3
LSD p = 0.05%		0.78		1046.3	15.98
r with number of seeds/fruit					r = +0.87
r with weight of seeds/fruit					r = +0.65
r with weight of 1000 seeds					r = +0.70

CONCLUSIONS

In indexes, the number of seeds and their weight in one fruit of tomatillo the genotypic differences were found. The percentage of fully developed seeds in each tested tomatillo genotypes is high. The differences of the tomatillo seed size have also been established.

Each vitality indicator of tomatillo seeds is characterized by high variation between the genotypes.

The differences in the fresh weigh of one seedling are more significant. Most often the type of deviations from the normal development of seedling included the lack of branches on embryo root and undeveloped cotyledons. Genotypic differences are also noted in insemination. The seed weight in one-kilogram tomatillo fruits as well as fruit: seeds ratio changes in a wide range.

The variations between individual genotypes about fruit and seed yield are significant. The average tomatillo seed yield between tested genotypes under South Bulgarian environmental conditions is 128.3 $kg.ha^{-1}$. Under these conditions, Mexican genotypes, 14-2013 and 10-2012, are performing with the highest productivity.

The strong positive correlations between the number and weight of the seed per fruit, as well as about seed yield with the number and weight of the seeds in one fruit and the weight of 1000 seeds and middle negative correlation between the seedling fresh weight and MGT, were established.

The conducted studies supplement the information on the overall development of tomatillo plants, especially by the seed development, the scope where the knowledge is quite limited.

The presented results are suitable to be applied for prediction of the expected yield of tomatillo seeds as well as to precise the realization price, depending on the genotyping insemination characteristics. It can be assumed that this will help be increasing the efficiency of the production of tomatillo seed.

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RESEARCHES CONCERNING THE BEHAVIOUR OF SOME CULTIVARS OF TOMATOES (*LYCOPERSICON ESCULENTUM* MILL.) IN VIDRA AREA, ILFOV COUNTY

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Abstract

During 2016, at the Research and Development Institute for Vegetable and Flower Growing Vidra, a monofactorial experiment was organized, placed in randomized blocks, with 15 experimental variants in 3 replications. The biological material used was constitued by 12 tomato varieties ('Kristinica', 'Darsirius', 'Măriuca', 'Romec' 554J, 'Chihlimbar', 'Viorica', 'Vipon', 'Pontica' 102, 'Missouri', 'Marmande', 'Heintz' and 'St. Pierre') and 3 hybrids ('Caspar' F1, 'Perfect Peel' F1 and 'Mirsini' F1). Under the climatic conditions of 2016, the fruits were attacked by pathogens: Alternaria porri f.sp. *solani, Phytophthora infestans and Phytophthora parasitica. The frequency of fruits attacked by the mentioned pathogens ranged from 6.2% in the 'Mirsini' F1 hybrid and 27.0% in the 'Heintz' variety. The highest yield was recorded at the hybrids 'Perfect Peel' F1 (5.87 kg/sq. m), 'Caspar' F1 (5.85 kg/sq. m) and the varieties 'Pontica' 102 (5.66 kg/sq. m), 'Missouri' (5.29 kg/sq. m), 'Viorica' (5.24 kg/sq. m).*

Key words*: cultivars, pathogens, yield.*

INTRODUCTION

In field tomato crops (Mandru et al., 2017; 2018) during the vegetation period, the following pathogens may appear on the foliage *Xanthomonas campestris* pv. *vesicatoria, Pseudomonas syringae* pv. *tomato, Alternaria porri* f. sp. *solani, Fulvia fulva, Phytophthora infestans* and *Phytophthora parasitica, Alternaria porri* f. sp. *solani, Colletotrichum coccodes* on the fruits.

Researches on the attack of pathogens *Botrytis cinerea*, *Alternaria porri* f. sp. *solani* were also carried out by the Buzatu et al. (2017) on eggplant crop.

Also, tomato diseases have been reported by Docea et al. (2012).

Drenth et al. (1993) studied the genotypic diversity of *Phytophthora infestans*, the first damage was recorded in potato crop.

In Romania was identified on tomatoes by Tr. Săvulescu et al., in 1940 (Velichi, 2012).

After Meng et al. (2014), *Phytophthora parasitica* is a soil born pathogen with a wide range of host plants that cause damage to crops. The attack occurs both in the field and in protected areas.

MATERIALS AND METHODS

During 2016, at the Research and Development Institute for Vegetable and Flower Growing Vidra, a monofactorial experience was organized, placed in randomized blocks with 15 experimental variants in 3 replications.

The biological material used was constitued by 12 varieties ('Kristinica', 'Darsirius', 'Măriuca', 'Romec' 554J, 'Chihlimbar', 'Viorica', 'Vipon', 'Pontica' 102, 'Missouri', 'Marmande', 'Heintz' and 'St. Pierre') and 3 hybrids ('Caspar' F1, 'Perfect Peel' F1 and 'Mirsini' F1).

In Table 1 we present data about the cultivars that was used in the experience: variety/hybrid, group of earliness, fruit shape, fruit color, fruit weight, destination of yield, resistance/ tolerance to pathogens.

The yield on variants and replications was recorded, the data obtained being processed with the ANOVA program.

Table 1. Description of the cultivars used in the experience (authors description) (Vidra, 2016)

Variety/hybrid	Group of earliness	Fruit shape	Fruit Colour	Fruit weight (g)	Destination of yield	Resistance/tolerance
1. 'Kristinica'	early (95 days)	round	intense red	100	industry and fresh consumption	Fol; Vd; Pi; Pst
2. 'Darsirius'	midseason (100-120 days)	pear-shaped	dark red	80	industry and fresh consumption	Fol; Vd; TMV
3. 'Măriuca'	mediumlate (130 days)	ovoid	dark red (intense)	120-150	fresh consumption	Fol; Pst; TMV; TSWV
4. 'Caspar' F1	early (110-115 days)	elongate	bright red	90-120	industry and fresh consumption	Fol; Vd
5. 'Romec' 554 J	mediumlate (120-125 days)	ovoid	bright red	50-60	industry and fresh consumption	Vd; Pst
6. 'Chihlimbar'	early (105-110 days)	round	orange yellow	185	fresh consumption	Pst
7. 'Viorica'	mediumlate (126 days)	round-ovoid	dark red	55-75	industry	As
8. 'Vipon'	midseason (110-112days)	ovoid	dark red	70-80	industry	Pst
9. 'Pontica' 102	mediumlate (110-120 days)	spherical-flattened	dark red	90-150	industry and fresh consumption	Vd; Pst
10. 'Perfect Peel' F1	early (110-115 days)	oval	dark red	55-65	industry	Fol; Vd; Sbl; As
11. 'Missouri'	early (75-80 days)	oval elongated	dark red	90-110	industry	Fol; Vd
12. 'Heintz'	midseason (110-115 days)	round	Red	120-150	industry and fresh consumption	Fol; Vd; TSWV
13. 'Marmande'	early (95 zile)	round costly	Red	150-180	fresh consumption	Fol
14. 'St. Pierre'	early (75-90 days)	round	Red	180-200	fresh consumption	Fol; Vd
15. 'Mirsini' F1	very early (64 days)	globular	bright red	210-230	fresh consumption	Fol; Vd; Sbl; Ff; TYLCV

Legend: TMV (*Tabacco mosaic virus*), TSWV (*Tomato spotted wilt virus*), As (*Alternaria solani*), Ff (*Fulvia fulva*), Fol (*Fusarium oxysporum* f. sp. *lycopersici*), Pi (*Phytophthora infestans*), Sbl (*Stemphylium botryosum* f. sp. *lycopersici*), Vd (*Verticillium dahliae*) (https://www.worldseed.org/).

RESULTS AND DISCUSSIONS

In Table 2 are presented the climatic data recorded in the Vidra area, in 2016 and in Table 3 the environmental factors which are favorable for the attack of pathogens on tomato crops.

For the attack evolution of pathogens, the abiotic factors (Mardare et al., 2015) have an important role (temperature, relative air humidity and rainfall mm).

Due to climatic conditions on eggplant crops was manifested the attack of *Alternaria porri* f. sp. *solani* and *Phytophthora parasitica* (Buzatu et al., 2018).

Table 2. Climate data in the field (Vidra, 2016)

Month	Temperature (^{0}C)			Relative air humidity (%)			Rainfall (mm)
	minimum	maximum	medium	minimum	maximum	medium	
June	16.1	28.4	21.9	56.8	89.5	71.3	33.5
July	17.0	31.0	23.7	48.1	78.3	59.7	2.0
August	17.5	30.6	23.4	49.5	77.2	60.6	110.0
September	13.1	26.3	18.9	50.9	80.2	62.8	43.5

Table 3. Environmental factors that are favorable for the appearance the attack of pathogens in tomato crops (Costache et al., 2018)

Pathogens	Conditions of manifestation		
	Temperature (^{0}C)	Relative air humidity (%)	Water on foliage
Alternaria porri f. sp. *solani*	25-28	>95	+
Phytophthora infestans	15-18	100	+
Phytophthora parasitica	20-25	-	water film

Under the climatic conditions of 2016 year, the tomato fruits was attacked by pathogens *Alternaria porri* f. sp. *solani*, *Phytophthora infestans* and *Phytophthora parasitica* (Table 4).

The frequency of attacked fruits on the studied varieties and hybrids, was between 1.8% for 'Mirsini' F1 hybrid and 8.3% for 'Chihlimbar' variety, for the pathogen *Alternaria porri* f.sp. *solani*. The frequency of attacking fruits for *Phytophthora infestans* pathogen, ranged between 0.3% for 'Mirsini' F1 hybrid and 2.7% for 'Pontica' 102 variety.

For *Phytophthora parasitica* pathogen the attack on fruits was between 4.1% for 'Mirsini' F1 and 20,4% for Haintz variety.

The total frequency of the attacked fruits was between 6.2% for 'Mirsini' F1 hybrid and 27.0% for Haintz variety.

Table 4. Frequency of attacked fruit (FA%) on the experimental cultivars (Vidra, 2016)

Variety/hybrid	Frequency of attacked fruit (%)			
	Alternaria porri f. sp. *solani*	*Phytophthora infestans*	*Phytophthora parasitica*	Total frequency of the attack (%)
1. 'Kristinica'	4.7	1.7	8.1	14.5
2. 'Darsirius'	2.7	0.7	17.9	21.3
3. 'Măriuca'	5.3	1.3	8.5	15.1
4. 'Caspar' F1	6.3	0.3	14.8	21.4
5. 'Romec' 554 J	6.7	0.7	19.3	26.7
6. 'Chihlimbar'	8.3	1.3	6.9	16.5
7. 'Viorica'	4.7	0.9	15.1	20.7
8. 'Vipon'	4.3	1.3	11.8	17.4
9. 'Pontica' 102	7.7	2.7	11.3	21.7
10. 'Perfect Peel' F1	5.7	1.6	12.3	19.6
11. 'Missouri'	5.3	1.3	9.2	15.8
12. 'Heintz'	5.3	1.3	20.4	27.0
13. 'Marmande'	2.5	0.9	11.2	14.2
14. 'St. Pierre'	2.7	1.7	10.2	14.6
15. 'Mirsini' F1	1.8	0.3	4.1	6.2
Avarage	4.9	1.2	12.1	18.2

In the Table 5 are prezented the yield data obtained at the varieties and hybrids studied.

It can be seen that the highest yield was obtained at 'Perfect Peel' F1 hybrids (5.87 kg/sq. m) and 'Caspar' F1 (5.85 kg/sq. m).

By the varieties were 'Pontica' 102 (5.66 kg/sq. m), 'Missouri' (5.29 kg/sq. m) and 'Viorica' (5.24 kg/sq. m) in comparation with varieties 'Kristinica', 'Darsirius', 'Măriuca', 'Romec' 554 J, 'Chihlimbar', 'Vipon', 'Heintz', 'Marmande' and 'St. Pierre', where yield ranged between 4.02 kg/sq. m to 4.92 kg/sq m.

The average yield of cultivars was 4.93 kg/sq. m. In the Figures 1 and 2 are presented aspects from the experience.

Table 5. Yield obtained from the tomatoes cultivars (Vidra, 2016)

Variety/hibrid	Yield		Difference from the average (kg/sq. m)	Signification
	kg/sq. m	relative yield %		
1. 'Kristinica'	4.73	95.9	-0.20	-
2. 'Darsirius'	4.76	96.5	-0.17	-
3. 'Măriuca'	4.92	99.67	-0.01	-
4. 'Caspar' F1	5.85	118.7	+0.92	***
5. 'Romec' 554 J	4.92	99.8	-0.01	-
6. 'Chihlimbar'	3.71	75.2	-1.22	-
7. 'Viorica'	5.24	106.3	+0.31	***
8. 'Vipon'	4.81	97.5	-0.12	-
9. 'Pontica' 102	5.66	114.8	+0.73	***
10. 'Perfect Peel' F1	5.87	119.0	+0.94	***

11. 'Missouri'	5.29	107.3	+0.36	***
12. 'Heintz'	4.59	93.1	-0.34	-
13. 'Marmande'	4.37	88.6	-0.56	-
14. 'St. Pierre'	4.02	81.5	-0.91	-
15. 'Mirsini' F1	5.24	106.2	+0.31	**
Avarage of cultivars	4.93	-	-	-
LD 5% = 0.175; LD 1% = 0.234; LD 0.1% = 0.306				

Figure 1. 'Măriuca' variety (original photo)

Figure 2. 'Caspar' F1 hybrid (original photo)

CONCLUSIONS

In the climatic conditions of 2016 year, on the fruits, at the tested cultivars was presented the attack of pathogens *Alternaria porri* f. sp. *solani* (early blight), *Phytophthora infestans* (late blight) and *Phytophthora parasitica* (fruit rot). The total frequency of fruit attacked fruits by mentioned pathogens, on the cultivars studied, was between 6.2% for the 'Mirsini' F1 hybrid and 27.0% for the 'Heintz' variety.

From the yield point of view, was mentioned 'Perfect Peel' F1 (5.87 kg/sq. m) and 'Caspar' F1 hybrids (5.85 kg/sq. m), the varieties 'Pontica' 102 (5.66 kg/sq. m), 'Missouri' (5.29 kg/sq. m) and 'Viorica' (5.24 kg/sq. m).

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'Camelia', A NEW GENOTYPE OF *SOLANUM MELONGENA* L. OBTAINED AT V.R.D.S. BUZĂU

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Abstract

The pedoclimatic conditions of the Buzau vegetable reservoir are favorable for the Solanum melongena species, therefore, begining with 1996 the researches aiming breeding Solanum melongena were made in an intensive approach at V.R.D.S Buzau. At this time, the unit owns a rich germplasm colection comprising over 200 genotypes that are in different breeding stages. The unit has pattented and registered in the Official Catalogue of Romanian Plant Crops the following cultivars: Dragaica, Zaraza and Rebeca F1. Begining with the year 2018, a new cultivar of white eggplants has been pattented and will be expanded widely in crop cultivation. While being tested at ISTIS Bucharest, V.R.D.S Buzau has offered seeds and seedlings promotionally for the growers and included in the internal program of conservative selection the seed production for this cultivar. The feedback received from the consumers was positive, the cultivar being distinguished by a great production capacity of a very high quality. In the present, in the internal program of conservative selection the seed had reached the stage of Basic seed, recording in fields a visible decrease of the main features variability starting with the Author' s seed until Basic seed. The cultivar is suitable for growing under protected spaces and open field conditions being distinguished by distinct phenotypic characteristics.

Key words*: white eggplants, cultivar, germplasm, variability, productivity.*

INTRODUCTION

In Romania, eggplant crop production is very common, this being practiced both in the household system and by the big farmers. (Lagunovschi & Vînătoru, 2016). The most important growing areas of this plant are located in the southern, south-eastern, and south-western parts of Romania and are usually cultivated on open fields as well as in unheated greenhouses.(Kovács et al., 2016)

Eggplants are mainly consumed in the summer-autumn season, and the way they are prepared is quite varied. Eggplant is cultivated for its fruit consumed upon maturity; it is used in the preparation of different dishes such as salads, moussaka, potlatch, and stuffed eggplants. (Posta et al., 2012)

V.R.D.S Buzau gave a special attention for breeding eggplants since April 1957, this being the year of its founding (Bratu et al., 2018) Considering that in Romania the eggplants are a very common vegetable grown and consumed seeing that the pedoclimatic conditions are extremely favourable for producing this species, the Laboratory of Genetic, Breeding and Biodiversity Preservation from Vegetable Research and Development Station Buzau oriented its activity towards obtaining new cultivars of eggplants in order to improve the range of varieties and hybrids that currently are produced and sold on the Romanian market. Consumers started to enrich and vary their taste for vegetables, therefore the farmers needed to vary their crops, species and most important needed to vary the cultivars used.

There is a great demand on the market for Romanian vegetables and people started to ask more and more for Romanian cultivars.

At the present the Research Station germplasm collection is extremely valuable, and has two cultivars and one variety registered in the Official Catalogue of Romanian Crop Plants under the name of Dragaica, Zaraza and Rebeca F1.

Beginning with the year 1996, new and improved programs in breeding eggplants were conducted in an intensive system by the Genetic, Breeding and Biodiversity Preservation Laboratory due to the varied

requests launched by the farmers and consumers. (Vînătoru et al., 2013) Although the climatic conditions allow us to produce vegetables that can supply the consumers requests there is a deficit for autochthonous vegetables. Only 60% of registered vegetable consumption nationwide is provided by domestic production. This means that 40% of consumption of vegetables is provided by imports (Soare et al., 2016).

The aim of the researches were mainly concerned in achieving a new eggplant cultivar that has the following characteristics: white fruits, high yield potential, superior quality production, great adaptability to the Romanian pedoclimate conditions, ecological plasticity, the capacity for being grown in open field and protected spaces conditions.

MATERIALS AND METHODS

The germplasm collection held by the Vegetable Research and Development Station Buzau at the present, consists of over 280 distinct genotypes that are in different breeding stages (Figure 1).

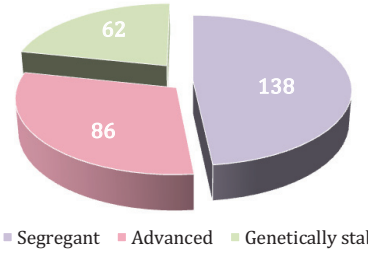

Figure1. Categories of genotypes studied at V.R.D.S. Buzau

Researches debuted with the consolidation and evaluation of the genetic material owned in the germplasm collection. After evaluation, the accession A10 that was introduced in the collection since 2006 was subjected to intensive breeding works. The breeding method used was the one specific to eggplants, respectively "repeated individual selection". In the year of 2015 the breeding program was finalised with the achievement of the desired cultivar, therefore, starting with 2016 the newly obtained cultivar was introduced in the conservative selection program, applying the following conservative selection scheme: first was established the Selection Field with plants obtained from the Author Seed from which 100 elite plants were retained; with the elite plants retained, the Field of Study of Descendants was established, and after careful evaluation of the descendants, 36 typical descendants plants were withheld and used in the establishment of the Pre-Basic field (Figure 2).

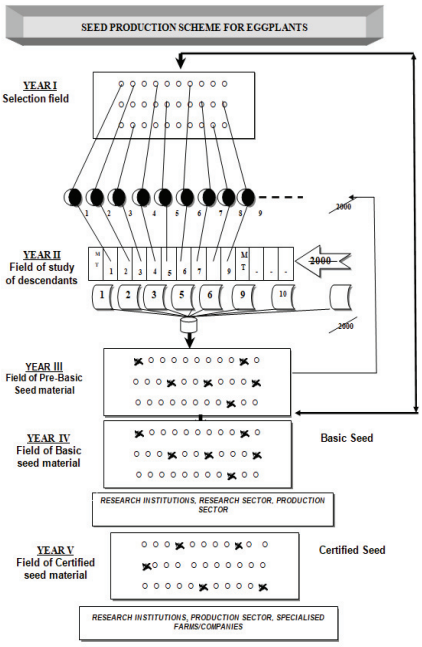

Figure 2. Selection scheme for eggplants

Biometrical and phenological determinations were made at two weeks after sowing and continued during the growing season until harvest, performing biological controls rigorously, eliminating from culture atypical and phonologically delayed plants, or the ones that showed sensibility to pests and diseases.

The crop technology used was the one specific for growing eggplants in open field.

The eggplant crop design was realised using the following distances: 70 cm between rows and 40 cm between plants per row (Figure 3).

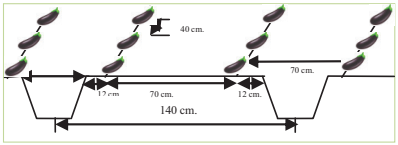

Figure 3. Eggplant crop design

RESULTS AND DISCUSSIONS

In order to obtain the established cultivar, from the Selection Field were harvested the plants that phenotypically showed the desired characteristics. Therefore, this fruit were analyzed from a biometric point of view. The collected results are synthetized in Table 1.

Table 1. Variability of the main characters at the 'Camelia' cultivar in the conservative Selection Field

Studied character	$\bar{X}$	S	CV %	$\bar{X} \pm S$
Fruit length (cm)	15	3	20	12-18
Median fruit diameter (cm)	9.5	1.7	18.4	7.8-11.3
Fruit weight (g)	592.5	263.5	44.4	329-856
Fruit no./plant	11	3	27.2	8-14
Total weight/plant(g)	4475	215	4.8	4260-4690

Table 2. Variability of the main characters at the 'Camelia' cultivar in the conservative Field of Study of Descendants

Studied character	$\bar{X}$	S	CV %	$\bar{X} \pm S$
Fruit length (cm)	16.5	2.5	15.1	14-19
Median fruit diameter (cm)	10.3	1.4	14.0	8.9-11.8
Fruit weight (g)	668	206	30.8	462-784
Fruit no./plant	11.5	2.5	21.7	9-14
Total weight/plant (g)	4695	115	2.4	4580-4810

Table 3. Variability of the main characters at the 'Camelia' cultivar in the conservative Pre-Basic Field

Studied character	$\bar{X}$	S	CV %	$\bar{X} \pm S$
Fruit length (cm)	18	2	11.1	16-20
Median fruit diameter (cm)	11.0	1.1	10	9.9-12.2
Fruit weight (g)	688.5	193.5	28.1	495-882
Fruit no./plant	14	2	14.2	12-16
Total weight/plant (g)	4946	157	3.1	4789-5103

The main indexes used are the ones recommended for realising these types of studies: arithmetic mean ($\bar{X}$), standard deviation (S), variation coefficient (CV %),

Amplitude variation, frequency on variation classes and standard selection interval (X ± S) (Potlog & Velican, 1971).

In Table 2 we registered the results collected in the second year, from the Field of Study of Descendants. As we can see, the stability of the plants and the fruit quality grew.

In Table 3 are synthesized the results collected from the Pre-Basic field, in the third year. As we can see, the selected genotype for the process of breeding became stable and phenotypically uniform. The variation coefficient value demonstrates that in this field we managed to obtain a relatively homogenous genotype.

Researches have resulted so far with the achievement of a new cultivar with distinct phenotypic expressivity. Also it has been registered and patented in the Official Catalogue of Romanian Crop Plants under the name of 'Camelia' (former A10).

The main characteristics of the new achieved cultivar are: the plant has a mean height of 96 cm in open field and 166 cm under greenhouse conditions; the main stalk has a mean height of 23 cm in open field and 28 cm in protected spaces; mean diameter of the main stalk is of 17 mm in open field and 19 mm in protected spaces. Near the ground the stalk becomes woodier and has a brownish colour.

It presents 3 lateral branches, two from the first division of the main stalk, and one from the second division of the main stalk that should be kept both in open field and protected spaces production.

The mean value regarding the length of the lateral branches is of 52 cm in open field and 92 cm in protected spaces. The bush diameter is of 86 cm in open field and 105 cm in protected spaces and has a large, globular constitution.

Leaf length is of approximately 29 cm in field crops and 36 cm in greenhouse crops. The mean values for width of the leaf are 21 cm in open field and 23 cm in protected spaces. The fruit peduncle has a length of 10.5 cm in field and 12 cm in protected spaces. The aerial vegetative organs were studied and concluded that there is no trace of anthocyanic coloration on them (Figure 4).

Figure 4. Plant aspect of A10

On the sepals, the cultivar has thorns that are not very aggressive. Thorns occurs both on plants grown in protected spaces and in the open field (Figure 5).

Figure 5. Thorns on sepals detail for genotype A10

The fruit has a medium length peduncle, with a mean value of 7.5 cm in open field plants and 9.5 cm in protected spaces. The peduncle and sepals are light green coloured. It has 5 sepals, 2 of which are conjoined (Figure 6).

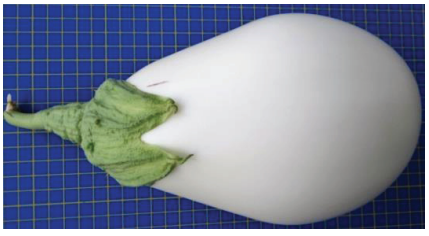

Figure 6. Sepals detail for genotype A10

The peduncle is thickened near the basis and has rare thorns that are not very aggressive.
The sepals are quite large and thick; they have a length with a mean value of 5 cm in open field and 7.5 cm in protected spaces.
The flower has a light-purple colour (Figure 7).
The fruit length is of approximately 16 cm in open field and 20 cm in the protected spaces plants. The fruits have a shape between obovate and pear-shape aspect. The fruit diameter near the peduncle is 5.5 cm in open field and 6.8 cm in protected spaces, the median diameter is 9.9 cm in open field and 12 cm in protected spaces and near the apex the diameter is 6.4 cm in open field and 7.8 cm in protected spaces. The fruit has an intended fruit apex; the pistil area is ellipsoidal, with dimensions between 0.4-0.6 cm in open field fruits and 0.5-1.1 cm in protected spaces fruits (Figure 8).

Figure 7. Flower detail of genotype A10

Figure 8. Indented fruit apex of genotype A10

The fruit weight varies between 495-882 g in open field and in protected spaces between 870-1200 g (Figure 9).

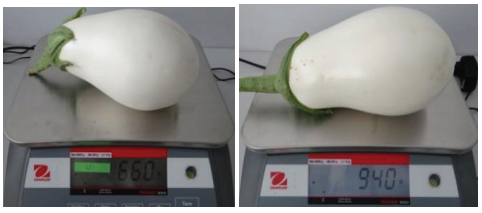

Figure 9. Fruit weight in open field and protected spaces for A 10

The fruits are white and shiny on the outside, with a pleasant commercial aspect and the mesocarp is buttery-white with a more aerated texture than the commonly known eggplants (Figure 10).
The fruits must be harvested while the exocarp is white and shiny.

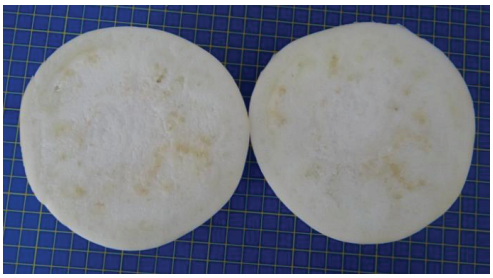

Figure 10. Cross section of eggplants fruits for genotype A10

The fruit has a reduced number of seeds, which are mainly placed towards the fruit apex. The seeds are brown, glossy and kidney-shaped (Figure 11). Thousand seed weight is 3.539 g and 1 g has 290 seeds.

Figure 11. Eggplant seed aspect for genotype A10

CONCLUSIONS

After prospecting the market, the Plant Breeding Laboratory of V.R.D.S. Buzau obtained a cultivar suitable for the pedoclimatic conditions of Romania.

The conservative selection programme applied for this new cultivar allowed us to obtain a relatively homogenous genotype, in the Pre-basic field, from a morphological point of view. After stabilization of the genotype, the accession A 10 was sent at ISTIS Bucharest for further examination in order to be registered in the Official Catalogue of Romanian Crop Plants under the proposed name as 'Camelia'.

While being tested at ISTIS Bucharest, V.R.D.S Buzau has offered seeds and seedlings promotionally for the growers and included in the internal program of conservative selection the seed production for this cultivar. The feedback received from the consumers is positive, the cultivar being distinguished by a great production capacity of a very high quality. In the present, in the internal program of conservative selection the seed had reached the stage of Basic seed, recording in fields a visible decrease of the main features variability starting with the Author' s seed until Basic seed.

The new achieved cultivar is suitable for growing under protected spaces and open field conditions with great yield productions and high quality fruit production. It mainly differentiates by distinct phenotypic characteristics, especially due to the white color of the fruits, the aerate fruit pulp texture and buttery- white pulp aspect.

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RESULTS AND PERSPECTIVES IN *OCIMUM BASILICUM* (BASIL) BREEDING AT VEGETABLE RESEARCH AND DEVELOPMENT STATION BUZĂU

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Abstract

The species Ocimum basilicum originates in Asia and is now widely spread throughout the globe. In Europe, it is assumed that it was introduced in 356-323 BC by Alexandru Macedon. Although the plant has been present in the crop for a very long time, it is grown on very small areas in the household system. In Romania, very few researches have been carried out that have targeted this species, although in the world, the plant is cultivated and widely used in food as well as decorative, ornamental, medicinal, aromatic, melliferous plant. But in our country, it has been used mainly in the cult of the church as sacred plant. At V.R.D.S. Buzau research was started for breeding this species in 1996, because at that time there was no registered autochthonous variety in the Official Catalog of Crop Plants in Romania, the only commercial variety being a local "de Radovanu" population. In 2006, the researches on this species ended with the first Romanian basil variety registered in the Official Catalog of Crop Plants under the name of 'Aromat de Buzau'.

Key words*: 'Aromat de Buzau', Macedon, germplasm, genotype, variety.*

INTRODUCTION

The basil plant (*Ocimum basilicum*) belongs to the *Ocimum* genus which is part of the *Lamiaceae* family, a family well-known for its aromatic and medicinal species. This family contains a large number of species, over 60 of whicht he most representative in our country is mint (*Menthapiperita*) widely used for a long time in our country.

Sweet basil (*Ocimum basilicum*) is one of the leading herb crops, used fresh or dry (Dudai et al., 2008).

Although, in Europe, basil was brought by Alexandru Macedon for a very long time (356-323 BC), in our country, this species was cultivated on small surfaces, in the household system, being used predominantly as sacred plant in the cult of the church. Lately, the interest in aromatic and medicinal plants has increased, a special emphasis being placed on this species, which is why the research has been extended since 1996 at V.R.D.S. Buzau, with a special emphasis on the breeding of this species. The main goals of breeding: high yield of herb, high content of essential oil, good yield of seeds. (Seidler-Lozykowska, 2001)

It should be noted that until then, in Romania, there was no variety approved for this species, but only one local population under the name "De Radovanu" was approved and registered in the Crop Plants Official Catalogue of Romania. In 2006 the first Romanian commercial variety was registered in the Crop Plants Official Catalogue of Romania under the name of 'Aromat de Buzau'.

Research has been focused on the genetic potential exploitation and use of the species.

Sweet basil is grown for culinary use for both fresh and dry consumption and as a source of essential oil and oleoresin for manufacturing perfumes, food flavors, and aromatherapy products (Wyenandt, 2010).

It is a valuable plant species with multiple uses in medicine, cosmetics and gastronomy (Burducea M., 2016; Lagunovschi et al., 2016).

Basil, being an entomophilous plant, shows great variability in the phenotypic expression of the characters, imposing special measures of selection and breeding.

There are many cultivars of basil which vary in their leaf size and colour (green to dark purple), flower colour (white, red, lavender, purple), growth characteristics (shape, height, flowering time), and aroma, making this plant an increasingly popular culinary and ornamental herb (Morales, 1993). Lately, besides the many possibilities of cultivating the species, research has been developed on new crop technologies.

MATERIALS AND METHODS

Currently V.R.D.S. Buzau has a rich and valuable collection in this species, composed of over 60 genotypes in various breeding phases. Depending on the stage of breeding, genotypes were divided into three fields: the collection field, that has the genetic stabilized genotypes in which the variability of the main characters are in the normal range, appropriate to crop varieties; the work field, in which are studied the advanced families whose characters are not well stabilized, but in a large proportion, the main characters expressivity is uniform; the work field in which are studied the segregating families where are the new introduced cultivars in the germplasm collection, or the ones that present a great variability of the main characters.

The undertaken researches for inventorying the genetic stability resulted with the following dispersion of the genotypes: the collection field has a number of 12 stabilized families which are presented in this work, the work field with 17 families that have advanced bred genotypes and the work field in which are studied a number of 32 segregant families. The researches undertaken by breading this species were achieved with difficulty due to the fact that this plant is entomophilous, its pollination being made by the wind and insects, it is preferred by bees, and for maintaining its genetic purity, safe isolators were used (divided greenhouses and textiles isolators) and in some cases, large distances between the genotypes undergone in the breeding program were applied.

The main genotypes bred belong to the following varieties: *Ocimum basilicum* f. *minimum*, characterized by a small size, small leaves and an intense scent in which we encounter the following types: *viridis* - with green leaves, *violaceum* - with violet leaves, *viridicrispum* - with green blistered leaves, *viola crispum* - with violet blistered leaves and also *Ocimum basilicum* f. *bulatum* - with blistered and very strong serration on margin.

As the main breeding method hybridization between families was used, then segregation, positive mass selection and negative mass selection, the stabilized families were isolated and self-pollinated for 6-7 generations, in order to be realized the homozygous of the main characters.

At the Biological Research Centre "Stejarul" Piatra Neamt were analysed volatile oils from two lines of basil, respectively the control variant, 'Aromat de Buzau' and L 12 using gas chromatography method coupled with mass spectrometry GC/MS by means of Gas Agilent Technologies type 6890N chromategraph coupled with mass detector (MSD) TYPE 5975 INERT xl Mass Selective Detector. Volatile oils were obtained by hydrodynamics.

The applied crop technology was species-specific, and seed production was performed on alveolar pallets with Klasmann TS 3 ground peat substrate.

The crop for all studied genotypes was set by seedlings and the planting was done using the following scheme (Figure 1).

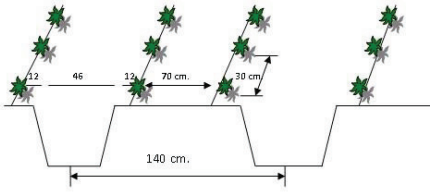

Figure 1. Planting scheme for basil

For most of the species, the crop was set using the following distances: 70 cm between rows and 30 cm between plants, an exception being L5 cultivar that has a small globular habitus, for which a smaller distance between plants was used, only 25 cm (Figure 2).

Figure 2. Basil seedlings

RESULTS AND DISCUSSIONS

A valuable germplasm of 60 genotypes was established within the research and, after their evaluation, 12 distinct phenotypic genotypes were genetically stabilized and retained for the work field. 'Aromat of Buzau' basil was used as control variant in the field.

During the vegetation period biometric and phenological measurements were made and the main plant features are presented in Table 1.

Table 1. The main plant features - average values

Character/Genotype	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
Plant height (cm)	60	57	120	76,3	28	42	48	45,5	89	107	75	96
Stem height to first branch (cm)	6	2	4	7.5	0.6	1.6	2.6	2.1	4	6	4	8
Bush shape	globular	globular	globular	globular	globular	erect	erect	erect	erect	erect	globular	globular
Internodes distance	6.3	6.8	7.2	12.5	1.7	4.4	3.2	6.2	8.1	8.2	7	8.5
Bush diam. (cm)	70	65	110	80	38	38	36	39	72	76	51	68
Stem diam. (cm)	2	1.6	2.4	1.8	1.2	1.2	1.3	1	1.6	1.4	1.4	1.2
No. of main shoots	3	7	15	18	7	9	8	7	3	24	8	12
Length of main shoots (cm)	29.3	33	82	59	72.5	38.5	40	39.5	77.1	63	39	64
No. of sec. shoots (cm)	15	46	163	76	119	69	78	87	68	196	34	72
Length of sec. shoots (cm)	11	16.5	36	44	11	14.4	19.5	8	75	18	20.5	22
Leaves colour	Light green	Light green	Medium green	Green	Green	Dark purple	Dark purple	Dark purple	Medium green	Gray-dark green	Light green	Dark greysh green
Leaf blade length (cm)	7.25	19	14	6.9	2.2	7.5	7.4	8	10.6	7.5	13	5.9
Leaf blade width (cm)	3.6	11	11	2.5	1.3	4.3	3.5	4.8	5.3	3.4	12.5	2.7
Peduncle length (cm)	2.7	7.5	6.5	2.6	0.8	2.3	1.7	3.5	4.1	3.5	4.8	2.9
Leaves area (cm^2)	22 714	28 572	30.008	17 196	14 084	17 917	18 693	17 820	24 538	18 925	29 143	7102
Flowers color	White	White	White	Purple-lilac	White	Purple-lilac	Purple-white	Violet	White	White	White	White slightly anthocyanin
Inflorescence length.(cm)	12	10	26	26	8,1	15	9,6	13,1	22,7	16,5	11	15
No. infl.	49	17	449	503	119	22	52	40	65	123	26	86
No. florets	8-10	7	20	12	6	13	10	9	14	9	9	11
Flavour	Typical basil	slightly sweet	cloves	anise	cloves	Sweet rosy	Slightly mint	cloves	Peppery	lemon	tasty	lemon

L1 belongs to *Ocimum basilicum* L., classic basil, with a mint flavour.

This cultivar is patented as 'Aromat de Buzau'.

This variety of spicy basil is late, with well-defined genetic structure, and adapted to the environmental conditions of our country.

This variety is easy to recognize due to its specific flavour, and also thru other distinct characters, such as: leaves colour, shoots length, the ability of retaining its characteristics during conservation.

The plant is distinguished by mean values in terms of the expressiveness of the main characters, recording a height of 60 cm, a diameter of 70 cm and a number of 48 inflorescences.

It is so typical of the classic basil plant (Figure 3).

Figure 3. 'Aromat de Buzau' (L1) variety - *Ocimum basilicum* L., classic basil

L2 belongs to *Ocimum basilicum* f. *bulatum* species, a valuable cultivar, genetically stabilized, with distinct phenotypic characteristics, a globular plant, with very large leaves that have a medium length of 19 cm and an average width of 11 cm. Due to its rich foliage, light green, juicy, this cultivar is suitable for salads and other dishes.
It is included in the earliness 02 group and has a specific slightly sweet flavour, mild to low intensity. Genotype is distinguished by the smallest stem, 2 cm but the largest leaf, 19 cm with the longest petiole, 7.5 cm (Figure 4).

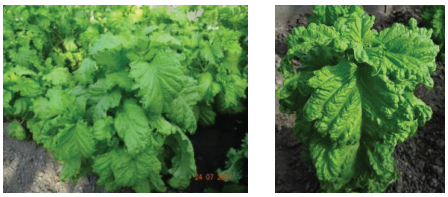

Figure 4. Plant detail of L2 - *Ocimum basilicum* f. *bulatum*

L3 belongs to *Ocimum basilicum* f. *viridi crispum* species, a cultivar characterized by a vigorous globular bush, that has a great foliage device, with an average foliar surface of 30 008 cm², the leaves are large, slightly corrugated and deeply toothed edges. The foliage colour is medium green and has a flavour that is specific to cloves. Because of the richness of the foliage, tender and juicy, this genotype can be used in salads and other dishes. This variety is the one with the highest port, 120 cm tall and 110 cm in diameter, and the main shoots are 82 cm high (Figure 5).

Figure 5. Plant detail of L3 - *Ocimum basilicum* f. *viridi crispum*

L4 belongs to *Ocimum basilicum* f. *thyrsiflora species,* a new cultivar obtained at V.R.D.S. Buzau by crossbreeding a variety of Thai basil with V.R.D.S Buzau's owned variety 'Aromat de Buzau'. This plant is globular and the inflorescence is corymb type. The foliage is green, the main ribs are slightly red and the inflorescence has white-lilac flowers with burgundy-red florets. The flavour is similar to anise released by the whole foliage give more value to this cultivar, recommended for various dishes and tea. This genotype is the one with the largest number of inflorescences, 503 with a length of 26 cm. Thus, it becomes even more productive (Figure 6).

Figure 6. Plant detail of L4 - *Ocimum basilicum* f. *thyrsiflora*

L5 belongs to *Ocimum basilicum* f. *minimum* species, a new cultivar genetically stable which has a shape of a small bush. Due to this characteristic, this cultivar is intended to be grown in pots or jardinière, outdoors like terraces and also indoors. The leaves are small, with a medium length of 2.2 cm and a width of 1.3 cm. It has a strong, fine flavour, slightly peppered, in which can be found a light scent of thyme, feature that recommends it to be grown in pots, for freshened air purposes. This variety records the lowest values for the leaf (2.2 cm long), the plant height (28 cm) and the inflorescence length (8.1 cm) due to the dwarf habit (Figure 7).

Figure 7. Plant detail of L5 - *Ocimum basilicum* f. *minimum*

L6 belongs to *Ocimum basilicum* f. *violaceum* species, a cultivar that has plants shaped as a slender bush. The defining characteristic is given by the colour of the foliage which is dark purple, with elliptical leaves, no hair, with acuminate top. It presents a strong flavour, sweet with scents of rose. It can be used in

various dishes, teas, juices and due to strong anthocyanin pigmentation it can be used as a natural food colouring.
This genotype is distinguished by its very strong violet-black foliage (Figure 8).

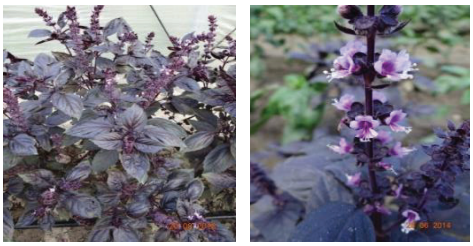

Figure 8. Plant detail of L6 - *Ocimum basilicum* f. *violaceum*

L7 belongs to *Ocimum basilicum f. violaceum* species, a new cultivar that has an erect shape, with great dark-purple colour foliage.
The shape of the leaves is oval-elongated, serrated and slightly wrinkled, a feature that gives distinctness to this cultivar.
The flowers are purple, discreet flavour is slightly sweet with scents of mint.
This genotype recorded the smallest bush diameter of only 36 cm (Figure 9).

Figure 9. Plant detail of L7 - *Ocimum basilicum* f. *violaceum*

L8 belongs to the *Ocimum basilicum* f. *violaceum crispum* variety, a cultivar that has an erect bush shape, the leaves are blistered, serrated on the margin, *crispum* type, a distinct characteristic of this family.
The flowers are purple and the flavour is specific, discrete, resembling with the flavour of cloves.
This plant has a long growing season, it blooms late and it is included in the 03 earliness group.
This genotype recorded the smallest stalk diameter of only 1 cm but also the smallest 2120 cm^2 foliar surface (Figure 10).

Figure 10. Plant detail of L8 - *Ocimum basilicum* f. *violaceum crispum*

L9 belongs to *Ocimum basilicum* L. var *genovese* species, a cultivar that has vigorous plants, with an average height of 89 cm and a diameter of 72 cm. Its medium green foliage has convex, tender, juicy leaves.
The flowers are white, with a length of 22.7 cm with a total number of 14 florets.
The flavour released by this cultivar is strong, peppery. The cultivar shows only 3 main shoots, the lowest number of main shoots being recorded among the 12 genotypes studied (Figure 11).

Figure 11. Plant detail of L9 - *Ocimum basilicum* L. var *genovese*

L10 belongs to the *Ocimum basilicum f. citriodorum* species, a cultivar characterized by an erect tall bush, with an average height of 107 cm and a diameter of 76 cm.
The foliage is rich, with a silver-green color, with a slightly pointed leaves that has both glandular hairs on the stalk and on the main and secondary ribs.
The color of the flowers is white, and the strong asset of this variety is its flavour that resembles with the lemon aroma, feature that makes it suitable for flavoring drinks and various dishes.
L10 has recorded the biggest number of main shoots, 24 and 194 secondary shoots, characterized by a rich vegetative mass (Figure 12).


Figure 12. Plant detail of L10 - *Ocimum basilicum f. citriodorum*

L11 belongs to the *Ocimum basilicum f. bulatum* species, characterized by a globular bush, an average height of 75 cm.
It has rich foliage with large leaves, slightly blistered, a length of 13 cm and a width of 12.5 cm.
Leaves are juicy and fleshy, suitable for salads.
Their flowers are white and the distance between florets is of 1.9 cm.
The plant presents a discreet flavour, specific to this species. This genotype is distinguished by the highest value of 12.5 cm leaf width (Figure 13).


Figure 13. Plant detail of L11 - *Ocimum basilicum f. bulatum*

The research was completed in 2018 with the approval of a new variety of basil, listed in the Official Catalogue of Crop Plants in Romania by the name of Macedon.
The researches undertaken at SCDL Buzau have demonstrated that this cultivar can be cultivated successfully in protected areas, in open field and in pots and jardinière, indoors.
The strongest asset of this cultivar is given by the specific intense aroma of lemon.
The plant has a globular shape, medium size, with a height of 96 cm the median diameter of the bush is 68 cm, having a number of 12 main shoots and a number of 72 secondary shoots.
The stem at the base is woody and as it evolves, growing turns from green to brown.
The height of the stem to the first branch is 8 cm and has a diameter at the base of 9.5 mm the main shoots have an average length of 64 cm and the secondary ones of 22 cm.
On the main stalk a number of 16 inflorescences are found and the length of the bloom of the inflorescence is on average 15 cm.
The diameter of a single floret is 2.2 mm.
The inflorescence has a diameter of 7.5 mm and the distance between the florets of the inflorescence is 2.8 cm.
The diameter of a floret is 1.6 cm and the height is 7.3 mm. The number of flowers in florets is 6. The colour of the flowers is white with light anthocyanin pigmentation.
The leaves are light green with a shade of grey.
The leaf has an average length of 5.9 cm and a width of 2.7 cm.
The length of the petiole is 2.9 cm, the thickness of the leaf is 0.4 mm. The thickness of the petiole is 1.3 mm, the leaf weight is 0.26 g.
On the whole plant, but more accentuated at the nodes and branches, there are glandular hairs that are responsible for secreting the specific lemon fragrance.
The plant has a high production potential, a single bush weighs 560 g, but production can increase if the young shoots are planted. Multiplication can be done by seeds, but also by sprouting.
It should be noted that the species generally has good seedlings and propagation capacity. Between 3-5 days the cuttings emit roots depending on temperature and the phenophase of the harvest.
The seeds are round, slightly oval, black with light hard tegument. There are 664 seeds per gram and MMB (1000 seed weight) is 1569 g (Figure 14).


Figure 14. Plant detail of L12, 'Macedon' variety

Table 2. Chemical composition of volatile oil in *Ocimum citriodorum* (%)

Compounds	S1	S1-C
β- myrcene	0.30	0.23
Eucalyptol	2.77	3.04
Trans-β-ocimene	0.69	0.47
Linalool	38.47	40.40
Camphor	1.03	1.18
Estragole	45.89	41.09
β- elemene	1.27	1.05
Caryophyllene	0.60	0.45
Germacren D	2.29	1.28
Elixene	0.47	0.35
δ- guaiene	1.99	1.31
ι- cadinene	1.26	1.11

S1- sample extract; S1-C-sample extract control

Following the analyses carried out, for the aromatic varieties of Buzau and 'Macedon' respectively, the chemical compounds of the volatile oils, which are presented in table 3 for *Ocimum citriodorum* ('Macedon') and 4 for *Ocimum basilicum*, ('Aromat de Buzau').
This genotype is marked by the study of biochemical analyses, with a high geranial content of 22.7% and carveol-17.7%. The results are consistent with the literature (Grayer, R., 1996).

Table 3. Chemical composition of volatile oil in *Ocimum basilicum* (%)

Compounds	S1	S1-C
Sulcatone	1.61	1.31
Cis-β-ocimene	1.03	0.51
Linalool	6.18	0.39
Cis-verbenol	1.13	1.31
Estragole	8.11	0.40
Nerol	9.65	11.04
Carveol	17.67	30.62
Neral	3.42	1.32
Geranial	22.67	37.50
β -caryophyllene	7.08	3.53
Trans-α-Bergamotene	2.22	1.12
α- caryophyllene	1.24	0.51
Cis-β-Farnesene	1.22	0.52
Germacren D	2.65	0.59
β- Bisabolene	6.61	3.31

S1- sample extract; S1-C-sample extract control

The volatile (essential) oils obtained by the hydrodynamics of the floral spice are characterized by a high content of estragon-45.9% and linalool-38.5%, the results are in line with those in the literature (Chalchat & Ozcan, 2008).

Both varieties are distinguished by a complex biochemical content of volatile oils that can be successfully used both for consumption and for industrial processing. 'Aromat de Buzau' has a balanced aroma from all points of view, being a classical basil and 'Macedon' comes with novelty within this species, adding value through its unique and special aroma of lemon, the aroma released by all its vegetative organs.

CONCLUSIONS

Twelve genetically stabilized genotypes were obtained, of which two were 'Aromat de Buzau' and 'Macedon' variety, a variety with a specific lemon flavour.
Biochemical analyses highlight the multiple possibilities of use the varieties and the qualities of volatile oils obtained from them.
The studies undertaken will be capitalized in the future by proposing the homologation of the genotypes that have been improved in the experience and that can meet the requirements of the DUS test (distinctness, uniformity, stability).
Along with new basil varieties, market studies have been carried out and the demands of consumers and growers and the demand for seeds and seedlings for the two new creations have increased significantly from one year to the next.

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DETERMINATION OF PRODUCTIVITY AND CHLORINE CONCENTRATION IN SOME BEAN CULTIVATION, FROM THE REGION OF MOLDOVA, UNDER SALT STRESS

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Abstract

Surfaces affected by excess salinity arrived at 200 million ha., at the level of the whole planet and to about 400.000 ha in Romania; around 7% of the world's total the surface area is affected by salinity. Salinization is a frequent process and is a major constraint for food production because it limits yield of the crops. Cl^- ion is dominant in saline substrates; it is essential as a microelement for all higher plants, but the high concentration of this ion leads to toxicity to the salt-sensitive plants; the stress caused by salinity is also a stress of dehydration. High salinity causes an internal water shortage, and plants that are affected by saline stress can sometimes develop xeromorphic structures. Therefore, in present study was pursued the influence of saline solutions of 100 mM and 200 mM NaCl on productivity of 7 local populations of common beans, collected from areas with saline soils, from North-East of Romania and grown under greenhouse conditions, in 2018. Thus, they were analyzed after 15 and 30 days of saline stress: the largest/ lowest amount of chlorine, the number of pods/plant and the weight of the grains/plant, elements that define productivity. As a result, the concentration of 100 mM and 200 mM NaCl negatively influenced the fruit weight against the control variant, to all the genotypes studied, and the Trușești 2, Blăgești, Săveni and Moșna genotypes recorded the highest number of grains/plants for the 100 mM variant and 200 mM.

Key words: *bean, correlations, NaCl, productivity.*

INTRODUCTION

Salinity is one of the major obstacles for increasing production in cropping areas throughout the world. The deleterious effects of salinity on plant growth are associated with: low osmotic potential of soil solution, nutritional imbalance, specific ion effect (salt stress) or a combination of these factors (Ashraf, 1994; Marschner, 1995). All of these cause adverse pleiotropic effects on plant growth and development at physiological and biochemical levels (Gorham et al., 1985; Levitt, 1980; Munns, 2002) and at the molecular level (Mansour, 2000; Tester & Davenport, 2003; Winicov, 1998). Crop plants will not grow in high concentrations of salt: only halophytes grow in concentrations of sodium chloride higher than about 400 mM (Marschner, 1996). Species resistant to excess chlorine are: beans, potatoes, tomatoes etc. (Șumălan, 2004; Covașă, 2016).

The bean (*Phaseolus vulgaris* L.) is an important source of dietary protein in many developing countries but is considered as sensitive species to salinity compared to other vegetables (Bouzid & Chaabane, 2012). For this reason, the purpose of the present paper was to determine the effect of excess NaCl on production of the plants as an indicator of salt stress tolerance.

Chlorine is an element present in all plants. The role of chlorine in plant metabolism is little known. Haas (1945) supported the influence of chlorine on enzymatic systems. It appears that $C1^-$ activates cytochrome oxidase.

It is accumulated in larger amounts in marine algae, ferns and halophilic plants. Higher plants take it from the ground through the root system and from the gaseous atmosphere through the stomachs of the leaves.

Nutritional insufficiency, causes leaf chlorosis, wrinkling of leaf edges and inhibition of root growth, deregulation of plant metabolism.

Such a insufficiency is also manifested by the wilting of the plants, stopping the growth, the easy breaking of the roots, which are generally small. The leaves have chlorotic spots, which later become yellowish.

The concentration of chlorine in the plant determines the yield and quality of production, for two reasons. First, chlorine is a mineral nutrient, and its deficiency induces metabolic problems that interfere with plant growth. However, due to the low requirements of most cultures, chlorine deficiency it does not appear hard in the field. Second, excess chlorine, an occurrence with salinity, results in serious physiological dysfunctions that affect both the quality and the yield of plants.

Most quality deficiencies are based on physiological dysfunctions that occur under toxic conditions (Zorb et al., 2018).

An important constraint on plants growing on saline soils is the toxicity of ions, especially those of Na^+ and Cl^-.

The ions can cause toxicity in different ways, as Bowen (1966) has shown, because they act as antimetabolites by binding or precipitating different metabolites, catalyzing the rapid breakdown of essential elements by combining with cell membranes and affecting thus permeability, but also embellish the essential elements, but cannot replace their functions (Marschner, 2002).

The experiment is part of a broader research, and the determination of chlor content is only one stage of this research.

We have tested the resistance of this species to a high degree of salinity, as there is currently an expansion of saline soils with high salt concentrations. Another reason for testing of these genotypes is that more and more, in agriculture, the use of seawater is increasingly desirable, for two reasons: the water crisis on the horizon, but also the nutritional value that seawater could provide by irrigating the crop plants.

As novelty, in the current study, new genotypes were found of bean, from Nord-East of Romania, tolerant to this factor. For this reason, the purpose of the present paper was to determine the effect of excess NaCl on production of the plants as an indicator of salt stress tolerance.

MATERIALS AND METHODS

Soil salinity is one of the best known stress factors of plants that can lead to crop yield reduction. Therefore, it is important to identify new tolerance varieties of plants that can grow on saline soils. The experience was done under greenhouse conditions, to "Vasile Adamachi" farm in Iasi, and the research took place in the Plant Physiology Laboratory, USAMV Iaşi. The biological material was represented by seven local bean populations (Blăgeşti 1, Blăgeşti 2, Blăgeşti 3, Blăgeşti 4, Truşeşti 2, Moşna and Săveni) collected from saline soils from the region of Moldova, known as the region of Moldova (Iaşi, Vaslui and Botoşani).

The bifactorial experience was set up in 12 liter seed pots, in randomized blocks with three repetitions. They were exposed to saline stress for a period of 30 days, being constantly watered with concentrations of 100 mM NaCl and 200 mM NaCl.

The concentration of chlorine was determined by potentiometric titration with silver ions, using the Analytical Chloride Titrator, as described by Slabu et al. (2009), and expressed as mg/100 g DW. The results were statistically interpreted using the Microsoft Excel - Data Analysis application, determining the correlation coefficient and magnitude of the effect in the linear correlation r (Pearson).

RESULTS AND DISCUSSIONS

Chlorine is involved in the photosynthesis process to remove harmful oxidants to photochemical systems and stimulate the transport of electrons. It also has the role of regulating the osmotic potential by maintaining the hydric cell level, ensuring the opening of stomates (Davidescu, 1988). The excess of this microelement influences negatively the growth of plants by the degradation of carbohydrate metabolism (Ciobanu & Şumălan, 2009). The chlorine ion is present in abundance almost everywhere in the world. It is required as a micronutrient for optimal plant growth, at a rate of only 0.3-1 mg/g dry matter (DW) in most plants (Marschner, 1986). The influnce of the chloride ion on plant growth depends on the plant variety (Tottingham, 1919). As an example, high salt - tolerant barley cultivars

cultured with 150 mmol/liter of NaCl produced 42-86% of the yields of the same cultivars grown under no saline conditions (Sopandie et al., 1993).

Analysis of the Cl^- content of bean leaves subjected to saline stress, over a period of 15 days reflects the fact that in the case of the control variant, values ranged from 0.97-3.37 mg/100 mg DW. For the variant treated with 100 mM NaCl, the values were higher compared to the control variant, so the maximum was recorded at the Săveni genotype (10.20 mg/100 mg DW) and the minimum value at the Blăgeşti 3 (3.90 mg/100 DW). The values are significantly higher than the control variant and for the 200 mM NaCl stress, genotypes with a maximum of 15.26 mg/100 mg DW is Săveni, followed by genotypes: Blăgeşti 4 (12.95 mg/100 mg DW), Moşna (7.89 mg/100 mg DW) and Truşeşti 2 (7.09 mg/100 mg DW) which denotes the resistance of these genotype to saline stres (Table 1).

Table 1. The content of Cl^- (mg/100 mg DW) of bean leaves under salinity stress for a period of 15 day

Population	Control	100 mM NaCl	200 mM NaCl
Blăgeşti 1	0.97	5.59	4.88
Blăgeşti 2	1.24	4.52	3.48
Blăgeşti 3	1.77	3.90	4.88
Blăgeşti 4	3.37	8.69	12.95
Moşna	2.32	6.65	7.89
Săveni	0.97	10.20	15.26
Truşeşti 2	1.33	5.23	7.09

Following analysis to variance in the seven saline stressed bean genotypes for 15 days, it was found that Cl^- accumulation was insignificantly influenced by genotype and very significantly by the applied NaCl concentration (Table 2).

Table 2. Variance analysis for bean genotypes subjected to NaCl stress for a period of 15 days with respect to Cl^- of the leaf content

Source of variation	SP	GL	MS	F	P-value	F crit	Influence
Genotype	97.4073	6	16.23.455	3.438095	0.032568	2.99612	NS
Concentration	151.8529	2	75.92643	16.07943	0.000402	3.8853	***
Error	56.66353	12	4.721961				
The total	305.9237	20					

*Anova Two-Factor: NS- statistic differences insignificant ($p \geq 0.05$); *Significant statistical differences ($p \leq 0.05$); ** Significantly significant statistical differences ($p \leq 0.01$); *** Very significant statistical differences ($p \leq 0.001$), F> F crit reject the null hypothesis.*

As a result of the T-test, it is noted that between the control variant and the variant subjected to a 100 mM saline stress, there are very significant statistical differences. There are statistically significant differences between the blank control and the 200 mM concentration. Significant distinct statistical differences are manifested between saline-treated variants (Table 3).

Table 3. Statistical differences between control (I) and variants treated with saline solutions: 100 mM (II) and 200 mM (III) in terms of Cl^- content, expressed as mg/100 mg DW

Comparative variants	t-stat	P two-tail	Significance
I-II	-5.51981	0.001487	***
I-III	-3.9656	0.007404	*
II-III	-1.91005	0.01047	**

*Test Paired Two Sample for Means: Ns-statistically insignificant differences ($p \geq 0.05$) between variants; *Significant statistical differences ($p \leq 0.05$) between variants; **Significant distinct statistical differences ($p \leq 0.01$) between variants; ***Very significant statistical differences ($p \leq 0.001$) between variants.*

Sensitivity to high Cl^- concentrations varies widely between plant species and cultivars. Generally, most nonwoody crops tolerate excessive levels of Cl^-, where as many woody plant species and beans are susceptible to Cl^- toxicity (Mass, 1986). The critical toxicity concentration is about 4-7 and 15-50 mg/g for Cl^- sensitive and Cl^- tolerant plant species. Critical toxicity concentration to bean is 1.0 mmol Cl^-/liter (maximum Cl^- concentration in saturated soil extracts without loss in yield) to an EC of 1.0 (dS/m) (Jing et al., 1992).

After 30 days, after application of saline treatments, in the case of the control variant, the Cl^- content of the leaves shows values ranging from 1.30-2.66 mg.u./100 mg s.u. For the variant treated with 100 mM saline, the values are higher compared to the control variant; the maximum was recorded to genotype Blăgeşti 1 (11.75 mg/100 mg DW) and the lowest value for Blăgeşti 4 genotype (3.19 mg/100 mg s.u.). In contrast, the values are significantly higher than the control variant for four populations subjected to a 200 mM saline stress; In this case, the maximum value reaches 14. 44 mg/100 mg s.c. to the Blăgeşti 2 genotype, and the minimum value is 5.56 mg/100 mg s.u. at Blăgeşti 4 (Table 4).

Table 4. The content of Cl^- (mg/100 mg DW) of bean leaves under salinity stress for a period of 30 days

Population	Control	100 mM NaCl	200 mM NaCl
Blăgeşti 1	2.43	11.75	18.67
Blăgeşti 2	1.30	12.21	14.44
Blăgeşti 3	2.64	5.45	11.73
Blăgeşti 4	2.53	3.19	4.56
Moşna	2.30	7.74	7.55
Săveni	2.66	3.75	6.81
Truşeşti 2	2.13	5.02	5.64

Following variance analysis, to bean genotypes subjected to saline stress for 30 days, it is noted that Cl^- accumulation was insignificantly influenced by genotype and significantly distinct from the applied NaCl concentration (Table 5).

Table 5. Analysis of variance to bean genotypes subjected to stress with NaCl over a period of 30 days, with respect to the Cl^- content of leaf

Source of variation	SP	GL	MS	F	P-value	F crit	Influence
Genotype	140.7583	6	23.45972	2.685111	0.068602	2.99612	NS
Concentration	207.6784	2	103.8392	11.88504	0.001426	3.8853	**
Erorr	104.8436	12	8.736966				
Total	453.2803	20					

Anova Two-Factor: NS- statistic differences insignificant ($p \geq 0.05$); *Significant statistical differences ($p \leq 0.05$); **Significantly significant statistical differences ($p \leq 0,01$); ***Very significant statistical differences ($p \leq 0,001$), F> F crit reject the null hypothesis.

This time, following the T-test, it is noted that between the control variant and the variant subjected to a 100 mM saline stress, there are very significant statistical differences. There are statistically significant differences between the control and the 200 mM variant. Significant distinct statistical differences are manifested between saline-treated variants (Table 6).

Table 6. Statistical differences between control (I) and variants treated with saline solutions: 100 mM (II) and 200 mM (III) in terms of Cl^- content, expressed as mg/100 mg DW

Compared variants	t-stat	P two-tail	Significance
I-II	-3.91308	0.007864	**
I-III	-2.97548	0.024781	*
II-III	-0.50368	0.0632443	**

Paired Two Sample for Means: Ns- statistically insignificant differences ($p \geq 0.05$) between variants; *Significant statistical differences ($p \leq 0.05$) between variants; **Significant distinct statistical differences ($p \leq 0.01$) between variants; ***Very significant statistical differences ($p \leq 0.001$) between variants.

Assessing the influence of saline stress on plant production is a very important aspect in determining how salinity affects productivity, and that is why the fructification process has been studied on the basis of determinations of different indices: average number of grains/ plant, and average weight of grains/pods.

Analyzing the average number of grains/plants and average weight of grains/pods, after treatment with 30 day saline solution, it was observed that, in comparison to the control variant, for all genotypes treated with 100 mM NaCl and 200 mM NaCl solutions, these parameters, decreased considerable**.** From Tables 7 and 8, it can be noticed that four genotypes recorded a constant number of grains and a constant grain weight, to all three analyzed variants (Truşeşti 2, Blăgeşti 4, Săveni and Moşna) and three of the genotypes had high values only at the control variant (Blăgeşti 1, Blăgeşti 2 and Blăgeşti 3); to the 100 mM treated variants, they had very low production, and to the variant treated with 200 mM was inexistent.

The results obtained are in full agreement with those presented in the literature, according to which high concentrations of NaCl have a negative influence on the production of cultivated plants (Giannakoula & Ilias, 2013; Covaşă, 2016).

Table 7. Effect of saline stress on the average number of grains/pod after 30 days of exposure to saline stress

Population	Control	100 mM NaCl	200 mM NaCl
Blăgeşti 1	17.40	11.40	0.00
Blăgeşti 2	11.60	0.00	0.00
Blăgeşti 3	7.00	2.40	0.00
Blăgeşti 4	6.20	4.20	2.40
Truşeşti 2	9.20	6.40	5.60
Săveni	6.40	5.00	1.60
Moşna	5.80	3.40	2.80

Table 8. Effect of saline stress on average weight of grains/pod after 30 days exposure to saline stress

Population	Control	100 mM NaCl	200 mM NaCl
Blăgeşti 1	0.92	0.59	0.00
Blăgeşti 2	1.21	0.00	0.00
Blăgeşti 3	0.51	0.12	0.00
Blăgeşti 4	0.48	0.28	0.12
Truşeşti 2	0.86	0.51	0.46
Săveni	0.90	0.64	0.32
Moşna	0.65	0.28	0.15

In nature, phenomena are in close association with the surrounding phenomena. That is why in practical applications we are interested in not only the presence and the meaning of the correlation, but also the extent to which it manifests itself; this grade is appreciated by statistical calculations. In this respect, the linear correlation coefficient Bravais-Pearson (Dragomirescu, 2003) was introduced to assess the correlation between two sizes. In this paper, in order to have statistical coverage, a correlation was made between two studied parameters, according to the rules established by Colton (1974).

The correlation between Cl^- content and average weight of grains/pod after 30 days of exposure to saline stress was found to be acceptable in accordance with the rules established by Colton (1974). Chlorine concentration at leaf level had a negative influence on the average number of grains/ pods, in this respect the excess of chlorine having a toxic effect (Figure 1).

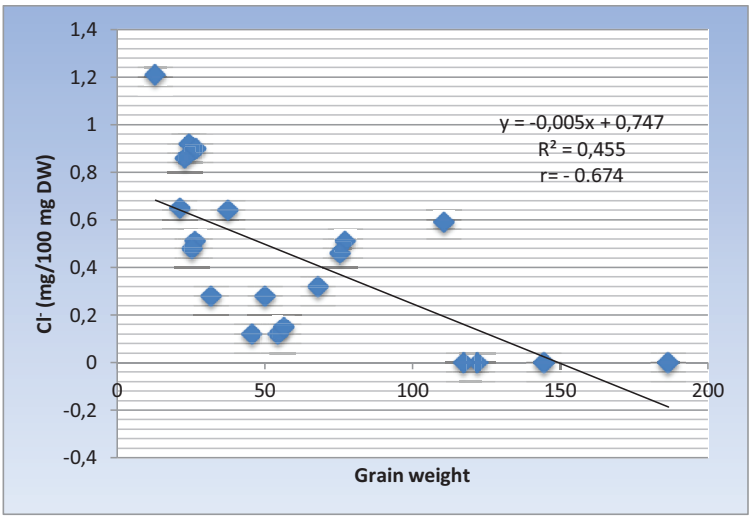

Figure 1. Correlation between Cl^- (mg/100 mg DW) content and average number of grains/pod after 30 days exposure to saline stress

Figure 2. Aspects on how to determine the Cl^- content of bean plants with the Analytical Chroride Titrator (original)

CONCLUSIONS

At the end of the treatment, the Cl^- content values were greater than to the control variant for the 100 mM genotypes and 200 mM NaCl. At the last treatment applied, the maximum value achieved was 14.44 mg/100 mg DW at Blăgeşti 2, and the minimum value was 4.56 mg/100 mg DW at Blăgeşti 4.

From the observations on the fructification process we can conclude the following: the number of grains exposed to saline stress over a period of 30 days decreased compared to the control variant for all genotypes soaked to 100 mM and 200 Mm NaCl; to the last variant there were genotypes to which no production was obtained, because salinity had an effect of inhibiting growth, photosynthesis and subsequent production (Blăgeşti 1, Blăgeşti 2 and Blăgeşti 3).

The genotypes Trușești 2, Săveni, Moșna and Blăgeşti 4 recorded the highest number of grains/plants for the 100 mM and for the 200 mM variant.

The concentration of 100 mM and 200 mM NaCl negatively influenced the weight of the fruit against the control variant, the values oscillating from 0.12 grams to 0.59 grams of berries/pods.

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CONTRIBUTIONS TO THE KNOWLEDGE OF SPONTANEOUS FLORA FROM THE PARK OF THE PELEŞ DOMAIN

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Abstract

Built by a series of extensive works that involved changes in topography, hydrography and vegetation of the Peleş Valley, the park of the Peleş Domain includes grassland areas whose vegetation coverage was achieved with the help of grass furrows brought from the pastures and meadows located in the surrounding area. Under the influence of local conditions, the original flora was replaced by phytocoenoses made of various species existing in the Bucegi Mountains, from the lower mountain region to the sub-alpine floor. This study presents the results of observations made on the herbaceous flora installed into the meadow in front of Peleş Castle.

Key words*: herbaceous flora, meadow, mountain regions, Peleş Domain, phytocoenosis.*

INTRODUCTION

The park of the Peleş Domain is composed of a succession of landscaped grounds designed according to the principles of landscape architecture where the meadow occupies a special place.

The establishment of this park, which began in the ending of the Peleş Castle building, has sought to integrate the highly modified site, *covered with debris, gravels, crushed bricks or woodcuts, all remains of the construction just finished* (Haret, 1924), into the natural landscape, characteristic of the area.

In this situation there was also the meadow in front of the terraces on which the castle is located, *result of topographical, hydrographical and vegetal transformations of the landscapes which, although concealed in the current landscape, which they have aesthetically enhanced can be deciphered especially at the level of documentary sources and also verified on the ground* (Huzui-Stoiculescu, 2015).

These transformations implied the filling of the gaps with soil brought from the top of Molomăţ mountain, the drainage of the springs and the spillage of the collected water in a ramification of the Peleş stream, the correction of the 30 degrees inclination of the slope and the covering of the terrain with grass furrows brought from the mountain meadows (Haret, 1924).

This solution for the setting up of the vegetal cover was imposed by the climate characterized by *frequent and rapid rains* that would have made useless sowing *directly on the stuffing* (Haret, 1924).

Also, for vegetation fixation, trees of different essences have been planted, few of wich, of considerable size, are still found today, holding a decisive role in shaping the phytocenoses formed on the meadows.

At this time, no further interventions have been identified to complete the vegetal cover with new herbaceous species after the initial moment of the meadow setting up. Local conditions - the 970 m altitude and the south orientation of the slope, have favored changes in the herbaceous carpet composition resulting in the disappearance of alpine flora and its replacement with subalpine grasses (Haret, 1924).

In this study we aim to show the composition of the phytocoenoses which form the meadow located in front of the Peleş Castle and to highlight the influence of the natural conditions in structuring the vegetation on an antropic initial site.

MATERIALS AND METHODS

Studies of the flora from the meadow located in front of Peleş Castle were made in May 2018.

The Peleş Castle is situated in Peleş Valley, at 970 m altitude, 25°34 '40' 'E and 45°21' 30 " N, surrounded by beech and spruce forests (*Fagus sylvatica* and *Picea abies*).

The meadow with a south exposition is crossed from north-west to south-east, on about half of its surface, by a ramification of the Peleş stream (Figure 1).

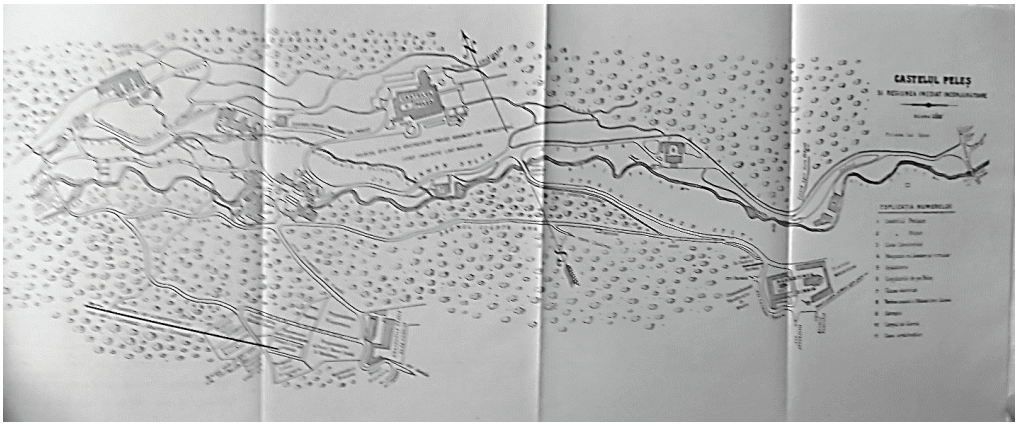

Figure 1. The map of the Peleş Domain (Haret, 1924)

Several springs marked by a characteristic vegetation can be traced from the middle of the slope to the base.

A hedge around 1 m high restricts public access. Till now, maintenance interventions consist of the hay annual harvesting.

Species of resinous or deciduous ornamental trees and shrubs are mainly found at the top or the bottom of the slope or along the ramification of the Peleş stream.

The meadow flora has been observed by crossing the land through certain segments selected according to some ecological factors such as soil moisture, using maps on which trees and shrubs were marked.

In order to achive the phytocoenosis structure, plants species were identified with field guides(Beldie, 1967; Ciocârlan, 2009; Sârbu et al., 2013) and listed in phytosociological tables.

RESULTS AND DISCUSSIONS

In the investigate territories there were 85 herbaceous species from 23 families (Table 1). Most species - 12, belong to the *Asteraceae* family, followed by *Poaceae* - 10 species and *Fabaceae* - 9 species. *Lamiaceae* and *Rosaceae* families are present with 5 species, *Boraginaceae*, *Brassicaceae*, *Juncaceae* together with *Cyperaceae*, with 4 species each, while *Liliaceae*, *Scrophulariaceae*, *Rubiaceae*, *Apiaceae*, *Polygonaceae* and *Ranunculaceae* are represented by 3 species each. From *Plantaginaceae*, *Primulaceae*, *Geraniaceae* and *Caryophyllaceae*, 2 species were recorded and one species were found from *Orchidaceae*, *Campanulaceae*, *Solanaceae*, *Euphorbiaceae*, *Urticaceae* and *Equisetaceae* families.

Species from *Poaceae* (Table 1), with the exception of the *Glyceria nemoralis* - specific for wetlands, form a mixture characteristic of *Agrostis capillaris* grasslands (Motcă, 1994), which are usually found in hilly areas up to 800 m altitude. In Bucegi, *A. capillaris* belongs to the sub-association *Festucetum rubrae agrostidetosum*, ass. *Festucetum rubrae fallax* (Puşcaru et al., 1956) - nowadays called *Scorzonero roseae - Festucetum nigricantis* (Sanda et al., 2008), which cover alluvial, relatively acidic soils, mostley located on the valleys.

Nearly stream ramifications there are phytocoenosis of *Petasites kablikianus*, and *Chaerophillum hirsutum*, *Petasites hybridus*, *Aegopodium podagraria* may be identified as accompanying species (Table 1). On the edge of the observed area, around *Fagus sylvatica* 'Atropurpurea' exemplars or near the forest, species from the *Symphyto cordati-Fagietum* association such as *Pulmonaria rubra*, *Poa nemoralis* or *Geranium robertianum* can be identified (Table 1).

Table 1. Plant species from the Park of the Peleș Castle Domain

Families	Species	Bioforms	Geoelements	Ecological factors			Frequency (Bucegi mountains)	Utmostalt. (m)	Frequency (Romania)
				U	T	R			
Poaceae (Gramineae)	*1. Arrhenatherum elatius*	H	Euras	5	X	6	sporadically	1650	frequent
	2. Festuca rubra	H	Circ.	4	4	5	common	1900	frequent
	3. Agrostis capillaris	H	Circ.	X	X	5	common	1920	frequent
	4. Trisetum flavescens	H	Euras	X	X	X	frequent	1600	frequent
	5. Dactylis glomerata	H	Euras	4	X	X	frequent	1900	frequent
	6. Poa pratensis	H	Cosm.	5	X	X	frequent	1800	frequent
	7. Holcus lanatus	H	Cosm.	5	5	4	sporadically	1650	frequent
	8. Elymus caninus subsp. *Biflorus*	H	Circ.	6	X	X	sporadically	1750	sporadically
	9. Briza media	H	Euras.	X	4	7	-	600-700	frequent
	10. Glyceria nemoralis	H	Centr. Eur.	9	6	7	sporadically	1500	sporadically
Juncaceae, Cyperaceae	*1. Juncus effusus*	H	Cosm.	7	X	X	common	600-700	common
	2. Carex humilis	H	Circ.	3	X	7	sporadically	1750	sporadically
	3. Carex sylvatica	H	Circ.	5	5	6	frequent	600-700	frequent
	4. Carex hirta	G	Circ.	6	X	X	sporadically	950	ordinary
Fabaceae (Leguminosae)	*1. Trifolium pratense*	H	Euras.	X	X	X	frequent	2120	frequent
	2. Medicago lupulina	T – H	Euras	4	X	X	common	600-700	frequent
	3. M. sativa	H	Eur.de est, Asia centr.	4	6	7	sporadically	600-700	naturalised
	4. Lotus corniculatus	H	Euras.	4	X	X	frequent	2 200	frequent
	5. Coronilla varia	H	Centr.eur. – submedit.	4	X	X	sporadically	1680	frequent
	6. Onobrychis viciifolia	H	Euras.	3	X	X	sporadically	600-700	frequent
	7. Vicia cracca	H	Euras.	4	X	X	sporadically	1600	frequent
	8. V. sepium	H	Euras.	5	X	7	sporadically	600-700	frequent
	9. Lathyrus pratensis	H	Euras.	6	5	6	frequent	1600	frequent
Equisetaceae	*1. Equisetum arvense*	G	Cosm.	6	6	X	common	-	frequent
Ranunculaceae	*1. Helleborus purpurascens*	H	Carp.-balc.-pan.	5	5	6	frequent	-	frequent
	2. Caltha palustris	H	Circ.	10	X	X	frequent	-	frequent
	3. Ranunculus acris	H	Euras.	6	X	X	frequent	1880	frequent
Urticaceae	*1. Urtica dioica*	H	Cosm.	6	X	X	common	2170	frequent
Caryophyllaceae	*1. Cerastium fontanum*	Ch-H	Euras.	5	X	X	frequent	600-700	frequent
	2. Lychnisflos-cuculi	H	Euras.	6	5	X	sporadically	600-700	common
Polygonaceae	*1. Polygonum bistorta*	G	Euras.	7	4	5	frequent	-	frequent
	2. Rumex acetosa	H	Cosm.	X	X	X	frequent	600-700	frequent
	3. R. alpinus	H	Alp.eur.	6	3	5	frequent	-	frequent
Rosaceae	*1. Fragaria vesca*	H	Euras.	5	X	X	common	600-700	frequent
	2. Geum rivale	H	Circ.bor.	8	X	X	frequent	-	frequent
	3. G. urbanum	H	Circ.	5	X	X	sporadically	600-700	frequent
	4. Filipendula ulmaria	H	Euras.	8	X	X	sporadically	600-700	frequent
	5. Alchemilla mollis	H	Carp.-balc.-cauc.-anat.	6	4	4	frequent	600-700	sporadically
Geraniaceae	*1. Geranium phaeum*	H	Centr. Eur.	5	X	6	frequent	600-700	frequent
	2. G. robertianum	T-Ht	Euras.	X	X	X	common	-	frequent
Euphorbiaceae	*1. Mercurialis perennis*	G (H)	Eur.	X	X	X	frequent	2000	frequent
Apiaceae (Umbelliferae)	*1. Chaerophyllum hirsutum*	H	Eur.centr.	8	4	5	frequent	1750	frequent
	2. Anthriscus nitida	Ht	Alp.-carp.-balc.	6	4	6	frequent	1780	frequent
	3. Aegopodium podagraria	H (G)	Euras.	6	X	X	frequent	1870	frequent
Brassicaceae (Cruciferae)	*1. Alliaria petiolata*	Ht-H	Euras.	5	6	7	sporadically	600-700	common
	2. Isatis tinctoria	Ht-H	Euras.cont.	3	X	8	sporadically	-	sporadically
	3. Cardamine amara	H	Euras.	9	5	6	sporadically	600-700	frequent
	4. Cardamine bulbifera	G	Cent.eur.	5	X	X	frequent	600-700	frequent
Primulaceae	*1. Primula elatior*	H	Eur.	6	4	5	frequent	2100	frequent
	2. Lysimachia nummularia	Ch	Am.de N.	6	6	X	frequent	600-700	frequent
Rubiaceae	*1. Galium mollugo*	H	Euras.	5	X	X	frequent	600-700	frequent
	2. Galium verum	H	Euras.	4	5	7	-	600-700	frequent

	3. Cruciata glabra	H	Euras.	5	6	6	common	2350	frequent
Boraginaceae	*1. Myosotis scorpioides*	H	Euras.	8	X	X	frequent	1100	frequent
	2. M. sylvatica	H	Eur.	6	4	4	frequent	-	frequent
	3. Symphytum cordatum	H	End.carp.	5	4	5	frequent	-	frequent
	4. Pulmonaria rubra	H	Carp.-balc.	6	4	5	frequent	2000	frequent
Lamiaceae	*1. Ajuga reptans*	H	Eur.	6	X	X	common	-	frequent
	2. Lamium maculatum	H (Ch)	Euras.	6	X	X	-	-	frequent
	3. Salvia nemorosa	H	Pont.-medit.-centr.eur.	4	X	X	-	1120	frequent
	4. Thymus pulegioides	Ch	Eur.	4	X	X	frequent	600-700	frequent
	5. Mentha longifolia	H	Euras.	8	X	X	frequent	600-700	frequent
Solanaceae	*1. Solanum dulcamara*	Ch	Euras.	9	X	X	frequent	600-700	frequent
Scrophulariaceae	*1. Scrophularia nodosa*	H	Euras.	6	6	6	frequent	600-700	frequent
	2. Veronica beccabunga	H	Euras.	10	X	X	frequent	-	frequent
	3. V. teucrium	H	Cont.euras.	3	6	X	frequent	600-700	frequent
Plantaginaceae	*1. Plantago lanceolata*	H	Euras.	X	X	X	sporadically	600-700	common
	2. P. media	H	Euras.	4	X	X	-	-	frequent
Campanulaceae	*1. Campanula abietina*	H	Carp.-balc.	6	4	4	frequent	2300	frequent
Asteraceae	*1. Bellis perennis*	H	Eur.	X	X	X	frequent	1950	common
	2. Telekia speciosa	H	Carp.-balc.-cauc.-anat.	7	5	6	frequent	-	frequent
	3. Achillea millefolium	H	Euras.	4	X	X	frequent	1600	frequent
	4. Leucanthemum vulgare	H	Euras.	4	X	X	frequent	2100	frequent
	5. Tussilago farfara	G	Euras.	6	X	X	common	1470	common
	6. Petasites hybridus	G	Carp.-sudet.-balc.	8	5	6	frequent	-	frequent
	7. P. kablikianus	G	Euras.	7	5	X	ordinary	1600	common
	8. Cirsium oleraceum	H	Euras.	7	5	6	sporadically	600-700	frequent
	9. Cirsium arvense	G	Euras.	X	X	X	-	600-700	common
	10. Centaurea phrygia subsp. pseudophrygia	H	Eur.centr.	5	4	5	frequent	600-700	frequent
	11. Taraxacum officinale	H	Euras.	5	X	X	common	2075	frequent
	12. Crepis biennis	Ht	Eur.	5	5	6	frequent	1520	common
Liliaceae	*1. Colchicum autumnale*	G	Centr. eur.	6	5	5	frequent	600-700	frequent
	2. Veratrum album	H	Euras.	6	4	3	frequent	600-700	frequent
	3. Polygonatum verticillatum	G	Euras.	5	4	4	frequent	1950	frequent
Orchidaceae	*1. Listeria ovata*	G	Euras.	6	5	6	rare	1000	frequent

Bioforms: H – Hemichryptophyta; G – Geophyta; T – Therophyta; Ht – Hemitherophyta; Ch – Chamaephyta.
Geoelements: Euras. – Eurasian; Eur. – European; Cosm. - Cosmopolite; Carp.balc.pan. – Carpatho-balcano-pannonic; E Eur., Centr. As – Est European, Central Asia; Alp. Eur. – Alpin European; Pont.medit. – centr.eur – ponto–mediteranean – central European; Circ. – cicumpolar; Centr. Eur. – Central European; Euras. Cont. – Continental Eurasiatic; Carp. balc.Cauc.anat. – Carpatho – balcanic – Caucaso - anatolic; Centr. Eur. Submedit. – Central European Submediterranian; Circ. bor. – Circumpolar boreal.
U – soil humidity: x – euryhydre; 3 – xero-mesophile; 4 – meso-xerophile; 5 – mesophile; 6 – meso-mesohygrophile; 7 – mesohygrophile;8 – hygromesophile; 9,10 – hygrofile.
T – air temperature: x – eurythermophil; 3– psihrothermophilic; 4 – microphilic; 5 – mesophilic; 6 – subthermophilic.
R – soil pH: x – euryionic; 3 – acidic soils; 4 – moderate acidic soils; 5 – moderate-weakly acidic soils; 6 – weakly acidic soils; 7 - neutral soils; 8 - calcarous soils

Most species - 75% are hemicriptophyte plants (Table 1, Figure 2) which emphasizes the character of the area as a meadow (Cristea et al., 2004).

Also in the group of perennial are included species of Geophyta - 14% and Chaemphyta - 3% (Table 1). Only 3 species out of a total of 85 are annual or annual hibernate plants (Table 1) (*Geranium robertianum*, *Anthriscus nitida*, *Crepis biennis*) which indicates a relatively high stability of phytocoenosis.

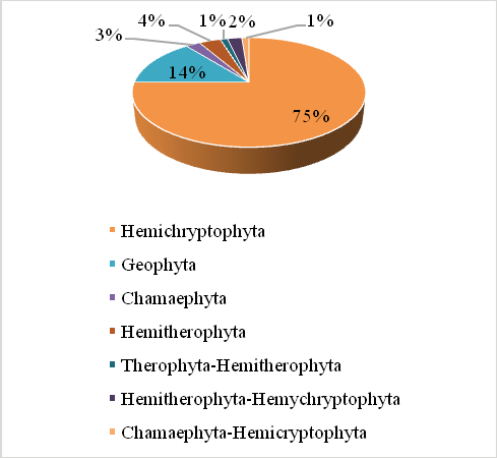

Figure 2. The bioforms spectrum

There is a reduced intervention of the anthropo-zoogenic factor, only two nitrophil species, *Urtica dioica* and *Rumex alpinus* were noted. Erosion areas are marked by the presence of *Tussilago farfara*.

From *Orchidaceae* family was found a couple of plants of *Listeria ovata* species on wet soils around the Peles brook ramification (Table 1, Figure 3).

Figure 3. *Listeria ovata*

51% of the total numbers of species (Table 1, Figure 4) are of Eurasian origin that is consistent the geoelements spectrum in our country.

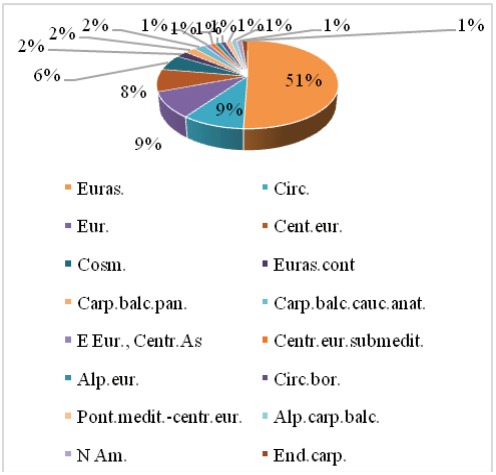

Figure 4. The Geoelements spectrum

Boreal circumpolar and circumpolar elements, representing 9% and 1% of all species, are mostly found around the watercourse, in damp and darklocations.

European, Central European and cosmopolitan elements are present in a proportion of 6%-9% (Figure 4).

Symphytum cordatum, a Carpathian endemism commonly found in the Bucegi Mountains in the median altitude mountainousareas, has been identified in areas at the base of the slope near the specimens of *Fagus sylvatica* 'Atropurpurea'.

In the field the distribution of species can be correlated with their preferred soil humidity (Figure 5).

Figure 5. The distribution in the field of species according to soil humidity

Out of the total number of the species are plants demanding high level of soil humidity, 5% are xero-mesophilic, 15% mezoxerofile, 24%

mezofile; the remainder 44% are plants growing in marshlandsand wetareas (Figure 6).

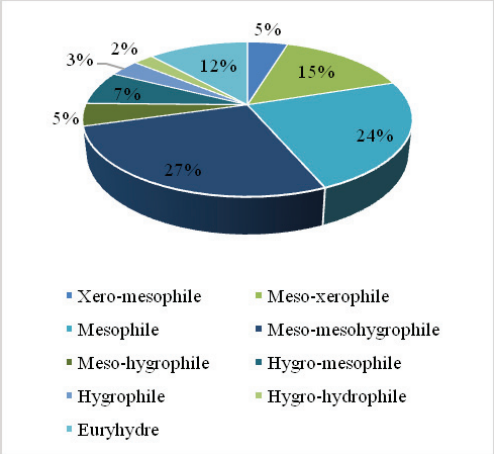

Figure 6. The soil humidity (U ecological factor) spectrum

58% of species manifest acceptance for a wide array of air temperature conditions (Figure 7) while 55% manifest it for soil pH conditions.

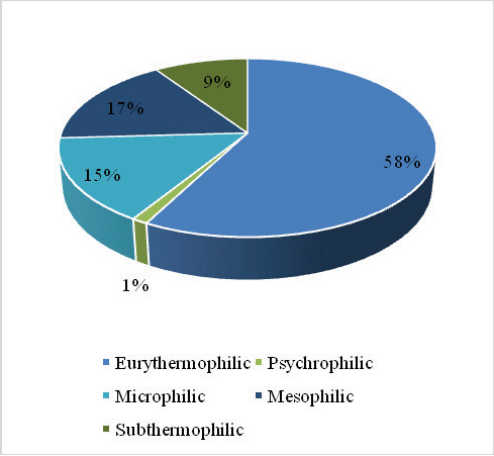

Figure 7. The air temperature (T ecological factor) spectrum

The species' overall indifference regarding surroundings factors allowed them to proliferated in all-over large areas containing multiple different micro-environments. Many of them are common species in the Bucegi Mountains and in our country (Table 1).

Although some species can be found at altitudes over 1900 m (Table 1), most of them most of them are specific to medianand lower mountainousaltitudes.

CONCLUSIONS

In Peleş Castle meadow it were identified species perteined to the following associations: *Petatisetum kablikiani*, *Scorzonero roseae - Festucetum nigricantis* and *Symphyto cordati-Fagietum*.

The majority of species presents requirements of high soil humidity while maintaining acceptance for a wide variety of air temperature and soil pH conditions.

The phytocoenoses encompass species often encountered in the median and lower altitude of the Bucegi Mountains.

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RESEARCH ON THE INFLUENCE OF THE AGE AND SIZE OF THE PLANTS ON GROWTH AND FLOWERING OF SOME CULTIVARS OF HERBACEOUS PEONY

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Abstract

The quality and the type of the planting material, the behavior in different conditions of culture, growth and blooming, as well as the appearance of the plant are important elements in choosing the variety and the cultivar or peony for plating in the landscape place. Also, the manner, appearance and duration of blooming, the earliness of the blooming, the aspect of the flower and the duration of the flowers on the plant or in vases are elements that render some qualities in the choice of peony varieties used in parks and gardens. In this paper, we present the results of the research on the influence of the age and size or the plants on the growth and blooming of some cultivars of herbaceous peony. The age of the plants had a major influence on the growth and blooming both in plants over 25 years and in young plants of over 2 years from planting. Likewise, the size of the plants had a different major influence on the growth and blooming both in older plants as well as in younger ones. The age and the size of the studied plants had a major influence also on the potential of reproduction of each studied cultivar. It analyzed the particularities of growth and blooming of the reproduction potential of each studied cultivar. The studied cultivars came from the collection of the University, as well as from the personal collection, having different ages such as 2 years, 4-5 years, 7 years, 8 years and over 25 years as well as various sizes of the ballot of the roots of herbaceous peony, i.e. 15-62 cm.

Key words*: age, breeding, flowering, growth, herbaceous peony.*

INTRODUCTION

This paper is topical and majorly important in the culture technology of the peony, providing new information, both to growers and to landscape artists in studying some important indicators regarding the choice of peony varieties and their cultivation. The indicators studied in this paper are important elements in the choice of the varieties of peonies and influence the growth, blooming and decorative appearance of the plant. The analyzed indicators are: the quality and type of the planting material, the behavior in various culture conditions, the growth particularities of growth and the particularities of blooming, the reproduction potential as well as the appearance of the plant. The herbaceous species of peony reproduce by the division of the bush, operation made by the mature plants, at the age of 4-5 years. After part of the soil is removed from the roots, the mother plants are cut by hand or with a very sharp knife in 3-6 parts, depending on the size of the mother plant (Toma, 2005).The division of the bush may be annual for flower-plants with intensive growth (*Aster, Chrysanthemum*), every 2 to 3 years (*Delphinium*) and every 5 to 6 years in slow growth plants or plants sensitive to separation (*Paeonia*) (Cantor, 2009).

Actual plants in the herbaceous peony are usually obtained by dividing the peony roots in 3-5 divisions, each division having 4-5 sprouts. The node stems and the underground rhizomes may be used for division (Wang & Zhang, 2005).

Cucu et al. (2009) mention that in the cultivars of the *Paeonia lactiflora* Pallas species it was observed the highest potential for reproduction. In plants older than 5 years, the maximum numbers of first quality divisions was 7 ('Candy Stripe' and 'Victoire de la Marne'). Plants aged 4, obtained a maximum number of 4 first quality divisions of the 5 of the cultivars subject to vegetative reproduction ('Albatre', 'Grover Cleaveland', 'Red Sarah Bernhardt', 'Top Brass', 'Wine Red').

As far as the flower production is concerned, Cucu et al. (2009), report that among the 5 years old plants (batch 1), 14 cultivars reached or exceeded 10 bloomed flower shoots/plant and there were over 50% bloomed flower shoots formed on a plant, during all the three years of the study. Among the 4 years old plants (batch 2), 19 cultivars reached or exceeded 10 bloomed flower shoots/plant and there were over 70% bloomed flower shoots formed on a plant, during all the three years of the study. The 33 cultivars were recommended for the production of cut flowers.

MATERIALS AND METHODS

The experiment developed in the research years 2016/2017 and 2017/2018 in two research locations: in USAMV Bucharest and in Singureni, Giurgiu County. The research took place in the field in the two locations studying the herbaceous peony cultivars in the University collection, as well as the cultivars existing locally. The presented results are obtained pursuant to the biometric measurements and the individual observations on the following indicators: quality and type of planting material, behavior of the plants in the culture, the particularities of growth and blooming and the decorative appearance of the plant.

The research is a two-factor experience consisting of two factors: factor A represented by the peony cultivars ('Festiva Maxima', 'Singureni pink', 'Singureni' with fringed scented flowers, Singureni pink, pink in the USAMV Bucharest collection, red in the USAMV Bucharest collection); factor B represented by the age of the plants (2 years, 5 years, 7 years, 8 years, and over 25 years); and the size of the plants (diameter of the root ballot). The combination of the two factors resulted in 13 experimental varieties (Table 1).

Figure 1. Unseparated bush stalks of 'Festiva Maxima' peony

Table1. Experimental varieties and quality of the materials used in the research

Variety	Cultivar	Plant age	Diameter of the root ballot min/max cm
V1	'Festiva Maxima' (white cultivar from Singureni)	over 25 years	54 - 62
V2	'Festiva Maxima' (white cultivar from Singureni)	7 years	35 - 47
V3	'Festiva Maxima' (white cultivar from Singureni)	2 years	15 - 22
V4	White flower peony from Singureni	over 25 years	57 - 61
V5	White flower peony from Singureni	7 years	40 - 48
V6	White flower peony from Singureni	2 years	17 - 25
V7	Pink cultivar with fringed perfumed flowers from Singureni	over 25 years	48 - 56
V8	Pink flower peony from the USAMV collection	8 years	36– 42
V9	Pink flower peony from the USAMV collection	5 years	28 - 33
V10	Pink flower peony from the USAMV collection	2 years	17 - 20
V11	Red flower peony from the USAMV collection	7 years	33 - 40
V12	Red flower peony from the USAMV collection	5 years	25 - 31
V13	Red flower peony from the USAMV collection	2 years	15 - 19

Figure 2. Pink peony from Singureni, undivided bush

Figure 3. Pink peony from Singureni, undivided bush

Figure 4. 'Festiva Maxima', undivided bushes

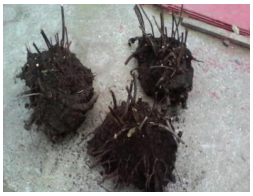

Figure 5. Pink peony from Singureni, undivided bushes

Figure 6. Pink peony from Singureni, undivided bushes

Figure 7. Red peony from USAMV Bucharest

RESULTS AND DISCUSSIONS

The results of the research are obtained pursuant to the analysis of the following indicators through visual observations and measurements of the ballot of roots, the potential of vegetative reproduction, the percentage of divisions, the particularities of growth and blooming, the percentage of flower shoots, the percentage of blooming, the dynamics of growth and blooming.

One can observe a minimum number of divisions of 7 and 8 and a maximum number of 9 and 10 divisions resulted from the cultivars ages over 25 years in the varietiesV1, V4 and V7. In the cultivars aged 7 it was noticed a minimum number of 4 and 5 divisions resulted and a maximum number of 6 and 7 divisions resulted in the varieties V2 and V5, and in the cultivars of 4-5 years a minimum number of 4 divisions and a maximum number of 5 and 6 in V9 and V11 (Table 2).

The diameter of the root ballot in the cultivars over 25 years has a minimum value of 5 cm and 8 cm, and a maximum value of 11 cm, 12 cm and 14 cm in varieties V1, V4 and V7. In the cultivars of 7 years it was observed a minimum diameter of 5 cm and maximum diameter of 8 cm. In the cultivars of 4-12 years a minimum diameter of 4 cm and a maximum diameter of 8 and 9 cm in the varieties V9 and V11 (Table 3).

Table 2. Number of divisions resulted on the plant of some herbaceous peony cultivars

Variety	Cultivar	Plant age	Number of resulted divisions	
			Min.	Max.
V1	'Festival Maxima' (white cultivar from Singureni)	over 25 years	7	9
V2	'Festival Maxima' (white cultivar from Singureni)	7 years	4	6
V4	White flower peony from Singureni	over 25 years	8	10
V5	White flower peony from Singureni	7 years	5	7
V7	Pink cultivar with fringed perfumed flowers from Singureni	over 25 years	7	10
V8	Pink flower peony from the USAMV collection	8 years	5	8
V9	Pink flower peony from the USAMV collection	12 years	4	5
V10	Red flower peony from the USAMV collection	8 years	4	8
V11	Red flower peony from the USAMV collection	12 years	4	6

Table 3. Diameter of the root ballot of the divisions in herbaceous peony cultivars

Variety	Cultivar	Plant age	Root ballot diameter (cm)	
			Min.	Max.
V1	'Festiva Maxima' (white cultivar from Singureni)	over 25 years	8	12
V2	'Festiva Maxima' (white cultivar from Singureni)	7 years	5	8
V4	White flower peony from Singureni	over 25 years	8	14
V5	White flower peony from Singureni	7 years	5	8
V7	Pink cultivar with fringed perfumed flowers from Singureni	over 25 years	5	11
V8	Pink flower peony from the UASVM collection UASVMB.	8 years	5	8
V9	Pink flower peony from the UASVM collection	12 years	4	8
V10	Red flower peony from the UASVM collection	8 years	5	9
V11	Red flower peony from the UASVM collection	12 years	4	9

Table 4 presents the particularities of growth of the peony plants depending on age and size. The following are confirmed: the older the plants, the later the phenophases compared to the younger plants. The length and the diameter of the shoot increase with age, as well as the number of shoots.

Table 4. Particularities of growth in some herbaceous peony cultivars, depending on the age of the plants

Variety	Sprouting	Shooting	Length of shoots (cm)	Diameter of shoots (mm)	Total shoots
V1	06.03	08.03	102	10	443
V2	27.02	01.03	89	9	276
V3	24.02	06.03	83	8	154
V4	05.03	07.03	107	11	667
V5	26.02	28.02	98	9	495
V6	24.02	05.03	93	8	352
V7	05.03	07.03	98	10	377
V8	03.03	05.05	86	6	197
V9	07.03	09.03	70	5	154
V10	04.03	10.03	65	4	98
V11	02..03	05.03	97	5	157
V10	09.03	11.03	66	5	114
V11	04.03	12.03	60	4	57

Figure 8. Pink peony bushes in the USAMV collection

Figure 9. 'Festiva Maxima' peony bushes

Figure 12. Red peony of the collection USAMV Bucharest, divided bushes

Figure 13. Ping peony, undivided bush, collection USAMV Bucharest

Figure 10. Undivided bush, ping peony from Singureni

Figure 11. Divided bushes of 'Festiva Maxima'

Figure 14. Red peony, undivided bush, collection USAMV Bucharest

Figure 15. Pink peony, divided bush, collection USAMV Bucharest

Figure 16. Red peony, collection USAMV Bucharest, divided bushes

Figure 17. Red peony, undivided bush, collection USAMV Bucharest

Figure 18. Pink peony, undivided bush, collection USAMV Bucharest

Figure 19. Divided bushes, pink peony, collection USAMV Bucharest

Figure 20. Pink peony, undivided bush, collection USAMV Bucharest

Figure 21. Red peony, undivided bush, collection USAMV Bucharest

Figure 22. Peony bushes, over 25 years, cultivar ‘Festiva Maxima’ and pink peony from Singureni

Figure 23. Peony bushes, 7 years, cultivar ‘Festivals Maxima’ and Singureni pink

Figure 24. Peony bushes, 7 years, cultivar ‘Festivals Maxima’ and Singureni pink

Figure 25. Bush of over 25 years, pink scented cultivar with double flowers

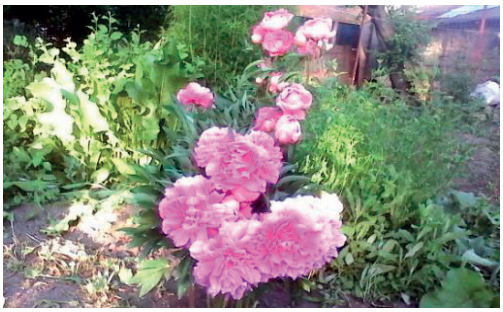

Figure 26. Appearance of the flower in pink perfumed cultivar, of over 25 years

Figure 27. Appearance of the blooming in bushes of over 25 years, pink and white cultivar

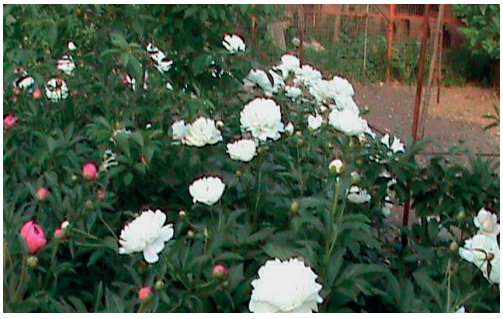

Figure 28. Appearance of the blooming, in 'Festivals Maxima' cultivar, aged 7

Figure 29.Appearance of the blooming in the pink cultivar from Singureni, aged 7

In Table 5 there is a drop in the percentage of flower shoots in plants of over 25 years as compared to the plants aged over 4 years and a growth of the plants of over 4 years as compared to the 2 years old plants.

Table 5. Number and percentage of shoots of varieties of herbaceous peony depending on the age of the age of the plants

Variety	Total shoots	Total flower shoots	Total bloomed flower shoots	Percentage of bloomed flower shoots
V1	443	224	102	45.53%
V2	276	270	268	99.25%
V3	154	135	85	64.96%
V4	667	336	177	52.67%
V5	495	492	489	99.39%
V6	352	292	221	75.68%
V7	377	175	112	64.00%
V8	275	268	266	99.25%
V9	154	148	145	97.97%
V10	98	42	12	28.57%
V11	221	210	202	96.19%
V12	114	102	96	94.11%
V13	57	36	3	8.33

As far as the percentage of plants with bloomed flower shoots, there is observed a decrease in the older plants and a maximum percentage in the plants aged 5 and 7 years, and the percentage of flowers decreases with the age of the plants, according to the data in Table 6.

Table 6. Percentage of plants with flower shoots and the percentage of blooming in varieties of herbaceous peony depending on the age of the plants

Variety	Percentage of plants with flower shoots	Percentage of plants with bloomed flower shoots	Blooming percentage
V1	100%	66%	45.53%
V2	100%	100%	99.25%
V3	100%	100%	66.05%
V4	100%	75%	52.67%
V5	100%	100%	99.39%
V6	100%	100%	97.59%
V7	100%	70%	64.00%
V8	100%	100%	99.39
V9	100%	100%	97.97%
V10	100%	15%	33.33%
V11	100%	100%	99.25%
V12	100%	100%	94.11%
V13	100%	10%	8.33%

Figure 30 presents the chart of the percentage of blooming flower shoots in the studied cultivars. The largest values being in the cultivars aged 5, 7 and 8 and the smallest in the cultivars aged 2.

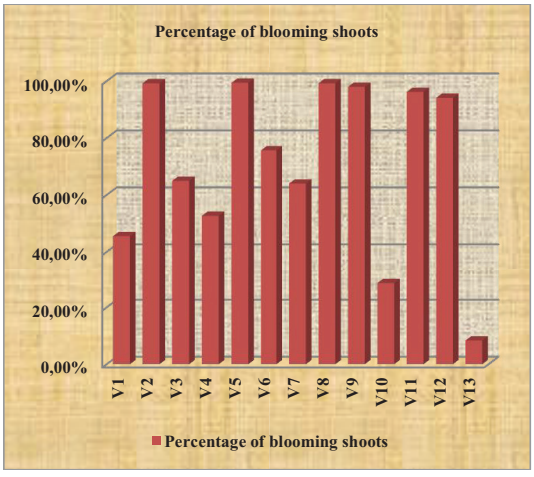

Figure 30. Percentage of blooming shoots in the peony cultivars studied depending on the research varieties

As far as the percentage of plants with flower shoots, it had the value of 100% in all the researched varieties according to the chart in the Figure 31.

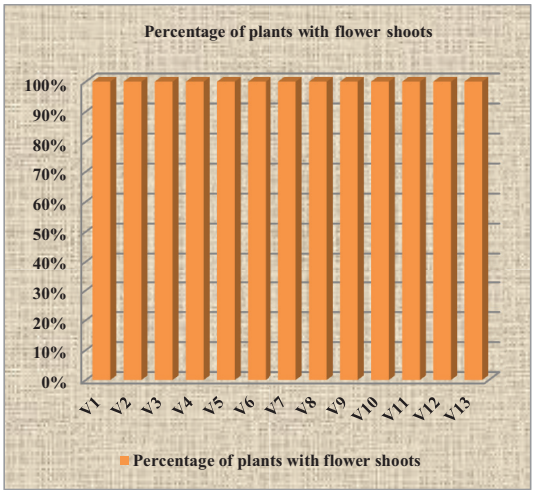

Figure 31. Percentage of plants with flower shoots in the studied cultivars

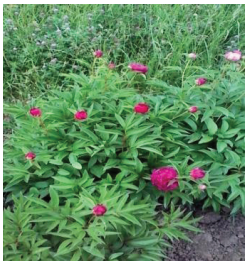

Figure 32. Appearance of the flower in the red cultivar, aged 12, collection USAMV Bucharest

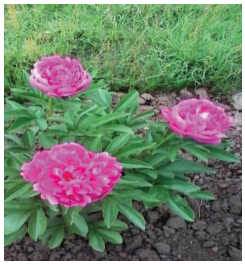

Figure 33. Appearance of the flowers in the pink cultivar, collection USAMV Bucharest, aged 2

In relation with the phenophases and the duration of the blooming there is an earlier occurrence of the bud and of the blooming in the young plants and a later occurrence in the plants over 25 years. As far as the duration of the blooming, there is a decrease of the duration in the older plants and an increase in the younger ones (Table 7).

Table 7. Phenophases and duration of the blooming in some peony cultivars, depending on the age of the plants

Variety	Budding	Blooming	End of blooming	Duration of blooming (days)
V1	15.04	17.05	22.05	5
V2	11.04	13.05	22.05	9
V3	08.04	11.05	18.05	7
V4	18.04	19.05	24.05	5
V5	14.04	15.05	23.05	8
V6	10.04	13.05	20.05	7
V7	14.04	18.05	24.05	6
V8	07.04	14.05	20.05	6
V9	09.04	17.05	24.05	7
V10	06.04	15.05	21.05	6
V11	05.04	14.05	20.05	6
V10	09.04	17. 05	23.05	6
V11	06.04	16.05	21.05	5

Figure 34. Appearance of the blooming in the pink cultivar, aged 12, collection USAMV Bucharest

Figure 35. Aspect of the blooming in the red cultivar, aged 2, collection USAMV Bucharest

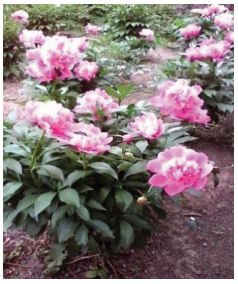

Figure 36. Aspect of the blooming in the pink cultivar, from Singureni, aged 2

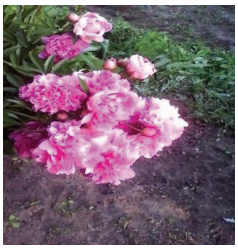

Figure 37. Appearance of the flower in the pink cultivar, from Singureni, aged 2

Figure 38. Aspect of the blooming in 'Festivals Maxima' cultivar, aged 2

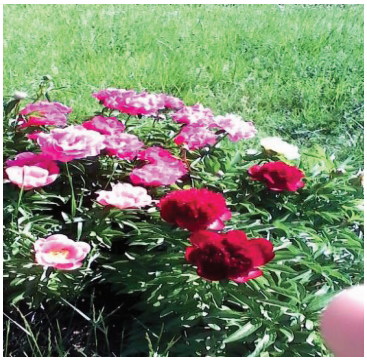

Figure 39. Appearance of the blooming in the red and pink cultivars, aged 8, collection USAMV Bucharest

Figure 40. End of the blooming in the pink cultivar, from Singureni, aged 7

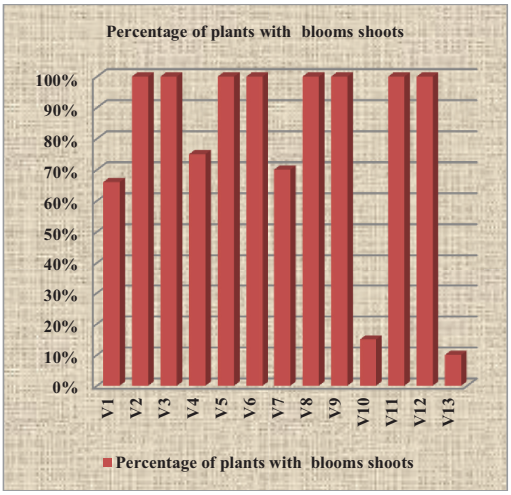

Figure 41. The percentage of plants with blooms shoots in the herbaceous peony cultivars studied

The percentage of plants with shoots bloom was over 50% to 11 variants of 13 and 66% at V1, 70% of V7, 75% in V4 and the percentage of 100% to V2, V3, V5, V6, V8, V9, V11, V12. Only two variants had a percentage below 50%, respectively the variants V10 and V13 (Figure 41).

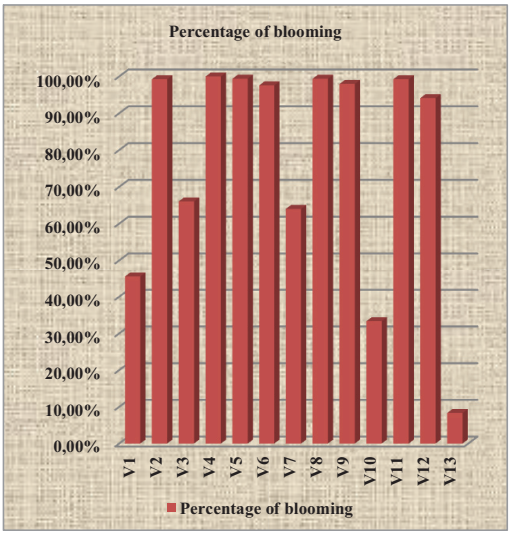

Fig.42. The percentage of blooming in the herbaceous peony cultivars studied.

The blossoming percentage shown in the chart in Figure 42, was over 50% for 10 variants of 13, starting from V4 with 52.67% to 99.39% for V5 and V8, and three variants had a percentage below 50% and 45.53% respectively at V1, 33.33% at V10 and 8.33% at V13.

CONCLUSIONS

The undertaken researches in this experience led to the following conclusions:

In the cultivars aged over 25 years there was a minimum number of 7 divisions and a maximum number of 10 divisions.

In the cultivars aged 7 years there was a minimum number of 4 and a maximum number of 7 divisions.

In the cultivars aged 4-12 years there was a minimum number of 4 and a maximum number of 7 divisions.

The growth phenophases have a delay as duration in the older plants as compared to the younger ones.

In older plants, there was a growth in height and the diameter of shoots, as well as their number.

The percentage of bloomed flower shoots decreases in older plants and it increases in younger plants, as well as the percentage of flower shoots.

The percentage of plants with flower shoots in all varieties is 100%.

The percentage of plants with bloomed flower shoots decreases in older plants and increases in plants younger than 3 years.

The blooming percentage decreases in older plants and increases in plants younger than 3 years.

The appearance of the bud and the beginning of the blooming is later in older plants and earlier in the younger plants.

The duration of the blooming is smaller in older plants as compared to the younger plants.

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RESEARCH ON THE PRODUCTION, BEHAVIOUR AND EVOLUTION IN TERMS OF GROWTH AND DEVELOPMENT OF SOME SUCCULENT PLANTS, IN ORIGINAL SUPPORT STRUCTURES

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Abstract

At worldwide scale, the tendency concerning the indoor decoration with plants is to use some original and functional structures to support them. The design and achievement of the support prototype may have not only an aesthetic role, but also of fixing and providing an optimal substrate, with subsequent implications in plants' growth and development. The chosen support together with different materials such as: moss, peat, tree bark, fragments of twigs or roots with special forms, sand, gravel or other materials arranged in harmony with plant material, amplify the decorative effect of the final product. Selection and association of plants in such structures are essential. These should not be based only on aesthetic principles and the size of individuals, but also on the requirements of plants for environmental factors, very close to the plants` growth rate and development, so they preserve decorative aspect as much as possible. The aim of this research was to conceive and realize the support prototype and then to investigate the behaviour and evolution of some succulent indoor plants regarding their growth and development after installation on support.

Key words*: succulent plants; concept; support; decorative.*

INTRODUCTION

Succulent species are highly appreciated plants by flower lovers (Stead, 2016; Toma, 2009). They are important floral assortment through their special decorative potential and very varied propagation, culture and use possibilities (Baldwin, 2017; Change, 2012; Maggio, 2016). Equally, succulent plants are the subject of numerous researches with a very broad subject. Enrichment of the assortment with new species and varieties having special decorative potential is frequently considered in studies (Pino, 2005; Thulin, 2002). Also, many studies and researches used prototype supports for a large number of succulent species in combinations that have decorative impact and in the same time are functional in many variants of interior spaces (Harvey, 2016; Starr, 2016). Other research is focused on the possibilities of propagation, including in vitro propagation methods (Cabahug et al., 2016). Researches on the behaviour of succulent plants in different types of climate (Zeevaart, 1982; Kelaidis, 2008) or the taxonomy of succulent plants (Gideon et al., 2017; Sandoval, 2017) are still modest.

The present research presents new and original supports prototype for succulent plants, which were then investigated indoors for their behaviour and development in time. Our work was based on many previous researches regarding the production and use of succulent plants in different crop varieties.

MATERIALS AND METHODS

I. Production of cuttings. Seventeen succulent plant species were selected to produce by cuttings the plant material necessary to cover different supports. The selected species were as follows: *Kalanchoe tomentosa* Baker, *Bryophyllum fedtschenkoi* Raym.-Hamet & H. Perrier, *Sedum nussbaumerianum* Bitter, *Sedum allantoides* Rose, *Sedum rubrotinctum* R.T. Clausen, *Sedum pallidum var. bithinicum* Gibson, *Sedum burrito* Walther, *Echeveria gibbiflora* DC, *Graptopetalum paraguayense* Walther, *Crassula perforata var. variegata* Thunb, *Aeonimum arboretum* Webb & Berth., *Aeonimum haworthii* Webb & Berth., *Cyanotis*

somalensis CB Clarke, *Senecio mandraliscae* Rowley, *Senecio kleiniiformis* Suess., *Messembryanthemum crystalinum* L., *Corpuscularia taylorii* Schwantes.

For all these species were made top shoot cuttings with length between 4 and 8 cm, depending on the species (Bala, 2012; Selaru, 2006). Once made, the cuttings were left to dehydrate in plastic crates for 6 days (Figure 1).

Figure 1. Cuttings left to dehydrate

Before planting in the rooting substrate, the height and the number of leaves of the cuttings of each species was noted (Table 1).

Table 1. The cuttings features

No crt.	Species	Height (cm)	Leaves no.	No. of cuttings
1.	*Kalanchoe tomentosa*	6	7	10
2.	*Kalanchoe fedtschenkoi*	8	8	10
3.	*Senecio mandraliscae*	6	8	10
4.	*Senecio kleiniiformis*	8	8	10
5.	*Echeveria gbbiflora*	4	5	5
6.	*Crassula perforata* var. *variegate*	5	10	5
7.	*Graptopetalum paraguayense*	5	11	5
8.	*Messembryanthemum crystalinum*	6	14	10
9.	*Corpuscularia taylorii*	7	6	10
10.	*Sedum nussbaumerianum*	5	14	15
11.	*Sedum allantoides*	5	25	10
12.	*Sedum rubrotinctum*	6	25	15
13.	*Sedum bithinicum*	4	15	10
14.	*Sedum burrito*	4	25	10
15.	*Cyanotis somalensis*	4	8	15
16.	*Aeonimum arboreum*	5	25	30
17.	*Aeonimum haworthii*	5	14	5
Total no. of cuttings		**185**		

Five substrate variants (Figure 2) were used to root the cuttings of all species, as follows: V1 - peat, V2 - sand, V3 - perlite, V 4 - peat (50%) + sand (50%), V5 - peat (50%) + perlite (50 %).

Figure 2. Substrates used for rooting the cuttings

Researches of various authors showed that the rooting time of the cuttings varies from a few days to a few weeks, depending on the species, the rooting substrate, climate or rooting stimulators applied (Cabahug et al., 2016; Cantor, 2016).

Eight weeks after planting, all the cuttings were rooted (Figure 3) and removed from the substrate.

Figure 3. Cuttings root 8 weeks after planting

The following observations and measurements were made: rooted cuttings height (cm), number of leaves, branches, branch length (cm), maximum root length (cm), number of main roots, length of main roots (cm), number of secondary roots, the length of the secondary roots (cm).

II. The growing of rooted cuttings in pots.

The eight-week-old rooted cuttings were planted individually in 5 cm pots (Figure 4). The substrate media used was consisted of celery soil, leaf soil, peat and sand in a ratio of 1: 1: 1/2: 1/3 plus chunks of 10% clay.

After planting, a set of observations and measurements were made: plant height, the number of leaves and shoots and the length of shoots (cm).

Figure 4. The aspect of plants after planting in pots

III. Designing and making structures with succulent plants. For several years, many publications and researches have been devoted to the creation and maintenance of different types of unconventional culture supports with succulent plants (Cammidge, 2019; Daigle, 2015). In this research we made and analyze three variants of such supports.

First support was represented by a **large decorative sphere (diameter 20 cm)** made up of two sections of rigid wire (with a diameter of 5 mm) and covered in wire mesh (Figure 5).

Figure 5. Forming the large sphere

The inner walls of the sphere were lined with vegetable moss and the core filled with peat. Peat was chosen because is a very light substrate, thus maintaining a low weight to the sphere. Then the two sections were fixed to each other with wire, clamped around the equator, resulting in the large sphere. After joining, a ring and a decorative chain needed for hanging the sphere was attached. The working time for this prototype was two and a half hours. A total of 187 cuttings of different succulent species were used to cover the sphere and the working time for planting was 6 hours (Table 2, Figure 6).

Figure 6. The planting on large sphere

Table 2. Species used for cover the large sphere

No. crt.	Species	No. of rooted cuttings
1.	*Kalanchoe tomentosa*	5
2.	*Kalanchoe fedtschenkoi*	15
3.	*Sedum nussbaumerianum*	10
4.	*Senecio mandraliscae*	12
5.	*Senecio kleiniiformis*	7
6.	*Echeveria gibbiflora*	2
7.	*Crassula perforate* var. *variegata*	13
8.	*Graptopetalum paraguayense*	3
9.	*Messembryanthemum sp.*	12
10.	*Corpuscularia taylorii*	10
11.	*Sedum allantoides*	26
12.	*Sedum rubrotinctum*	4
13.	*Sedum bithinicum*	4
14.	*Cyanotis somalensis*	13
15.	*Aeonimum arboretum*	40
16.	*Aeonimum haworthii*	7
Total rooted cuttings: 187		

The second support was a **small decorative sphere (diameter 10 cm).** The construction technique of the sphere was identical with that presented previously at the large sphere, the single difference being the size (10 cm diameter). In this case, before join together the sections these were covered first with succulent plants (Figure 7).

Figure 7. Forming and planting the small sphere

The working time for making this prototype was two hours. For the small sphere, we used 78 rooted cuttings belonging to 12 species, and the working time for planting was 4 hours (Table 3).

Table 3. Species used for the small sphere

No. crt.	Species	No. of rooted cuttings
1.	*Kalanchoe tomentosa*	3
2.	*Sedum nussbaumerianum*	6
3.	*Senecio mandraliscae*	10
4.	*Echeveria gibbiflora*	2
5.	*Crassula perforate* var.*variegata*	3
6.	*Graptopetalum paraguayense*	2
7.	*Corpuscularia taylorii*	5
8.	*Sedum alantoides*	7
9.	*Sedum rubrotinctum*	8
10.	*Cyanotis somalensis*	11
11.	*Aeonimum arboreum*	18
12.	*Aeonimum haworthii*	3
Total rooted cuttings: 78		

The third support was **a decorative photo frame with succulents.** For this support it was used a wooden photo frame of 20 x 15 cm behind which was attached another wooden framework of 5 cm height and a wooden plywood, necessary to support the substrate. Also, a wire mesh was fixed in the space for photography. Over this it was supplemented with muscle and then peat was used as substrate (Figure 8).

Figure 8. The appearance of decorative photo frame

Before attaching the back plywood, a plastic foil was placed over the peat to protect it from moisture generated by the water used for watering.

The working time for making this prototype was one and a half hours. It was necessary a 33 cuttings of 14 species of succulent plants to cover the frame. The working time necessary for planting the support was 1 and a half hours (Table 4).

Table 4. Species used in the decorative photo frame

No crt.	Species	No. of rooted cuttings
1.	*Kalanchoe tomentosa*	3
2.	*Kalanchoe fedtschenkoi*	1
3.	*Sedum nussbaumerianum*	3
4.	*Senecio mandraliscae*	5
5.	*Echeveria gibbiflora*	1
6.	*Crassula perforate* var.*variegata*	3
7.	*Graptopetalum paraguayense*	1
8.	*Sedum alantoides*	1
9.	*Sedum rubrotinctum*	2
10.	*Sedum bithinicum*	1
11.	*Cyanotis somalensis*	2
12.	*Aeonimum arboreum*	3
13.	*Sedum burrito*	5
14.	*Aeonimum haworthii*	2
Total rooted cuttings: 33		

The height of the plants, the number of leaves, the number and length of shoots, the diameter of the plants and the moment of flowering were

determined to establish the degree of growth of the plants.

RESULTS AND DISCUSSIONS

I. Production of cutings

The data shows that the percentage of rooting varied between 72.90% at variant V4, substrate peat (50%) + sand (50%) and 94.50% at variant V3, perlite.

The rooting quality varied with species substrate used (Table 5, Figures 9-10).

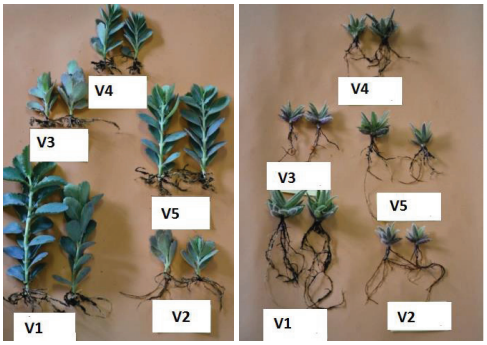

Figure 9. Differences between cuttings in terms of length and rooting at *Kalanchoe fedtschenkoi* (left side) and *Cyanotis somalensis* (right side)

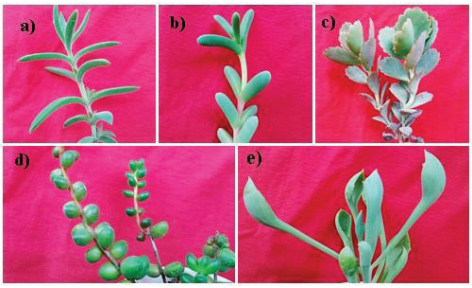

Figure 10. Species with indefinite growth:
a) *Messembryanthemum crystalinum;* b) *Corpuscularia taylorii*; c) *Kalanchoe fedtschenkoi*; d) *Sedum bithinicum*; e) *Senecio kleiniiformis*

From the 17 initial species, only the cuttings of *Aeonimum haworthii* did not rooted on any substrate tested.

II. The growing of rooted cuttings in pots.

The results obtained by the rooted cuttings of the 16 succulent plant species, after planting in pots, showed a different reaction in their growth (Table 6-7). At planting time, the average height of plants was 5.23 cm and after nine months this achieve a value of 17.98 cm (Figure 11).

Table 5. Cuttings roots characteristics on different substrate

Species	Var.	Length of roots (cm)	No. of primary roots	No. of secondary roots
Kalanchoe tomentosa	V1	7.00	9.0	12.0
	V2	3.75	13.5	11.5
	V3	3.20	21.0	27.5
	V4	2.75	10.0	-
	V5	2.50	9.5	-
Senecio kleiniiformis	V1	11.00	5.5	19.0
	V2	-	-	-
	V3	3.50	6.0	15.0
	V4	5.50	5.0	7.0
	V5	9.00	6.0	12.5
Kalanchoe fedtschenkoi	V1	11.25	10.0	8.0
	V2	10.75	7.5	35.0
	V3	6.75	7.0	27.5
	V4	7.75	9.5	17.5
	V5	9.00	10.5	10.5
Sedum nuss-baumerianum	V1	2.50	2.5	3.5
	V2	3.00	5.0	6.5
	V3	2.75	5.0	8.0
	V4	-	-	-
	V5	2.50	3.5	-
Cyanotis somalensis	V1	17.00	10.5	5.0
	V2	13.25	3.5	3.5
	V3	14.50	2.5	4.5
	V4	12.25	5.5	6.0
	V5	11.75	7	4.0
Senecio mandraliscae	V1	-	-	-
	V2	2.00	2.0	-
	V3	4.50	2.5	6.5
	V4	2.00	2.5	3.0
	V5	0.85	3.0	3.0
Sedum rubrotinctum	V1	4.25	1.0	3.0
	V2	5.25	8.5	14.5
	V3	3.25	5.5	9.0
	V4	3.00	3.5	10.0
	V5	4.75	7.0	13.0
Aeonimum arboreum	V1	2.75	2.5	2.0
	V2	4.00	6.5	7.5
	V3	2.50	5.0	2.5
	V4	3.25	5.5	3.0
	V5	3.25	5.0	5.0
Messem-bryanthemum crystalinum	V1	3.25	2.5	-
	V2	6.75	7.0	6.5
	V3	12.00	4.5	6.5
	V4	2.25	2.0	-
	V5	12.00	6.5	5.0
Grapto-petalum paraguayense	V1	4.50	7.0	5.0
	V2	3.50	7.0	-
	V3	2.00	8.0	-
	V4	2.20	5.0	-
	V5	3.50	6.0	-
Sedum bithinicum	V1	11.00	2.5	-
	V2	6.40	2.5	-
	V3	6.00	3.0	2.0
	V4	8.75	3.0	2.5
	V5	10.50	3.5	3.0
Sedum burrito	V1	4.50	0.5	-
	V2	4.25	1.0	-
	V3	4.75	1.0	-
	V4	3.75	1.0	-
	V5	3.50	2.0	-
Crassula perforata variegata	V1	6.00	3.0	-
	V2	4.50	4.0	-
	V3	5.50	3.0	-
	V4	4.00	5.0	-
	V5	5.00	2.0	-
Echeveria gibbiflora	V1	10.00	4.0	-
	V2	3.50	3.0	-
	V3	3.50	1.0	-
	V4	11.00	4.0	-
	V5	-	-	-
Corpuscu-laria taylorii	V1	11.50	5.0	-
	V2	11.00	5.0	-
	V3	9.50	5.0	-
	V4	-	-	-
	V5	23.50	6.5	-
Sedum allantoides	V1	2.50	3.0	-
	V2	7.25	8.5	-
	V3	8.25	4.5	-
	V4	-	-	-
	V5	-	-	

a

b

Figure 11. The aspect of plants at planting (a) and after nine months (b)

In nine months, five species - *Kalanchoe fedtschenkoi, Senecio kleiniiformis, Mesembryanthemum crystalinum, Corpuscularia taylorii* and *Sedum bithinicum* reached considerable heights of 20-45 cm (Figure 10).

Consequently, these succulent plant species, with indefinite growth, were considered inappropriate for use in such decorative systems (sphere and photo frame).

Table 6. Height of potted plants (cm)

Species	*h* at planting	*h* after nine months
Kalanchoe tomentosa	5.50	14.45
Kalanchoe fedtschenkoi	13.25	44.50
Sedum nussbaumerianum	6.60	12.75
Senecio mandraliscae	3.90	15.00
Senecio kleiniiformis	9.25	20.50
Echeveria gibbiflora	3.50	10.50
Crassula perforata var.*variegata*	2.75	13.50
Graptopetalum paraguayense	3.50	19.00
Messembryanthemum crystalinum	5.95	39.50
Corpuscularia taylorii	4.70	21.50
Sedum alantoides	3.25	13.50
Sedum rubrotinctum	7.50	18.50
Sedum bithinicum	5.25	21.50
Cyanotis somalensis	3.00	5.25
Aeonimum arboreum	4.07	11.25
Sedum burrito	1.75	10.25

After nine months of growing in pots, most of the succulent plants branched and considerably increased the number of leaves (Table 7). The most thickest and compacted plants were *Sedum alantoides*, *Sedum rubrotinctum* and *Cyanotis somalensis*.

Table 7. Plants measurements after nine months

Species	Leaves no.		No. of branch.		Length of branch (cm)	
	at planting	after 9 months	at planting	after 9 months	at planting	after 9 months
Kalanchoe tomentosa	8.5	34.5	-	4.0	-	4.1
Kalanchoe fedtschenkoi	16.5	36.5	-	2.5	-	10.0
Sedum nussbaumerianum	16.5	41.5	-	2.5	-	3.8
Senecio mandraliscae	5.5	29.0	-	0.5	-	4.0
Senecio kleiniiformis	13.5	41.0	-	3.5	-	6.3
Echeveria gibbiflora	5.0	20.5	-	0.5	-	2.0
Crassula perforata var.*variegata*	14.0	45.0	-	1.5	-	4.0
Graptopetalum paraguayense	9.5	44.0	-	1.0	-	9.2
Messembryanthemum crystalinum	14.5	98.0	3	55.0	1	23.5
Corpuscularia Taylorii	6.0	19.5	-	6.5	-	1.5
Sedum alantoides	15.5	70.5	-	6.5	-	6.5
Sedum rubrotinctum	21.5	88.0	4	13.5	2	6.6
Sedum bithinicum	18.5	93.0	3	5.5	1.8	4.9
Cyanotis somalensis	9.0	78.5	5	10.5	2	4.8
Aeonimum arboreum	15.0	98.0	1	3.0	1	4.5
Sedum burrito	19.5	73.5	-	1.5	-	1.1

Regarding the viability of plants after nine months after planting, it was establish that only 7% from the total plants were lost (Table 8).

Table 8. Plants viability in potted culture

Species	Initial no.	No. after nine months	% of viability
Kalanchoe tomentosa	10	10	100
Kalanchoe fedtschenkoi	10	10	100
Sedum nussbaumerianum	12	12	100
Senecio mandraliscae	7	5	71
Senecio kleiniiformis	7	7	100
Echeveria gibbiflora	4	4	100
Crassula perforata var.*variegata*	5	5	100
Graptopetalum paraguayense	5	5	100
Messembryanthemum crystalinum	10	10	100
Corpuscularia taylorii	8	3	37
Sedum alantoides	6	2	33
Sedum rubrotinctum	15	15	100
Sedum bithinicum	10	10	100
Cyanotis somalensis	14	14	100
Aeonimum arboreum	26	26	100
Sedum burrito	8	8	100

In several months after planting, five of sixteen species flowered (Figure 12).

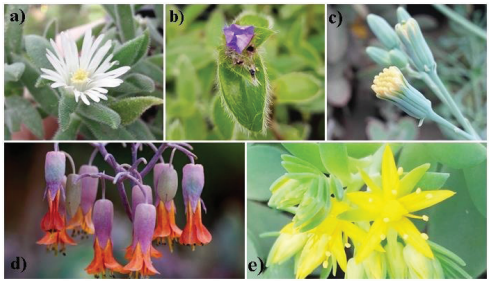

Figure 12. Flower details at: a) *Messembryanthemum crystalinum;* b) *Cyanotis somalensis;* c) *Senecio kleiniiformis;* d) *Kalanchoe fedtschenkoi;* e) *Aeonimum arboreum*

The length of the flower stems varied between 3.5cm at *Aeonimum arboreum* and 45cm at *Senecio kleiniiformis* (Table 9).

Table 9. The variation in the quality of the floral elements and the flowering time

Species	Height of stem flower (cm)	No. of flower stems	No. of flowers/stem	Color of flowers	Time of flowering
Senecio kleiniiformis	45	1	10	yellow	XI
Messembryanthemum crystalinum	-	-	1	white	X
Kalanchoe fedtschenkoi	10	1	10	orange	XII
Aeonimum arboreum	3.5	5.5	11	yellow	XII
Cyanotis somalensis	-	-	3.5	violet	I

Two of the species, *Mesembryanthemum crystalinum* and *Cyanotis somalensis*, bloom directly on shoots, consequently they do not have flower stems. The number of stems per plant ranged from 1 to 5-6 flower stems/plant. The number of flowers on a stem varies from one single flower and reaches up to 11 flowers. The colour of the flowers was: white, yellow, orange and purple. For most of the species, flowers do not smell, except *Senecio kleiniiformis*, whose odour is strong and unpleasant. The flowering period was between October and January.

III. Designing and making structures with succulent plants. Results of studies on the **large decorative sphere (diameter 20 cm)** showed that after planting, the viability of the cuttings of the 16 species had a percent of 88.7%. Losses of cuttings were noted at *Senecio kleiniiformis, Mesembryanthemum crystalinum* and *Corpuscularia taylorii,* the last one, with the most severe losing (100%).

In this system, the association of plants proved to be approximately successful, since only two of the species - *Kalanchoe tomentosa* and *Crassula perforata variegate*, were partially covered by the rest of the species (Figures 13-14).

Figure 13. Initial form of the large decorative sphere

Figure 14. The large decorative sphere after one year

Leaf decay and loss was also observed in the case of the species*: Aeonimum arboretum,*

Sedum rubrotinctum, Graptopetalum paraguayense, Sedum alantoides and *Echeveria gibbiflora*.

In December, some of the species, such as *Kalanchoe fedtschenkoi, Aeonimum arboreum* and *Mesembryanthemum crystalinum* have blossomed.

Data results in the case of the **small decorative sphere (diameter 10 cm)**, the cuttings survived better than previously structure. For this sphere of smaller size covered with 12 species, the viability of cuttings had a percent of 93.5% (Figures 15-16).

Figure 15. Initial form of the small decorative sphere

Figure 16. The small decorative sphere after one year

Only the cuttings of *Corpuscularia taylorii* were entirely lost, therefore we considered that the association of plants in this system was successful. Plants developed harmoniously, without invading each other. Still, similar with the plants of large sphere, the plants of smaller sphere presented leaf decay and loss at *Aeonimum arboretum, Sedum rubrotinctum, Graptopetalum paraguayense* and *Sedum allantoides* (Figure 17).

Figure 17. Leaf loss at sphere system: a) *Aeonimum arboretum;* b) *Sedum alantoides and Sedum rubrotinctum;* c) *Graptopetalum paraguayense;* d) *Echeveria gibbiflora*

The only species that developed flowers in this system was *Aeonimum arboretum*, in December.

The observations made in the case of **the decorative photo frame with succulents**, indicated an excellent survival of the cuttings. All the 14 species cuttings rooted and developed without any lost (Figures 18-19). Consequently, the association of plants in this system was considered successful. During one year, plants developed harmoniously, without invading each other.

Figure 18. Initial form of the decorative photo frame

Figure 19. The decorative photo frame after one year

The species that developed flowers were *Aeonimum arboreum* and *Kalanchoe fedtschenkoi*. The leaf decomposition was noted at five species: *Sedum alantoides, Sedum rubrotinctum, Sedum bithinicum*, *Graptopetalum paraguayense* and *Kalanchoe fedtschenkoi* (Figure 20).

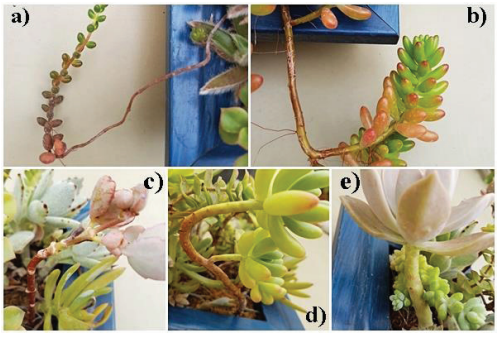

Figure 20. Leaf loss at the photo frame system: a) *Sedum bithinicum*; b) *Sedum rubrotinctum*; c) *Kalanchoe fedtschenkoi*; d) *Sedum allantoides*; e) *Graptopetalum paraguayense*

CONCLUSIONS

I. Production of cutings. Data of this experiment showed slightly variation between species and substrate used for rooting. The best rooting results obtained for the cuttings of 16 species were on two substrates: pearlite (94.5%) and peat+perlite (89%). The substrates with smaller rooting percent were: sand - 86%, peat 81% and peat+sand - 72.9%. One of the species (*Aeonimum haworthii*) failed to root on all five tested substrates.

II. The growing of rooted cuttings in pots. Regarding the evolution of the selected species, it was found that not all the 16 species selected originally are suitable for planting in structures. This occurs because *Kalanchoe fedtschenkoi, Senecio kleiniiformis, Mesembryanthemum crystalinum, Corpuscularia taylorii and Sedum bithinicum* had a faster growth rate, reaching in a relatively short period of time 20 cm height or more. Therefore, these species exceed, invading and compete the other plants and finally changing the shape and the harmony of plant association of the structures.
A percent of 31.25% from the tested species initiated and developed flowers.
After nine months of planting, the cuttings viability was of 92.9%.

III. Designing and making structures with succulent plants. The design and construction of sphere-type structures is recommended to be done in two sections, which can be easily fill and assembled. Also, such systems must be made of durable materials, wire and wire mesh, in order to maintain the spherical shape. Regarding the realization of the structure of the photo frame, it was noted that the wood used as material for sustain the substrate and plants, did not deteriorate over time. Anyway, it is recommended to protect structure on the outside at the time of spraying water.
The percentage of viability of the non-rooted cuttings planted in the three decorative structures was different: 88.7% at the large decorative sphere, 93.5% at the small decorative sphere and 100% at decorative photo frame. The species *Corpuscularia taylorii* recorded a plant loss of 100% for both types of spheres. Another two species, *Senecio kleiniiformis* and *Mesembryanthemum crystalinum* were lost in the case of large sphere.
Regarding the evolution of the species used in sphere-type systems, it was noted that five species, *Mesembryanthemum crystalinum, Sedum bithinicum, Sedum rubrotinctum, Sedum alantoides* and *Graptopetalum paraguayense*, require a guidance of shoots among the wire at

the moment where they exceed the spherical contour of the structure.

The development of the species in the decorative photo frame was remarked to be much faster at *Sedum alantoides, Sedum rubrotinctum* and *Sedum bithinicum.* So, we recommend to plant these species at the base of the structure, so that the subsequent growths did not cover and compete the other species.

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MISCELLANEOUS


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ASPECTS REGARDING THE IMPLEMENTATION OF HACCP (HAZARD ANALYSIS AND CRITICAL CONTROL POINTS) SYSTEM ON THE "FOURTH GAME" HORTICULTURAL PRODUCTS

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Abstract

HACCP is the abbreviation for the English expression "Hazard Analysis Critical Control Points". To obtain high - quality products, capable of meeting the consumer's demands and complying with the food safety standards, certain risk-prevention and control methods should be applied. In the horticultural products processing company, the application of an HACCP system allows the identification of the key-elements of the technological process. The system analyses the hazard related to the product and the process, indicating the critical control points to the hygienic quality of the product. Starting with the fact that some of these products are ready to eat (e.g. the „fourth game" horticultural products), there are major concerns regarding the level of pesticides and other chemical contaminants, the maintenance of hygiene during harvesting, handling, processing, storage, and commercialisation. In order to prevent or reduce the above-mentioned hazards, the big specialised companies and small producers must apply HACCP prevention methods, not methods based on the final product control (which may affect consumer's health and may lead to important economic loss). Therefore, this paper contains a HACCP study, characteristic for "ready to eat" vegetable products and explains the management of the identified CCPs.

Key words*: CCPs, HACCP, food safety.*

INTRODUCTION

The sanitary status of food products is a foremost quality attribute, even though it is among the less discernible traits for the consumer (Arvanitoyannis I.S., 2009).

Fresh horticultural products are the main source for diet fiber and vitamins, but can convey chemical, physical, and microbial hazards and contaminants or some synthetic pollutants (residues of fertilizers, herbicides and pesticides). Food safety management systems like ISO 22000:2005, IFS and BRC (all containing the Hazard Analysis and Critical Control Points-HACCP) can secure food safety by preventing potential hazard at source points of the process (Varzakas T. et al., 2008).

In order to guarantee the food safety products, it would be desirable to structure the production according to HACCP principles. In the minimal processing ("ready to eat") of horticultural products, the critical control points can be detected mainly for microorganisms (Chira A. et al., 2007).

MATERIALS AND METHODS

The studies were carried out on the processing of ready to eat vegetables salad (cabbage and carrots), according to the flow diagram described in Figure 1.

For each step of the process the risk analysis was performed, in order to identify the chemical, physical and biological hazards correlated to the product and process and also the preventive actions and control measures which are necessary to keep these hazards under control (Chira A. et al., 2000).

In order to implement specific control measures regarding food safety wherever it is possible, the CCP decision tree was applied.

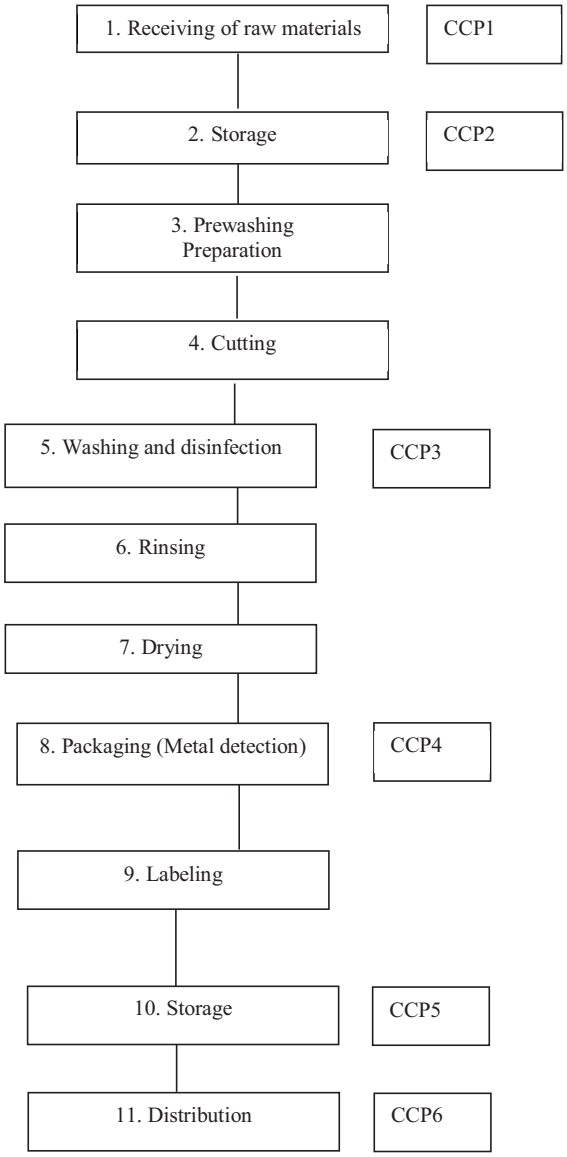

Figure 1. Flow diagram for the processing of the "fourth game" horticultural products

The control of each CCP, according HACCP principles are planned in the HACCP plan and the implementation of the control measures is shown by specific records.

RESULTS AND DISCUSSIONS

Minimally processed horticultural products are in danger of increasing the metabolic activity which causes their degradation. The natural degradation of freshly cut products determined by processing increases respiration and ethylene production very quickly and the accumulation of phenol substances.

Following these processes, the biochemical reactions are responsible for colour change, taste and nutritional composition (loss of vitamins).

All these aspects could be minimised by cooling the products before the production process. Also the temperature control following production process is very important in order to decrease the negative effects in metabolic activities. Other preventive measures involved in the reduction of the minimal processed horticultural products degradation are: the correct use of sharp knives and their cutting quality, the strict hygienic rules and effective washing and drying (removal of water excess quantity from the products surface).

This kind of products maintains a big part of the microflora after processing. A part of the microflora is represented by pathogenic microorganisms like *Listeria monocytogenes, Escherichia coli enterohemoragic, Clostridium botulinum* and *Vibrio cholerae*.

One measure involved in keeping under control the growth of microorganisms regard the packaging of "ready to eat" horticultural products, under modified atmosphere. So, the level of O_2, reduced from 21% to 2-6% and CO_2 level increased from 0.03% to 3-8%; correlated with cooling and respecting hygiene results the respiration rate and microbial growth are reduced and the shelf life of the products is extended.

Starting from these aspects, in Table 1 the process step and related potential hazards are presented, including the preventive actions and the control measures, from which one of the most important is the temperature control.

Table 1. Hazard analysis regarding the processing of "fourth game" horticultural products

Process step	Hazard	Preventive actions/ Control measures
1. Receiving of raw materials	B- Pathogenic micro organisms C- Pesticides residues - Heavy metals - Fertilizers Ph - Foreign matter	Visual control Supplier assessment *Temperature control of the product*
2. Storage	B - Idem	Sanitation programme Regular maintenance FIFO system *Temperature control during storage*
3. Prewashing Preparation	B- idem Ph - Metal piece from cutting machine	Cleaning programme Right equipment Personal hygiene rules
4. Cutting	B - idem Ph - idem	Cleaning programme Right equipment Personal hygiene rules
5. Washing and disinfection	B - idem C - Disinfectant in excess Ph - Foreign bodies	Cleaning programme for equipment Control of the mechanism for removal of foreign matter; Personal training; *Disinfection to reduce microbial load*
6. Rinsing	B - idem Ph - Foreign bodies	Water specifications Control of the mechanism for removal of foreign matter Cleaning programme for equipment
7.Drying by centrifugation	B - idem	Regular maintenance of equipment Visual control Personnel training

8. Packaging (Metal detection)	B - idem Ph - Metallic foreign bodies C - Unacceptable substances from packaging materials	Cleaning programme for equipment Quality certificates of packaging materials; Personal hygiene rules Equipment maintenance *Removal of metallic objects by metal detector*
9. Labelling	B - idem	Visual control Personnel training
10. Storage	B - idem	Cleaning programme *Temperature control of the storage*
11. Distribution	B - idem	Right transportation vehicles Cleaning programme; Personnel training *Temperature control of transportation vehicle*

By using the CCP decision tree, in Table 2 the six CCPs identified are presented, out of which, five are focused to keep the microorganisms under control and one for the metallic foreign bodies, although during the packaging process the cooling temperature has to be respected.

Table 2. CCP determination during processing of "fourth game" horticultural products

Process step	Hazard	Decision tree questions				CCP Nr.
		Q1	Q2	Q3	Q4	
1.Receiving of raw materials	B - Pathogenic organism	Yes	No	Yes	No	CCP1
2. Storage	B - idem	Yes	No	Yes	No	CCP2
3.Washing and disinfection	B - idem	Yes	Yes	-	-	CCP3
4. Packaging (Metal detection)	Ph - Metallic foreign body	Yes	Yes	-	-	CCP4
5. Storage	B - idem	Yes	No	Yes	No	CCP5
6. Distribution	B - idem	Yes	No	Yes	No	CCP6

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

As we know, the monitoring of finished food product is no guarantee of safety because unsafe samples may be not analysed. Because of that, the HACCP system is a structured approach to hazard identification, associated with the processing of "ready to eat" horticultural products.

Table 3. HACCP plan regarding the processing of "fourth game" horticultural products

Process Step	CCP Nr.	Critical limits	Monitoring process				Corrective action	Verification
			Responsable	Method	Frequency	Records		
Receiving of raw materials	CCP 1	Temperature products lower than 6°C	Warehouse supervisor	Products temperature control	Each receiving	Receiving sheet	Immediate cooling Return the nonconforming product to the supplier	Laboratory analysis of raw materials Vehicle recording paper
Storage	CCP 2	T <6°C UR = 95-100%	Warehouse supervisor	Visual control of temperature and relative humidity	Every 3 hours	Storage sheet	Adjustment of faults Hold nonconforming lots	Laboratory analysis of raw materials
Washing and disinfection	CCP 3	Water temperature 1-6°C Disinfectant conc. in water 90-100 ppm	Production supervisor	Temperature control and disinfectant conc. of washing water	Every product change and during washing	Liner washer sheet	Prewashing check and disinfection of nonconforming lots Adjustment of water temperature	Laboratory analysis of water and final products
Packaging (Metal detection)	CCP 4	Absence of metal objects >1 mm	Production supervisor	Operation control with metal tester with diameter <1mm	Every 3 hours	Packaging control sheet	Adjustment of metal detector Check suspicious products using another detector	Daily verification
Storage	CCP 5	Storage temperature lower than 6°C	Storage supervisor	Visual control of storage temperature	Every 3 hours	Storage sheet	Adjustment of faults Hold nonconforming lots	Laboratory analysis of final products – monthly
Distribution	CCP 6	Temperature during distribution lower than 8°C	Vehicle driver Quality supervisor	Control of temperature	At each loading and every hour during distribution	Loading control sheet Temperature recording during transport	Idem	Idem

CONCLUSIONS

The use of the adequate working techniques and hygiene programmes according to the HACCP principles assures the food safety of minimal processing ("ready to eat") horticultural products.

Products that are safe for consumption can be obtained only by keeping the identified CCPs under control.

The temperature control of the process and the product are the main issues in order to assure the food safety of minimally processed ("ready to eat") horticultural products.

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OBSERVATIONS RELATED THE LONG-EARED OWL (*ASIO OTUS* L.) FEEDING IN TOW HORTICULTURAL ECOSYSTEMS

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Abstract

Long-eared Owls (Asio otus L.) are very important in the ecosystem because they control rodent populations that especially in anthropogenic ecosystems are very often harmful. In this context, the study of the ecology and ethology of this night raptor species in anthropogenic habitats is an important task. The present paper includes observations made in winter agglomerations (roost) of Long-eared Owl in two horticultural ecosystems: a periurban private garden in Ilfov County and an urban green space, between apartment buildings in Bucharest. Birds pellets were analysed in order to determine the pray composition. The Long-eared Owls feeding were significantly influenced by the horticultural ecosystem. In the pellets collected from the green space in Bucharest, 94% were rodents, the Brown Rat (Rattus norvegicus) being predominant, by 59%, then the House Mouse (Mus musculus) by 32%, both species being considered as pests. In the pellets collected from Ilfov County, the House Mouse (Mus musculus) has the largest share, by 58%, followed by the Brown Rat by 30%. Useful information regarding the favourite prey of this night predator.

Key words*: periurban ecosystems, urban ecosystems, rodents, roost, night raptors.*

INTRODUCTION

The Romanian Biodiversity Directorate draws attention to the fact that populations of Long-eared Owl (*Asio otus* L.) in the country are threatened by habitat alteration and loss, use of long-standing pesticides, poaching, loss and deterioration of breeding and roosting birds that also shelter specimens from other parts of Europe (Petrovici, 2015).

According to Theodor Mebs and Wolfgang Scherzinger, from 19237 of preys found in pellets, were identified rodents - 95%, birds - almost 5% and a small number of insectivorous mammals (Mebs & Scherzinger, 2006).

Luminița Laiu observed that Long-eared Owls from Amara, Ialomita county, Romania, on over one year were fed with the following mammals: *Crocidura suaveolens* P. 2.14%; *Microtus arvalis* P. 41.03%; *Apodemus sylvaticus* L. 4,27%; *Apodemus agrarius* P. 0.43%; *Mus musculus* L. 50.85%; *Fringilla coelebs* L. 0.43%; *Passer domesticus* L. 0.85%. It also stands up that in Bucharest, Long-eared Owl consume also *Rattus rattus* L. and *Rattus norvegicus* B. (Laiu, 2010).

As shown, Long-eared Owl controls small rodents populations (Pirovano et al., 2000) Hadjisterkotis (2003), Balčiauskienė et al. (2006), Dupal and Chernyshov (2013), most of them producing significant damages to horticultural, agricultural or even households crops. It is necessary to know the behavior of this night predator, but also the threats from different ecosystems, especially those anthropic.

METHODS AND MATERIALS

For this study it was used the qualitative method (Papadopol & Petrescu, 2006; Laiu, 2010) to prey identification from pellets. It was collected 317 samples from two horticultural ecosystems.

The first horticultural ecosystem in the study was the roosting birds on Făurei Street in Bucharest. Practically the Long-eared Owls gathered in the trees in the green space between the blocks (Figure 1). The colony consisted of 17 birds of *Asio otus* L. According to the inhabitants, the birds gather in the area for at least 5 winters. Most of the specimens are sheltered in Lime Trees *(Tilia platyphyllos* S.) and some of them used as shelted Thuja *(Thuja orientalis* L.), Black Poplar *(Populus nigra* L.) and Spruce *(Picea abies* L.).

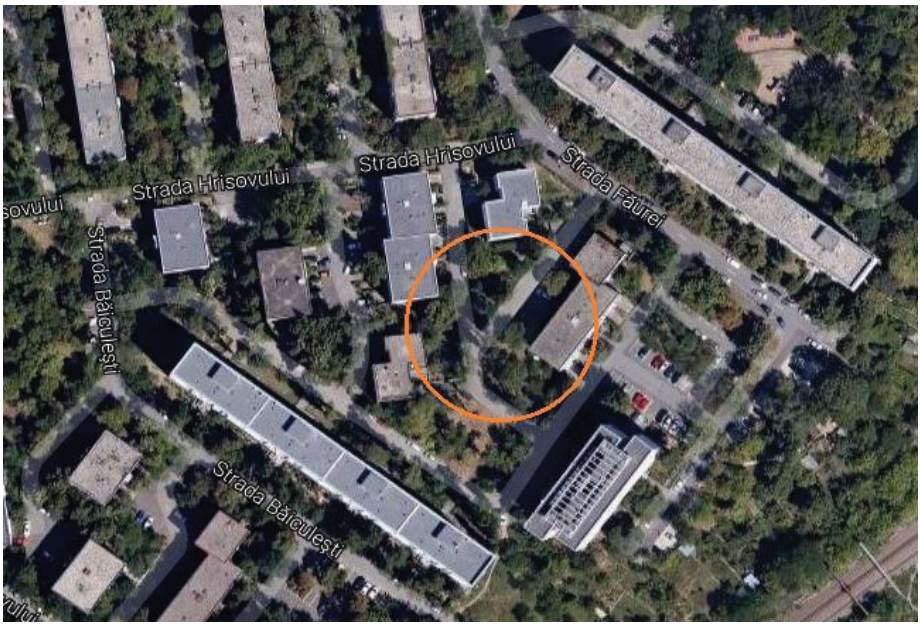

Figure 1. Localization of the Long-eared Owls roosting sites from Făurei Street in Bucharest (Google Maps)

More than 14 specimens of the colony are resting in lime trees, most likely because they are camouflaged with linden limestone inflorescences. From here, we took 150 pellets on January 19, 2016.

The second roosting of Long-eared Owls is in Moara Domnească village, Ilfov County, inside the Didactic Farm Moara Domnească (Figure 2). The farm has over 500 ha, having agricultural land, lake, orchard, arboretum nursery, farm domestic animals and green spaces.

A teacher living there for 29 years mentioned that in every winter between 3 and 15 long-eared owls gather.

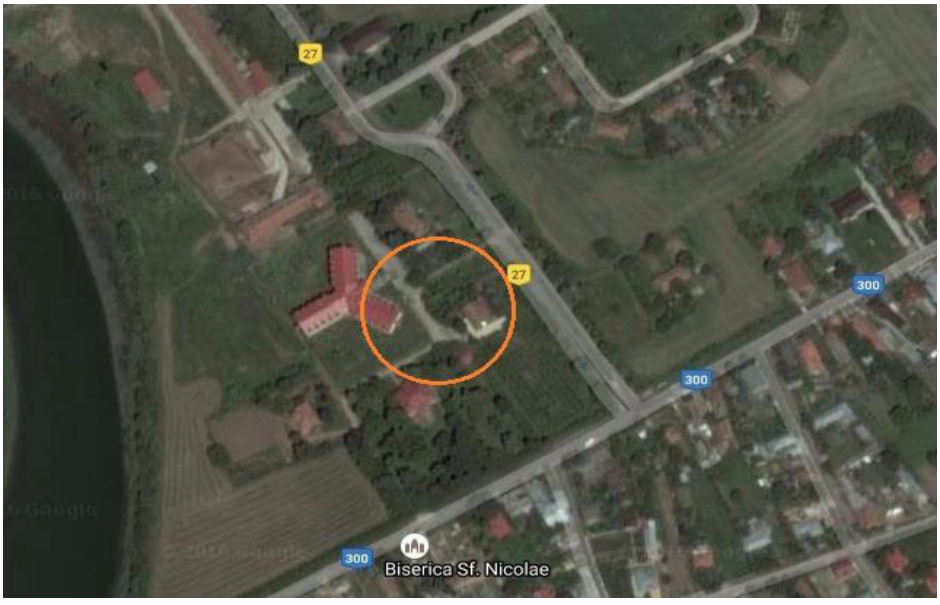

Figure 2. Localization of the Long-eared Owls roosting sites in Moara Domnească, Ilfov county (Google maps)

There were found 15 specimens of Long-eared Owl *(Asio otus* L.) in two plants 8-9 meters of Thuja *(Thuja orientalis* L.). We noticed that all the specimens were sheltering into the Thuja *(Thuja orientalis* L.), being the same place where they were hiding in the day time Magpies (*Pica pica* L.), which were aggressing them every time they were flying during the day. On February 10, 2016, they we picked up 167 pallets from this roosting birds.

Species and preys were identified based on the determination keys from the following: Barbu and Popescu (1999), Macdonald and Barrett, (2007), Humphries et al. (2007), Mullarney et al. (2009).

That materials used for the pellets determination were: metallic ruler, electronic caliper Dexter, magnifying glass, tweezers, gloves and ethanol, and the identification of pray from pellets was based on skulls, mandibles and jaws (Figure 3).

Figure 3. Ruler measurement for prey identification

RESULTS AND DISCUSSIONS

From the first location, on Făurei Street (Figure 1), from Bucharest, we collected 150 pellets and we were able to identify the prays in 64 cases; the remaining 86 of them did not contain identifiable elements.

In the 64 cases we identified 100 prays (Figure 4) as follows:

- Rodents: 3% Black Rat (*Rattus rattus* L.), 59% Grey Rat (*Rattus norvegicus* B.), 32% Domestic Mouse (*Mus musculus* L.). A total of 94% rodents.
- Insectivorous mammals: 4% Bicolored Shrew (*Crocidura leucodon* H.). In total 4% insectivorous mammals.
- Granivorous birds: 2% European Greenfinch (*Chloris chloris* L.). In total 2% of granivorous birds.

There have been pellets with two or three skulls or mandible, giving a large number of prays identified in the 64 pellets.

We noticed that the birds were placed in a quiet area, behind the blocks, near the garbage can (Figure 5), a source of food for the Black Rat (*Rattus rattus* L.), the Grey Rat (*Rattus norvegicus* B.) and Domestic Mouse (*Mus musculus* L.).

Unfortunately, at only 300 meters, on the main road, exaggerated maintenance prunning were made on trees in the green space.

These exaggerated shortening of branches, carried out during the roosting period of long-eared owls, are extremely dangerous because they can scare the birds that are nesting in these trees.

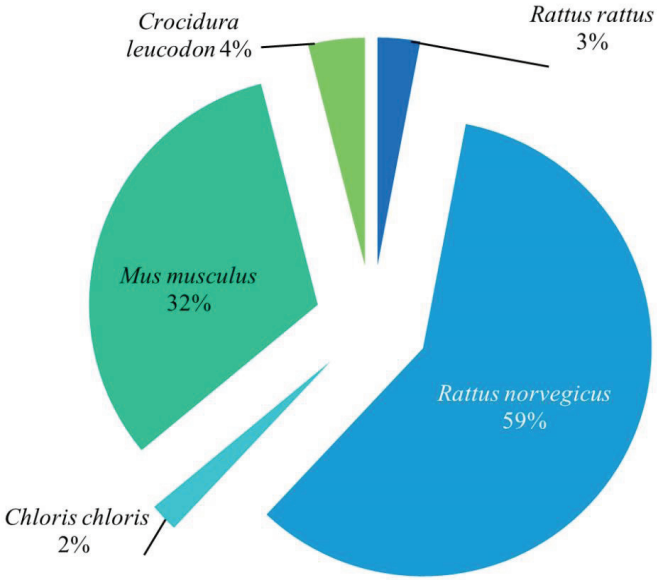

Figure 4. The preys identified in pellets. Făurei Street, Bucharest

Figure 5. The garbage dump between the blocks on Făurei street near the agglomeration of long-eared owl

From the second location, from the Didactic Farm Moara Domnească (Figure 2), Ilfov County, we collected 167 pellets and we were able to identify the preys in 70 pellets; of the other 97 of them we could not identify the preys, due of lack of identification elements.

In the 70 pellets we identified 84 prays (Figure 6) as follows:

- Rodents: 1% Hazel Dormouse (*Muscardinus avellanarius* L.), 30% Gray Rat (*Rattus norvegicus* B.), 58% Domestic Mouse (*Mus musculus* L.). Total: 89% rodents.

- Insectivorous mammals: 3% Common Shrew (*Sorex araneus* L.), 2% Bicolored Shrew (*Crocidura leucodon* H.). In total: 5% insectivorous mammals.
- Insectivorous birds: 1% tit (*Parus* sp.). In total 1% insectivorous birds.
- Granivorous birds: 4% Sparrow (*Passer* sp.), 1% Goldfinch (*Carduelis carduelis* L.). A total of 5% of granivorous birds.

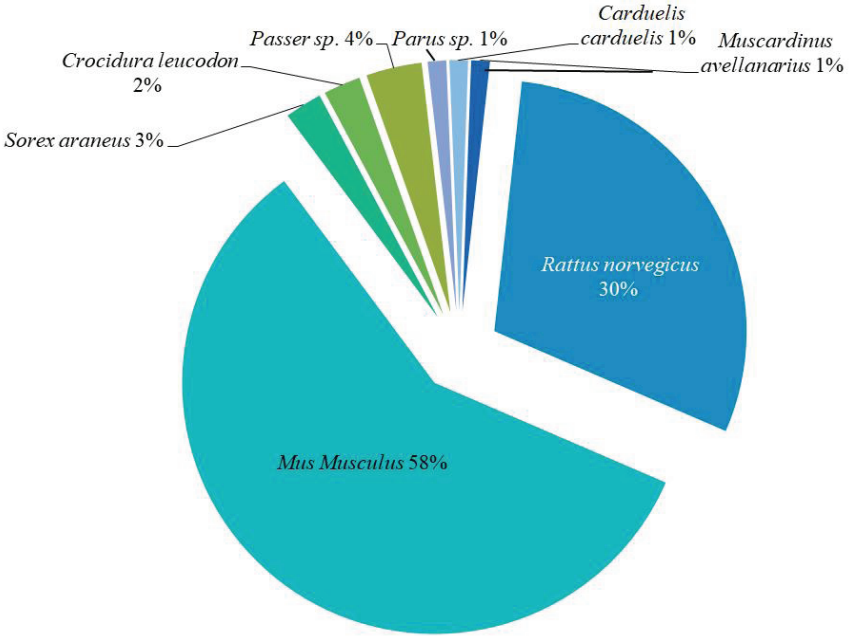

Figure 6. The preys identified in pellets. Moara Domnească, Ilfov County

CONCLUSIONS

Into the pallets collected from the green space from Bucharest, we identified 94% rodents, the biggest percentage being of Gray Rat (*Rattus norvegicus* B.) with 59% and Domestic Mouse (*Mus musculus* L.), with 32% - both rodent species being considered harmful.

We also identified three skulls of Black Rat (*Rattus rattus* L.), indigenous species, showing that this species it is still present in Bucharest, despite the competition he has with the Gray Rat (*Rattus norvegicus* B.), exotic and invasive species;

Into the pallets collected from the Didactic Farm Moara Domnească of the Ilfov county, the biggest percentage were on the Domestic Mouse (*Mus musculus* L.) with 58% and Gray Rat (*Rattus norvegicus* B.) by 30%.

The Long-eared Owls from the Didactic Farm Moara Domnească yard had a variety of seven species that were fed to those in Bucharest, with only five species - the diversity of food being affected probably by ecosystem.

Into the both locations, percentage of another mammalian and insectivorous birds percentage it is insignificant. We can appreciate that the Long-eared Owl (*Asio otus* L.) being a very helpful species into the control of rodent populations.

Roosting birds from the Făurei Street from Bucharest it is inside the city, 5 km from the periphery of Bucharest city. These testify that these species can do wintering colonies into big cities, between blocks, not just at the periphery or in small cities.

The long-eared owls from the cities can bring special services to the ecosystem through rodents consumption (mice and rats), but they are linked with the horticultural ecosystem quality.

For protection Long-eared Owl, we recommend administration of raticides with caution or even avoidance, maintenance of diversity, limitation

of disturbance into the roosting birds areas on winter, limit of heavy pruning and avoidance execution into the winter in the roosting long-eared owls areas.

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DISTRIBUTION AND MORPHOLOGY ASPECTS OF EXTRAFLORAL NECTARIES IN *PRUNUS AVIUM*

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Abstract

Plants secrete nectar to achieve two important mutual interactions with animals, namely pollination and indirect defence. Floral nectar is secreted inside flowers and attracts pollinators. Extrafloral nectar is generally secreted on vegetative parts of plants and attracts members of the third trophic level as a method of indirect protection against herbivores. Extrafloral nectaries are morphologically diverse and include glandular structures that differ in location, size and shape. The purpose of this study was to determine the morphology and position of extrafloral nectarines on petiole and limb in several cherry tree varieties, namely: 'Giorgia', 'Giant Red', 'Ferrovia', 'Kordia', 'Regina', 'Skeena' and 'Lapins'.

Key words*: cherry tree, extrafloral nectaries, morphology.*

INTRODUCTION

Plants secrete nectar to achieve two important mutual interactions with animals, namely pollination and indirect defence. Floral nectar is secreted inside the flowers and attracts pollinators.

Extrafloral nectar is generally secreted on vegetative parts of plants and attract members of the third trophic level as a method of indirect protection against herbivores (Escalante-Pérez et al., 2012).

Extrafloral nectaries are secretory glands that have no connection with the process of pollination. They are morphologically diverse and include glandular structures that differ in location, size and shape. They can be found on almost all plant organs, including leaves, petiole, bracts, cotyledons, fruits and on the exterior of sepals.

Also, extra-floral nectaries are found in varied forms such as unicellular forms, nectar secretion hairs, amorphous glandular tissue, secretory channels, which can be strongly vascularized or completely devoid of vascular system (Rodríguez-Morales et al., 2016).

The presence of extrafloral nectaries in plants has been reported around the world for about 25% of angiosperms. Most plants with such glands belong to the families *Asteraceae, Euphorbiaceae, Fabaceae, Lamiaceae, Melastomataceae, Orchidaceae* and *Rubiaceae* (Weber & Keeler, 2013).

Nectars can be defined as plant secretions, which mediate mutual interactions with a wide variety of animals, which from an ecological point of view can be divided into two main groups: pollinators rewarded with floral nectar and defenders against herbivores rewarded with extrafloral nectar.

It is well established that nectar from the extra-floral nectariferous glands, by mutual association with ants mainly, provides the plant with an indirect defence against herbivores. There is also a wealth of evidence that the ecological effects of these nectar sources are much deeper, knowing that they not only mediate interactions between several species along the food chain but can also be induced by herbivores (Tilney et al., 2018).

A large number of plants exhibit extrafloral nectaries that are not associated with

reproductive functions but are intended to attract ants and other arthropods. Extrafloral nectars are common and widespread in many vascular plants and are generally considered to be a tool used by plants to attract animals for defensive purposes (Grasso et al., 2015).
The defensive action of the ants is so visible that there is a long history of using these animals as biocontrol agents, and there are numerous studies reporting the protection of plants by ants in a wide variety of habitats, from the temperate to the tropical climate (Grasso et al., 2015).
The purpose of this paper was to determine morphology and position of extrafloral nectaries on petiole and limb for 7 varieties of cherry. The results obtained are presented below.

MATERIALS AND METHODS

Materials

The varieties analysed in this study are: 'Giorgia', 'Giant Red', 'Ferrovia', 'Kordia', 'Regina', 'Skeena' and 'Lapins'. For each variety, 9 to 12 leafs were collected on 27th April 2018 from the USAMV experimental fields in Bucharest.

Methods

The dimensions of the biological materials were adapted for the microscopic study.
Microscopic images were acquired using the Leica S8 APO stereomicroscope, which is connected to the LAS Core software that controls the Leica DFC295 camera installed on the microscope.

RESULTS AND DISCUSSIONS

For each studied variety, a series of images was made to determine the morphology and position of extrafloral nectaries on petiole and limb (Figures 1 to 7).

Figure 1. Extrafloral nectaries for the 'Giorgia' variety

Figure 2. Extrafloral nectaries for the 'Giant Red' variety

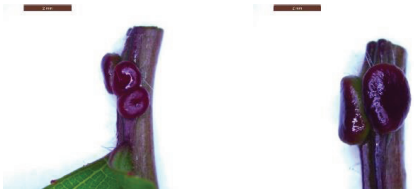

Figure 3. Extrafloral nectaries for the 'Ferrovi'a variety

Figure 4. Extrafloral nectaries for the 'Kordia' variety

Figure 5. Extrafloral nectaries for the 'Regina' variety

Figure 6. Extrafloral nectaries for the 'Skeena' variety

Figure 7. Extrafloral nectaries for the 'Lapins' variety

After the images were acquired, it was observed that the glands are found on both the

petiole and the limb, their number ranging from 2 to 4, for the studied samples. Also, their position on the petiole differs from one variety to another.

The position, number and dimensions of the extrafloral nectaries of the analysed varieties are presented in Tables 1 to 7.

Table 1. Position, number and dimensions of the extrafloral nectaries for the 'Giorgia' variety

No.	Distance to the limb (mm)	Length (mm)	Width (mm)	Number of glands on the limb	Total number of glands
F1	2.488	1.692±0.187	1.062±0.061	0	2
F2	1.577	1.867±0.061	1.139±0.049	0	2
F3	2.255	1.553±0.113	0.948±0.056	0	2
F4	3.717	1.550±0.354	1.056±0.306	1	3
F5	3.155	1.983±0.015	1.250±0.042	0	2
F6	1.796	2.113±0.342	1.032±0.051	0	2
F7	2.377	1.531±0.028	1.072±0.045	0	2
F8	2.418	1.935±0.064	1.184±0.127	0	2
F9	1.614	1.655±0.017	1.050±0.043	0	2
F10	1.770	1.298±0.092	0.932±0.144	0	2

Table 2. Position, number and dimensions of the extrafloral nectaries for the 'Giant Red' variety

No.	Distance to the limb (mm)	Length (mm)	Width (mm)	Number of glands on the limb	Total number of glands
F1	0	1.225±0.400	0.736±0.322	1	2
F2	0	1.316±0.117	0.755±0.0007	0	2
F3	0	1.251±0.088	0.752±0.125	0	2
F4	0	0.650±0.612	0.463±0.361	1	2
F5	0	0.733±0.132	0.592±0.022	1	2
F6	0	1.173±0.519	0.721±0.155	1	2
F7	0	0.645±0.106	0.491±0.132	2	2
F8	0	0.805±0.379	0.604±0.300	2	2
F9	0	1.254±0.158	0.838±0.065	0	2
F10	0.335	1.005±0.197	0.795±0.002	0	2

Table 3. Position, number and dimensions of the extrafloral nectaries for the 'Ferrovia' variety

No.	Distance to the limb (mm)	Length (mm)	Width (mm)	Number of glands on the limb	Total number of glands
F1	0.841	1.538±0.450	1.034±0.364	1	3
F2	0.000	2.346±0.191	1.596±0.176	0	2
F3	3.862	2.936±0.012	1.986±0.090	0	2
F4	1.582	2.079±0.059	1.308±0.116	0	2
F5	2.889	2.559±0.074	1.516±0.061	0	2
F6	0.964	1.610±0.147	1.210±0.140	0	3
F7	3.049	1.546±0.559	1.162±0.483	1	3
F8	2.680	2.174±0.018	1.511±0.023	0	2
F9	3.487	2.307±0.041	1.432±0.018	0	2

Table 4. Position, number and dimensions of the extrafloral nectaries for the 'Kordia' variety

No.	Distance to the limb (mm)	Length (mm)	Width (mm)	Number of glands on the limb	Total number of glands
F1	1.385	1.504±0.090	1.09±0.142	0	2
F2	2.191	1.016±0.648	0.772±0.522	2	4
F3	0.000	1.4385±0.045	1.034±0.002	0	2
F4	0.000	1.2035±0.092	0.709±0.141	0	2
F5	0.946	1.284±0.091	1.117±0.005	0	2
F6	1.301	1.796±0.070	1.414±0.049	0	2
F7	2.423	2.2±0.062	1.486±0.058	0	2
F8	0.000	1.107±0.047	0.824±0.062	0	2
F9	0.000	0.958±0.209	0.804±0.155	0	2

Table 5. Position, number and dimensions of the extrafloral nectaries for the 'Regina' variety

No.	Distance to the limb (mm)	Length (mm)	Width (mm)	Number of glands on the limb	Total number of glands
F1	0.000	2.839±0.420	1.937±0.175	0	2
F2	1.215	1.333±0.089	1.083±0.027	0	2
F3	0.000	2.538±0.326	1.934±0.234	0	2
F4	3.346	2.166±0.314	1.507±0.028	0	2
F5	0.625	1.933±0.058	1.621±0.164	0	2
F6	0.880	2.030±0.145	1.240±0.035	0	2
F7	0.000	1.171±0.092	1.330±0.012	0	2
F8	1.121	2.026±0.041	1.381±0.009	0	2
F9	0.000	1.359±0.376	0.850±0.060	0	2
F10	0.000	1.262±0.113	1.118±0.073	0	2

Table 6. Position, number and dimensions of the extrafloral nectaries for the 'Skeena' variety

No.	Distance to the limb (mm)	Length (mm)	Width (mm)	Number of glands on the limb	Total number of glands
F1	1.456	1.961±0.0071	1.391±0.135	0	2
F2	0.805	2.157±0.013	1.511±0.292	0	2
F3	3.213	2.571±0.057	1.514±0.226	0	2
F4	1.767	1.624±0.301	1.031±0.406	1	3
F5	2.290	2.213±0.092	1.393±0.032	0	2
F6	1.406	1.646±0.259	0.835±0.033	0	2
F7	3.278	2.354±0.245	1.466±0.094	0	2
F8	2.959	1.132±0.050	0.684±0.202	0	2
F9	2.182	1.115±0.135	0.723±0.146	0	2

Table 7. Position, number and dimensions of the extrafloral nectaries for the 'Lapins' variety

No.	Distance to the limb (mm)	Length (mm)	Width (mm)	Number of glands on the limb	Total number of glands
F1	0.723	0.983±0.270	0.759±0.138	0	3
F2	0.000	1.523±0.179	1.096±0.062	0	2
F3	1.64	1.721±0.038	0.964±0.256	0	2
F4	0.87	1.281±0.462	1.391±0.393	0	2
F5	3.044	1.528±0.173	0.783±0.282	0	2
F6	4.287	2.354±0.203	1.701±0.038	0	2
F7	0.891	2.024±0.357	1.291±0.126	0	3
F8	0.000	1.676±0.338	1.312±0.295	0	4
F9	2.848	1.846±0.790	1.204±0.704	1	3
F10	2.226	1.754±0.096	1.245±0.375	0	2

Following microscopic analysis of different cherry varieties, there are some major differences in position, number and size of extrafloral nectaries as follows: the 'Giant Red' variety consistently has a total of 2 glands which are predominantly positioned on the edge of the limb.

At the same time, this variety has the smallest size of the glands (both length and width).

Regarding the 'Regina' variety, it has no more than 2 glands, which are always positioned on the petiole. The variety with the largest extrafloral nectaries is 'Ferrovia'.

Figures 8 to 10 show the distance to the limb, the total number of extrafloral nectaries and the number of glands present on the limb for all studied cherry varieties.

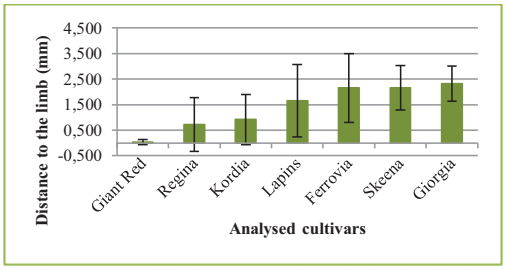

Figure 8. Graphical representation of gland-to-limb distance (mm) for analysed cherry varieties

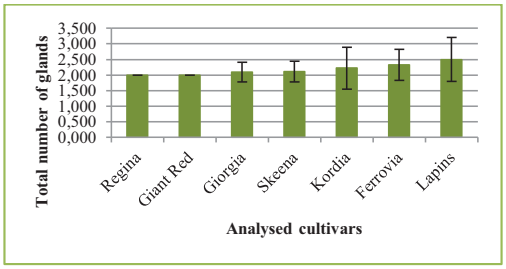

Figure 9. Graphic representation of the total number of glands for the analysed cherry varieties

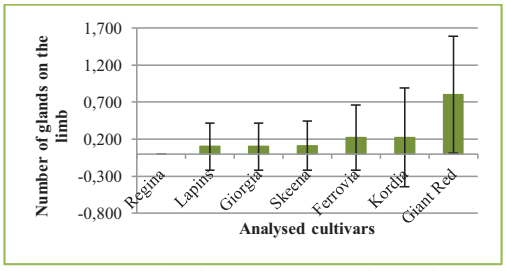

Figure 10. Graphical representation of the number of glands placed on the limb for the analysed cherry varieties

CONCLUSIONS

The largest distance between the glands and the limb was recorded on the 'Giorgia' variety leaves, while the smallest distance was recorded for the 'Giant Red' variety. As for the total number of extrafloral nectaries, it was between 2 and 4 per leaf for the analysed varieties. Thus, the largest number of leaf glands was recorded for the 'Lapins' variety, and the lowest number of leaf glands was recorded for 'Regina' and 'Giant Red' varieties. The presence of extrafloral nectaries on the limb was predominantly observed on the analysed leaves of the 'Giant Red' variety, while for the 'Regina' variety these glands were present only on the petiole.

Overall, the present study is unique, as no literature data was found on the correlations among cherry tree variety and extrafloral nectaries morphology and further attention has to be paid on research related to the influences of these on the pathology and pest resistances as well as on productivity of the same cultivars.

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MORPHOLOGICAL AND ANATOMICAL STUDY OF *PSIDIUM GUAJAVA* LINN. (GUAVA) - A NEW FRUIT TREE AND MEDICINAL PLANT RESEARCHED IN ROMANIA

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Abstract

Guava - Psidium guajava Linn. (Myrtaceae) is an evergreen tree cultivated for its precious fruit and high therapeutic properties of the whole plant. Guava is native to the Caribbean, Central America and South America. In 2011, guava was propagated by seed and it was analysed in 2018 at the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The research pointed that in Romania guava, as container plant, can produce fruit every year, starting with the fourth year after sowing. The morphological analyses showed that the leaves of the main shoots varied in length between 11.1-12.5 cm, in width between 5.6-6.5 cm and the length of the petiole was between 0.7-1cm. The leaves of the suckers varied in length between 8.7-10.5 cm, in width between 4.1-5.3 cm and the length of the petiole was between 0.6-0.9 cm. The anatomical analyses of the leaf showed a dorsoventral mesophillum with a multiseriate upper epidermis. Biochemical analyses were done on fresh and dried leaves of 8 years old guava tree.

Key words*: anatomy, guava, medicinal plant, morphology.*

INTRODUCTION

Psidium guajava Linn. commonly called guava belongs to the family *Myrtaceae* and genus *Psidium*, described as a genus by Linnaeus in 1753. Genus *Psidium* includes 96 accepted species. Most important is *Psidium guajava,* known as "the apple of the tropics". It is native to Central and South America, West Indies, Mexico, Florida, Louisiana, Arizona and naturalized in parts of Africa, Indian sub-continent and on numerous oceanic islands (Ho & Long, 2016). *Psidium guajava* is a large dicotyledonous shrub or small evergreen tree, generally 3-10 m high with many branches. Guava plants are hardy and produce good yield. Nutritional values of guavas are often included among super fruits, being rich in dietary fibres, vitamins A, C, B3, B5 and B6, potassium, copper and manganese (Jimenez et al., 2001). Guava fruits are grown for their excellent health benefits and they can be consumed as fresh fruits or used in beverages, jams, candies, purees, powder, jelly, ice cream, dried snacks, frozen pulp, fruit bars, yoghourts and desserts. It has a pleasant aroma and flavour, varying from medium soars till very sweet. Propagation is done by seeds, cuttings, grafting and air layering. Guava trees are cultivated in orchards but, they can be grown very well in pots/containers, back yards, greenhouses and poly houses. Trees reach full bearing after 5-8 years, depending on growing conditions and spacing. Guava is not a long-lived tree (about 40 years) but, the plant may bear heavily for 15-25 years (Meidell et al., 1998). It exceeds the majority of tropical and subtropical fruit trees in adaptability, productivity and tolerance to mild cold and light frosts (Yadava, 1996). Guava seems indiscriminate to soil conditions, it is somewhat salt-resistant and tolerates a pH range from 4.5 to 9.4 (Singh et al., 2017). *Psidium guajava* Linn is an amazing medicinal plant (Grigore et al., 2016; Hobert & Tietze, 1998) studied from centuries. Whole plant of guava, mainly its leaves and bark have a long history of medicinal uses that are still employed today and validated by scientific research on animal and human subjects (Gutierrez et al., 2008). Toxicity studies in mice and other animals, as well as controlled human studies (Rizo et al., 2014; Deguchi et al., 2000) show

that both, leaf and fruit, are safe without any side effects (Teixeira et al., 2003). The metal analysis of powdered sample of *P. guajava* showed the presence of calcium, magnesium, manganese, zinc, iron, sodium and potassium (Okunrobo et al., 2010). Guava leaves have antioxidant action (Chen & Yen, 2007), anti-ageing properties (Edwin et al., 2007), antimicrobial effects (Buvaneswari, 2011). Reported pharmacological activities include diarrhoea (Ojewole et al., 2008), dysentery, gastrointestinal disorders (Lozoya et al., 2002), infantile enteritis caused by *Rotavirus* (Wei et al., 2000), diabetes (Cheng & Yang, 1983; Oh et al., 2005), obesity (Deguchi & Miyzaki, 2010), high cholesterol (Singh et al.,1993), bronchitis, laryngitis (Jaiarj, 1999), acne and skin infection (Qadan et al., 2005), wounds, boils, bites, soft tissue infectious site (Abubakar, 2009), hair loss (Sim et al., 2016), allergy (Han et al., 2011), asthma (Batick, 1984), epilepsy (Meckes et al., 1996), fever (Olajide et al., 1999), flu-H1N1, (Sriwilaijaroen et al., 2012), malaria (Nundkumar & Ojewole, 2002), periodontal diseases (Ravi & Divyashree, 2014), rheumatism (Ayensu, 1978). The leaves of *Psidium guajava* have anti-inflammatory and analgesic effects (Jewole et al., 2006), liver protective activity (Roy et al., 2006), antihypertensive and cardio protective (Sakanashi et al., 2003), anti-ulcer (Edwin et al., 2007), anti-stress (Lakshmi & Sudhakar, 2009), immuno-stimulatory activity (Laily et al., 2015); the anticancer effects of guava (breast, cervix, colon, mouth, prostate, stomach, thyroid) have variously been reported (Lee & Park, 2010; Chen et al., 2010). In several studies, guava showed significant antibacterial activity against *Citrobacter* sp. (Gupta & Birdi, 2015), *Escherichia coli* (Geidam et al., 2015), *Salmonella* sp. (Etuk & Francis, 2003), *Shigella* sp., *Vibrio* sp. (Chulasiri et al., 1986), *Staphylococcus aureus* and β-*streptococcus* group A (Jaiarj et al., 1999), etc. The essential oil of guava leaves shows anticancer activity (Manosroi et al., 2006), anthelmintic (Tangpu & Yadav, 2006) and antibacterial activity against *Toxoplasma* sp. (Lee et al., 2013). Entire plant of guava has a huge therapeutic potential and the above list of diseases and disorders is not exhaustive.

MATERIALS AND METHODS

The sowing material is originated to a private garden from India, Uttrachand State, Sherkothi district, Roorkee town, 33/104 Civil lines. The seeds were isolated from ripe fruits of *Psidium guajava*, variety "Safed" (white-yellow pulp and light green-yellow skin) in March 2011 and sown in July 2011 in Romania, Bucharest into the own balcony. The seeds germinated after 18 days and the seedlings growth in the first year was quite slow. Guava, in the temperate climate of Romania, grew in the open-air condition between April and October and indoor - balcony condition between November and March. It reached near 250 cm height and 120 width, as is shown in the Figure 1.

Figure 1. Habitus of guava (14.11.2018)

Pruning was done once a year. Four years after sowing, in August 2015, the container guava started to bear flowers and fruits. Since 2015, guava produced fruits every summer, as is clearly seen in the Figure 2. Its fruits are very delicious and they represent a huge attraction for birds, especially when their size is small, until an olive size. For this reason, it is necessary to protect them with bird net, over the canopy. Relating pest and disease control, guava was attacked in 2017 by *Cossus cossus* (goat moth) and the damaged branch was removed from the tree.

Figure 2. Different aspects of *P. guajava* growing: A. Flower after pollination – 25 July 2016; B. Small fruits – 16 August 2017; C. Habitus in the garden – 12 July 2018; D. Fruiting under birds net – 7 August 2018

Other form of infection or infestation was not observed. Guava was fertilized with N:P:K-16:16:16 and alternatively with Vitaflora, containing macro & micro minerals or Cropmax-foliar fertilizer, containing amino acids, enzymes, macro & micro minerals and other nutrients.

The best results were obtained with Cropmax.

The analyses were done on the eight years old plant shoots and its suckers. Transverse sections were made in the fresh material: mature, unlignified and lignified stem, leaf and petiole.

The sections were manually made with the razor blade, clarified with chloral hydrate for 24 hours, then washed and stained with carmine and green iodine (Savulescu & Hoza, 2010; Georgescu et al., 2015).

The analyses and observations of these sections were carried out at the Centre for the Study of Food and Agricultural Products Quality at U.SAMV Bucharest and the images and measurements were made using Leica DM 1000 LED, Leica DFC 295 - Video Camera and S8 APO - Stereo Microscope, SEM Fei inspects 50 and Digital camera Sonny.

RESULTS AND DISCUSSIONS

As cultivation requirements, guava is partially rustic, resistant to temperatures up to 0°C. In case the tree is affected of late hoar-frost, it has a very good capacity to regenerate (Hoza, 1998). It is cultivated outdoors only in frost free climates but, it grows and fruits very well, even in containers. It prefers south facing for a generous amount of light because guava likes full sun or partial shade. In the summer time, guava must stay in sunny place and put indoor to protect it from winter cold, near a bright window or in greenhouse. It is fairly cold-hardy and can survive as low as 5°C for short periods of time at night (Wei, 2008). At low temperatures, it loses all or a part of the leaves. Guava requires medium care and is relatively easy to cultivate. Sowing time is all year round in green-house and from spring to autumn outdoor in repaired place. For sowing, a small and large pot must be filled with a quality potting medium. The seeds must be soaked near 5-8 hours, if they are fresh and 2-3 days, in case they are older than 1 year. The seeds must be sown approximately 2 cm bellow the medium potting surface. It is important do not allow the potting medium to dry out but, at the same time, it needs a caution do not have the mix soggy or standing in water. An even moistness throughout the pot is the most desirable. The seeds germinate easily if sown in potting soil and kept warm, optimum temperature of 25-28°C.The germination takes between 2-12 weeks, averagely 4-6 weeks, depending on the temperature, humidity, seed quality and cultivar (Padilla-Ramirez et al., 2012). The recommended pH should be light acid to neutral, because with alkaline pH, it manifests iron chlorosis. Even if guava is not fussy on the soil quality, it prefers light soils, although it can also live on those heavy but, well drained-sandy loam or clay loam. Guava will produce better in rich soils, high in organic matter. After approximately 2-3 months, the seedlings with substantial thickened stems will be ready for transplanting. It means when the seedlings get large enough to handle, reaching near 25 cm, it's the time to transplant them to larger pots. At the temperature of -1°~ -2°C the seedlings will freeze to death (Salazar et al., 2006).

Suitable growth temperature in summer is above 15°C. After the fifth year of growing in temperate climate, guava tree becomes fairly cold - hardy and it can survive temperatures colder than 4°C for short periods of time but, younger plants will likely freeze to the ground. Heavy rain fall close to maturation time will damage the fruit and its aroma will be diminished. From the fourth year, guava gets smooth, thin copper coloured bark that flakes of showing the greenish layer beneath. Its trunk has a "bony" aspect. Guava grows rapidly from second year. Yearly pruning is important to control its size. The root sucker, water sprouts and cross branches should be removed. Guava pruning is relatively similar to apple pruning; it bears fruit on the shoots of one year. The flowers will appear solitary or 1-3 at the leaf axil. Because the flowers are hermaphrodites, guava has self - pollination. The fruit is a near round or pear-shaped berry of 4/12 cm, exuding a strong, musky odour when ripe, with thin, light-yellow skin. Guava is a refreshing fruit with a sweet taste, somewhere between pear and strawberry. In the Figure 3 is represented the growth's dynamics of *Psidium guajava* on 20 November 2018.

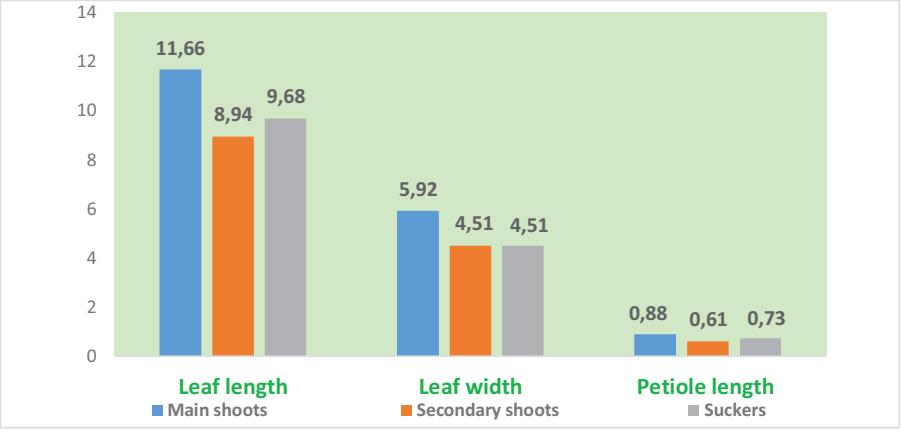

Figure 3. Growth dynamics of *Psidium guajava* leaves and petioles (averages) - 20.11.2018

Thus, the length average of the main shoots leaves was 11.66 cm, the length average of the secondary shoots leaves was 8.94 cm and the length average of the suckers leaves was 9.68 cm. The width average of the main shoots leaves was 5.92 cm, the width average of the secondary shoots leaves was 4.51 cm and the width average of the suckers leaves was 4.51 cm. The length average of the main shoots petiole was 0.88 cm, the length average of the secondary shoots petiole was 0.61 cm and the length average of the suckers petiole was 0.73 cm. The suckers are very strong, making competition with the 3 years old branches but, they can be used for vegetative multiplication of guava.

Macroscopic characteristics

Root system is branched, quite superficial. It has some deep roots but, no distinct taproot. The stem is erect, woody, solid and branched; the bark is light to reddish brown, thin, smooth and continuously flaking. The leaves of *P. guajava* are oval to oblong - elliptic, 9-12 cm in length, bright green, simple, alternate, short petiolate, 4.5-7 cm in width. Lamina is pubescent on the underside with prominent veins. The leaves have entire margins, oil bearing glands that release a pleasant fragrance, a common feature of all plants of *Myrtaceae*. The petiole is short (0.6-0.8 cm in length and 0.2-0.3 cm in diameter), green, showing a groove on the upper surface and hairy. The flowers are 1-3, white, large and fragrant that grow in the leaf axils; they are pedicellate, bracteate, complete and hermaphrodite.Corolla: 5 petals; Calyx: 5 sepals; Androecium: 250 stamens tipped with pale-yellow anthers; Gynoecium: 4-5 carpels, inferior ovary. The fruit is a many-seeded berry and consists of a fleshy pericarp and seed cavity with pulp. (Pandey, 1999)

Microscopic characteristics

Anatomical properties are indices used in taxonomical studies and a lot of anatomical characters of some dicotyledonous families (Sandulescu et al., 2016), were followed by Metcalfe and Chalk, in 1950 who gave a synthesis of their previous works and own investigations on the family *Myrtaceae.* Different studies on plants anatomy of *Myrtaceae* were done by Onaran and Bayan, 2016, Ali et al., 2009, Kantachok et al., 2007, Tantawy, 2004, etc. Morphological and anatomical studies of *Psidium guajava* L. were done by Metwally et al., 2011.

Leaf anatomy

Transverse section in the leaf lamina (Figure 5) shows upper (16.43-17.13 µm) and lower epidermises (7.41-7.90 µm), hypodermis and a dorsoventral mesophyll (61.20 µm). The hypodermis consists of 2-3 layers of collenchymatous cells.

The palisade tissue consists of two rows of columnar cells and is discontinuous in the midrib region. The spongy tissue is formed of 5-8 rows of more or less spherical cells. Small vascular bundles and the oil glands may be embedded within the spongy tissue. The midrib is more prominent in the lower side and shows bi-collateral arc-shape vascular bundle. In the midrib are present calcium crystals and few crystal sheaths (Figure 7). The cortical tissue of the midrib consists of 2-3 rows of collenchymatous cells beneath the upper epidermis and 3-4 rows abutting the lower epidermis, followed by 3-4 layers of thin walled parenchyma with distinct intercellular spaces. The parenchyma cells contain few prisms and numerous clusters of calcium oxalate. Oil glands are also present. The pericycle is formed of two arcs of lignified fibres above and below the vascular bundle. The fibres are fusiform with wavy lignified walls, rather wide lumen and more or less acute apices. The upper epidermis of lamina (Figure 9) consists of polygonal, nearly iso-diametric or slightly elongated cells. The lower epidermis (Figure 10) consists also of polygonal, nearly iso-diametric cells. Stomata are present on the lower epidermis only and is the paracytic type. Trichomes are present in both epidermises, being more numerous in the upper epidermis (Figure 9-F). They are non glandular unicellular wooly straight, curved or twisted, arising from a cicatrix surrounded by radiating epidermal cells. The vascular tissue consists of an arc-shaped bi-collateral vascular tissue which is formed of xylem and two arcs of phloem above and below it.

Stem anatomy

Transverse sections in both, lignified and unlignified stem were made. In the transverse section of unlignified stem are present: epidermis (consisting of thin or thick layer of cuticle), multi - layered cortex (chlorenchyma, collenchyma and parenchyma), vascular continuous bi-collateral bundle and, in the centre of stem, the pith composed of parenchymatous storage cells. There are also many secretory cavities and tanniniferous cell (Figure 8). The lignified stem consists in secondary vascular xylem (vessels, fibres and parenchyma) and vascular secondary phloem (sieve tube, companion cells, parenchyma and fibres). Very clearly the annual rings are seen (Figure 6).

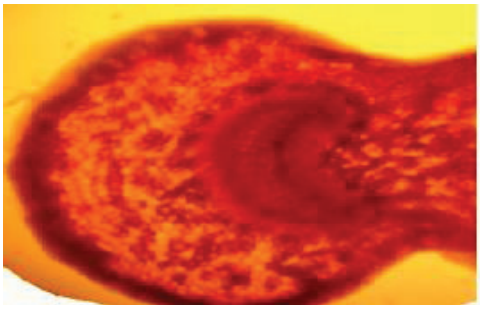

Figure 4. U-shaped cell in guava's petiole

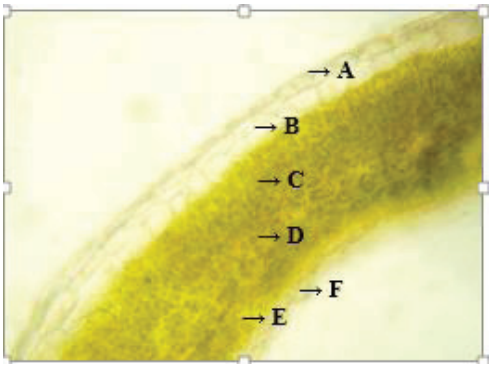

Figure 5. Cross section of mature leaf lamina (20X): A. Upper epidermic; B. Hypodermis; C. Palisade layer; D. Spongy layer; E. Palisade layer; F. Lower epidermis

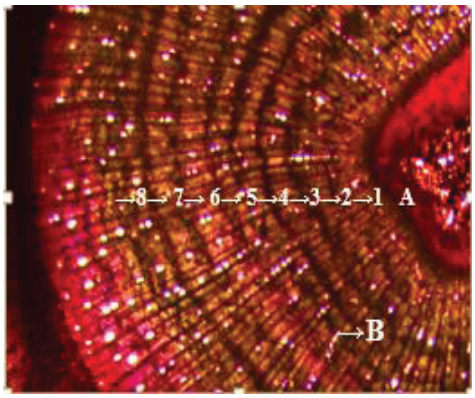

Figure 6. Section of guava lignified stem (4X)
(1⟶8) Annual rings; B. Vessels of xylem

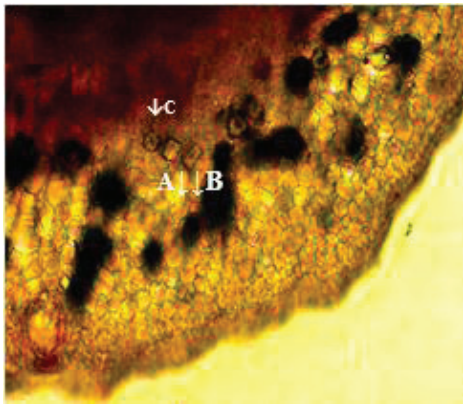

Figure 7. Prismatic crystals of guava leaf
A., B., C. Prismatic crystals of calcium oxalate

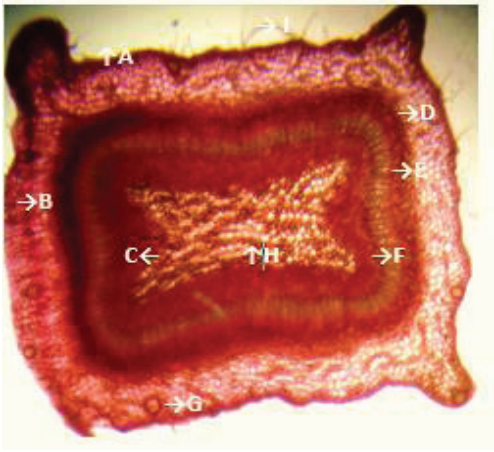

Figure 8. Transverse section of unlignified stem (10 X)
A. Epidermis; B. Cortex; C. Vascular bundle;
D. Phloem; E. Xylem; F. Phloem;
G. Secretory oil cavity; H. Pith; I. Trichomes

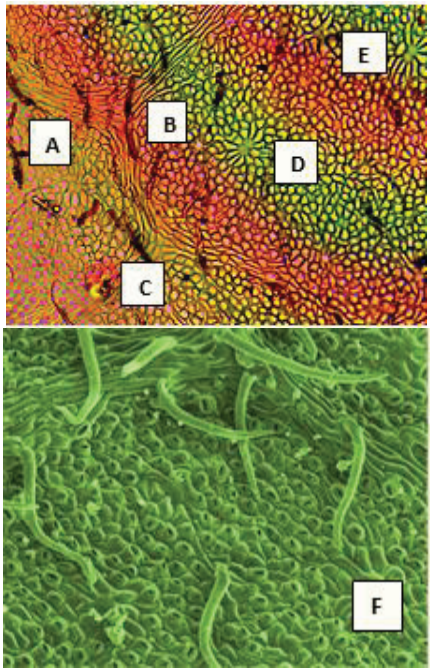

Figure 9. Upper epidermis of guava leaf (20X)
A,B. Trichomes; C.Oil gland; D.,E. Cells in cicatrix;
F. View by SEM

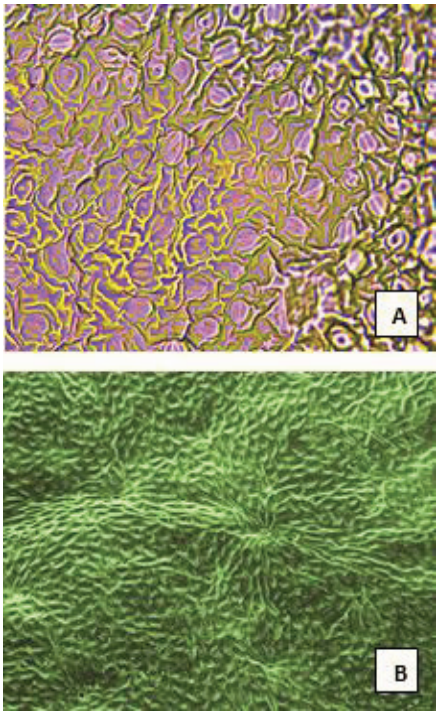

Figure 10. Guava lower epidermis with stomata:
view by (20 x) microscope (A); view by SEM (B)

Petiole anatomy

Transverse section in the petiole (Figure 4) is semicircular and presents epidermis, cortex, vascular bundle. Epidermis is one layered with hairs, square or rectangular shaped, covered with a layer of cuticle.

Cortex of 2-3 rows of collenchyma, 2-5 rows of chlorenchyma and 3-5 rows of parenchyma. In the cortex are presented secretory cavities and prismatic-rectangular crystals.

Vascular bundle is U-shaped; the main arc-shaped vascular strand is widely open. Vascular bundle is surrounded by sclerenchymatic tissue.

A trans-verse section in the petiole is planoconvex, slightly grooved on the upper part. It is formed of an parenchy-matous tissue with prisms and clusters of calcium oxalate and one row of oil glands situated in the outer region of the cortex.

The cortex is traversed by crescent U-shaped vascular tissue similar to that present in the leaf.

Biochemical compounds analyses

The analyses were done on the 25 g of dried leaves of *P. guajava*, collected in January 2019, at the "Chem-Analyst" private laboratory, 101 L Timisoara Avenue, district 1, Bucharest and they showed that, at the humidity of 10.3%, the amount of total polyphenols was 85.48 mg %, flavones 22.46% and caffeic acid - a very important antioxidant - 0.574 mg %.

The test report done on 25 g of fresh leaves of *P. guajava* collected at the above date and analysed at the same laboratory showed that, at the humidity of 42.87%, the amount of total polyphenols was 63.84%, flavones 29.59 mg % and caffeic acid 0.190 mg %.

The dried powdered leaf is light green in colour with an aromatic taste and a characteristic odour.

The analyses of container guava's polyphenols and flavones revealed the same or very close values of those of guavas grown in the orchards or forest from tropical and subtropical regions (Mital & Sumitra, 2011; Metwally et al., 2011; Rattanachikunsopon & Phumkhachorn, 2010; Gutierrez et al., 2008; Bala, 2006; Lapik et al., 2005; Abdel et al., 2004; Arima et al., 2002; Kandil et al., 1997; El-Khadem et al., 1958).

CONCLUSIONS

Microscopic analyses showed that the growth and development of container guava follow the dimensions of the leaf, stem and petiole, as those of its origin places.

The suckers are very strong, making competetion with the 3 years old branches but, they can be used for vegetative multiplication of guava.

P. guajava responded very well to the foliar fertilizer - Cropmax.

It was resistant to the attack of pests and diseases, for this reason guava is highly recommended for bio cultivation.

In the temperate climate of Romania, the container guava beard fruit four years after sowing. It resisted outdoor till 5°C temperature and needed indoor protection between November and March.

It shows a very good adaptability of *P. guajava* in our country, making guava an amazing choice to grow it on a large scale in Romania, for fruits and leaves, as well.

The analyses of container guava polyphenols (quercetin, caffeic acid) and flavones revealed guavas grown in the orchards or forests from tropical and subtropical regions. It demonstrates that the medicinal proprieties of container guava remain very precious and, for this reason, guava must be researched more extensively in our country and used for prevention and as an adjuvant in the treatment of numerous disorders. *Psidium guajava* Linn. is a very valuable candidate to offer efficient solutions to the medical problems of our modern society.

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SOME MORPHOLOGICAL AND ANATOMICAL PARTICULARITIES IN *VITEX AGNUS-CASTUS* L. SPECIE GROWN IN PROTECTED SPACE

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Abstract

The assessed species, Vitex agnus-castus L., was taken from Thasos, Greece and cultivated in pots for acclimatization in the botanical garden, to be studied for its medicinal and ornamental use. During the vegetation period, the morphological and anatomical observations were made. It is an aromatic shrub, with opposite leaves, palmate-composed of 3-5 (7) lanceolate leaflets, entire margins or serrate-dented, 3-7 cm long and 1.5 cm width. The transversal sections were made in the root, stem and leaf. In the root and stem, it was observed that the vascular bundle had a secondary structure. In the epidermis of young stems there are non-glandular hairs and very rarely secretory hairs. In the pith of stem there were seen the starch granules and calcium oxalate crystals. On the epidermis of rachis there are non-glandular and secretory hairs. The epidermis of the leaflets is unistratified, with the corrugated walls and covered by thin cuticle. On the both epidermis of the leaflets there were observed the non-glandular hairs. The secretory hairs were identified mainly on the lower epidermis. The type of stomata is anomocytic, identified only on the lower epidermis. The mesophyll is bifacial with palisade tissue toward upper epidermis and spongy tissue toward lower epidermis.

Key words*: Vitex agnus-castus, morphology, anatomy, secretory hairs.*

INTRODUCTION

In the first taxonomic classifications, the genus *Vitex* L. is included in the *Verbenaceae* family, with approx. 250 species of trees and shrubs (Linnaeus, 1753; Jussieu, 1806; Backer, 1965; Griffiths, 1994; Takhtajan, 2009).

In some recent classifications, the *Vitex* L. genus has been transferred to the *Lamiaceae* family following morphological, micromorphological, anatomical and phytochemical investigations on pollen, petiole, stems, fruits, between *Vitex* and *Verbenaceae* species, which have provided useful characteristics in taxonomy (Abbas et al., 2006; Jamzad et al., 2006; Chantaranothai, 2011).

It grows naturally in Mediterranean countries, southern Europe, tropical area, West and Central Asia (Hegi, 1966; Tutin et al., 1972; Davis, 1982; Donald, 1994; Griffiths, 1994; Wasson, 2003).

The name *Vitex agnus-castus* derives from the Greek word "Chastity" which means virgin, being used in popular medicine for a variety of gynecological dysfunctions for over 2000 years in ancient Greece, Egypt and Rome (Christie & Walker, 1997; Chen et al., 2010).

From the plant are uses the fruit, sometimes the leaves, from which is obtained infusion, mixture, extract for different applications.

From the chemical point of view, the fruits of *Vitex agnus-castus* contain: flavonoids, iridoid glycoside, p-hydroxybenzoic acid, alkaloids, essential oils, fatty oils and essential oils such as linoleic acid, diterpenoids and steroids (Adams, 2004; Hajdu et al., 2007; Marongiu et al., 2010; Borges et al., 2012; Toplan, 2015; Hanane et al., 2016; Hina et al., 2016; Tģn et al., 2017; Allison et al., 2018; Levchyk et al., 2018).

The fruit composition have therapeutic effects for the regulation of the menstrual cycle, for the treatment of premenstrual syndrome (PMS), gynecological disorders, support the progesterone secretion, infertility in women (Mehrangiz et al., 2012; Schellenberg et al., 2012; Mohammad et al., 2015; Berrani et al., 2018; Mina et al., 2018). In addition, it has been used to reduce the symptoms of menopause such as hot flashes, depression, and

sleep disturbances, headaches, rheumatism, corpus luteum insufficiency, disrupted lactation, acne, antimicrobial, antifungal, antiepileptic, antioxidant, antitumoral, diuretic, digestive, insect repellant, larvicidal (Doll, 2009; Sarikurkcu, 2009; Khaled, 2013; Shenghong et al., 2013; Sakhavar et al., 2013; Miguel et al., 2014; Franciele et al., 2015; Katiraee et al., 2015; Yilar et al., 2016; Abeer et al., 2017; Zinat et al., 2017; Keikha et al., 2018).

The plant also has economic importance. Aromatic leaves can be used as spices and the fruits, due to their quick taste, are used as a substitute for pepper (Hanelt et al., 2001; Novak et al., 2005). Some studies show that essential oils obtained from different parts of *Vitex agnus-castus* have acaricidal potential, being tested on *Tetranychus urticae* (Hamid et al., 2010; Roberta et al., 2016).

The anatomy of vegetative organs is reconfirmed in the literature (Metcalfe, 1963; Metcalfe & Chalk, 1983; Toma & Rugină, 1998; Schweingruber et al., 2013; Garner, 2017).

From the data analysis of literature, the Vitex species have anatomical differences (Abbas et al., 2006; Bejenaru et al., 2013).

The *Vitex agnus-castus* species being little known in Romania, the purpose of this study is to identify certain anatomical features at the plants grown in protected space that will be acclimatised for planting in the field as a medicinal and ornamental species.

MATERIALS AND METHODS

The plants of *Vitex agnus-castus* of about 50 cm high were brought from Thasos, Greece at the beginning of August 2018 and planted for acclimatization in the pots, in the greenhouse of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV).

The Bucharest is located at 44º24′49″ North latitude and 26º5′48″, East longitude, 90 m altitude and temperate-continental climate, with 585 mm/year rainfall and 10.86°C the average of the temperature.

During the vegetation period, morphological, micromorphological and anatomical observations were made. Biometric measurements were performed at the leaves, and some anatomical measurements. The average was obtained from 10 measurements. The both epidermis were exfoliated and assessed.

For anatomical analysis of cross-sections were performed in very young vegetative organs (roots, stems, leaves) which were clarified with Chloral-hydrate coloured with the Carmine - Alaunte and Iodine Green (Luchian et al., 2018).

The observations, images and measurements of the anatomical structures were made with the optical microscope Leica DM1000 LED, Camera video Leica DFC295 the Stereomicroscope Leica S8 APO, and SEM, belonging to the Laboratory of Microscopy and Plant Anatomy of the USAMV of Bucharest.

RESULTS AND DISCUSSIONS

After planting, the plants were formed new composed leaves with five, rarely three leaflets, elliptical-lanceolate, with 3-7 cm long and about 1.5 cm wide with the entire margin or finely toothed in the middle part.

Analysing the internal structure of the vegetative organs this is similar to the literature data.

Anatomy of root

The contour of the cross section is circular, with a secondary structure for the most part.

From the outside to the inside there are observed: the peridermis (suber, phellogen, phelloderm), primary cortex and the central cylinder (Figure 1).

The rhizodermis is early exfoliated and replaced on the outside by the suber generated by the felogen. The suber consists of 2-5 rows of elongated, suberified cells with the first rows being exfoliated.

The phelloderm is parenchymatous tissue, consisting of 2-4 rows of isodiametric cells with thin walls. The cortex of primary origin is reduced to a row of cells. The vascular tissues are collateral, of secondary origin, generated from the cambium and consisting of two concentric rings.

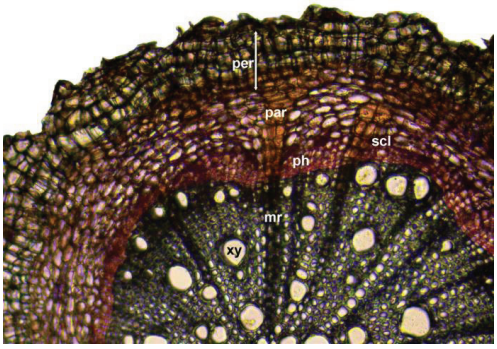

Figure 1. Cross section in the root of *Vitex agnus-castus*: per - peridermis; par - parenchyma; ph - phloem; scl - sclerenchyma; xy - xylem; mr - medullary rays

The ring of secondary phloem, located on the outside of cambium, is very thin at about 22.73 µm, consisting of sieved vessels, annexe cells and parenchyma. At the exterior of the phloem were observed small and thin fragments of sclerenchyma of primary origin, originating from pericycle.

The ring of secondary xylem is thick, approx. 210.53 µm, with a lot of libriform, strongly lignified, disorderly dispersed and metaxylem vessels with large diameter in average of 30.37 µm.

Anatomy of stem

The stem structure corresponds to the data from the speciality literature.

In the annual stems, the contour of the cross section is oval, with 4 edges, consisting in the epidermis, cortex and central cylinder (Figure 2).

The epidermis is formed by one layer of cells with about 25 µm thick and covered by a thin cuticle. In the epidermis were observed non-glandular, unicellular or multicellular hairs, with a conical to sharp peak of about 36.4 µm long.

On the epidermis of the young stems were sporadically identified the multicellular secretory hairs with 2 (3) cells, with spherical secretory cell (Figures 3 and 4).

In the literature have not been reported the secretory hairs in the epidermis of the stem (Yunus et al., 2008).

In the young stems, under the epidermis is the collenchymatous tissue more developed on the

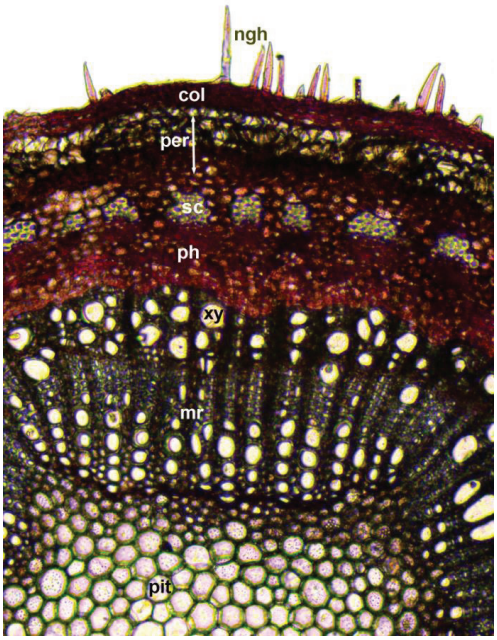

Figure 2. Cross section in the stem of *Vitex agnus-castus*: ngh – non glandular hairs; col – collenchyma; per – peridermis; ph – phloem; sc – sclerenchyma; xy – xylem; mr – medullary rays; pit – pith

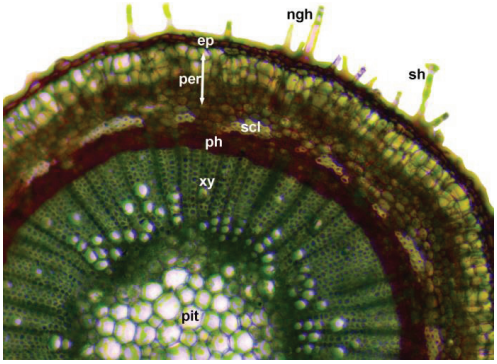

Figure 3. Cross section in the stem of *Vitex agnus-castus*: ep – epidermis; ngh – non glandular hairs; sh – secretory hairs; per – peridermis; ph – phloem; scl – sclerenchyma; xy – xylem; pit – pith

edges followed by the suber, phellogen and phelloderma.

The primary cortex is reduced to 1-2 rows of cells.

In some stems there were observed also the lenticels (Figure 5).

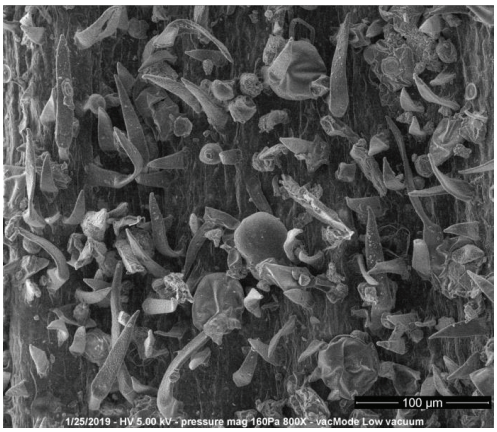

Figure 4. Micromorphological analysis of the stem (SEM 800X)

In the mature stem, the epidermis is replaced by the suber generated by the phellogen, consisting on 2 (3) rows of elongated cells.
In the central cylinder, the vascular tissues are collateral, consisting of two concentric rings, generated by the cambium.
At the outside of cambium is a thin ring of phloem and inside of it is a thick ring of the xylem.
At the base of the secondary xylem there was observed a thin primary xylem from place to place.
The ring of secondary phloem is very thin at about 52.70 µm, consisting of sieved vessels, annexe cells and thin layer of parenchyma. At the exterior of the phloem were observed fragments of sclerenchyma.
The ring of secondary xylem with a thickness of 181.17 µm with irregularly dispersed vessels in the basic libriform is separated by the medullary rays. The metaxylem vessels have an average diameter of 30.80 µm.
The pith is a well-developed parenchymal tissue in which have been observed starch granules and prismatic calcium oxalate crystals (Figure 6).

Anatomy of leaf rachis
The shape of leaf rachis is semicircular in cross section in accordance with the data from the literature (Figure 7).
The structure of the rachis consists of the epidermis, collenchyma and fundamental parenchyma with vascular tissues.

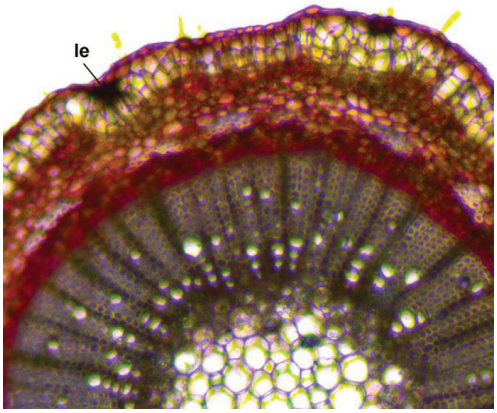

Figure 5. Cross section in the stem of *Vitex agnus-castus*: le – lenticels

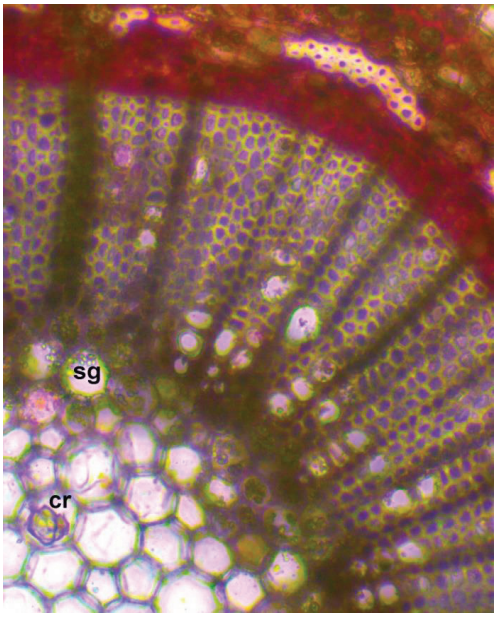

Figure 6. Cross section in the stem of *Vitex agnus-castus*: cr - calcium oxalate crystal; sg - starch granules

The epidermis is represented by the single layer of isodiametric cells of approximate 25 µm and covered by thin wax layer.
From place to place, on the epidermis there are observed the typically multicellular non glandular hairs with an average length of about 80 µm and also secretory multicellular hairs 2 (3) cells of approx. 20 µm long with the spherical secretory cell.

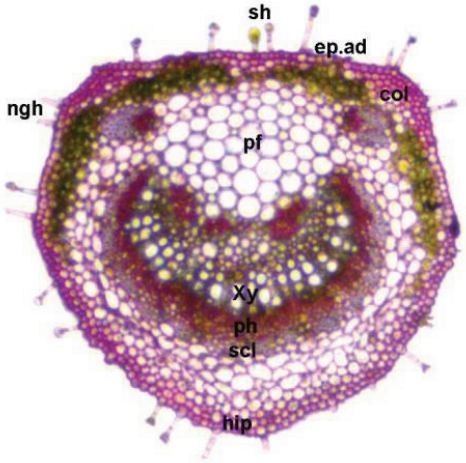

Figure 7. Cross section in the leaf rachis of *Vitex agnus-castus*: ep.ad – adaxial epidermis; hip – hypoderma; ngh – non glandular hairs; sh – secretory hairs; col – collenchyma; pf – fundamental parenchyma; ph – phloem; xy – xylem; scl – sclerenchyma

Under the epidermis there is one layer of collenchymatous cells but it is multi layered on the adaxial corners of the rachis.

Into the fundamental parenchyma it was observed one big central vascular bundle and two minor vascular bundles in the adaxial corners.

The vascular bundles are collateral with a primary structure, delimited by sclerenchyma tissue.

The phloem is thin, consisting of sieved vessels, annexe cells and little parenchyma. The xylem has vessels arranged in ray strings, separated by cellulosic parenchyma.

Anatomy of the leaflets

The epidermis, seen from the front is made up of cells with strongly corrugated walls (Figure 8).

The leaflets are hypostomatic of anomocytic type and annexe cells similar to epidermal cells, reconfirmed by the data from the literature (Figures 9 and 10).

In the cross-section of the leaflet, the midrib is strongly prominent on the underside with a unistratified epidermis and a thin cuticle. On the epidermis of midrib are observed non-glandular hairs and very rarely the secretory hairs (Figure 11 A).

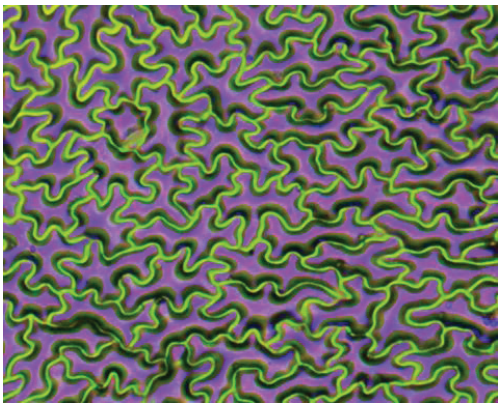

Figure 8. Analysis of upper epidermis (Ob. 10X)

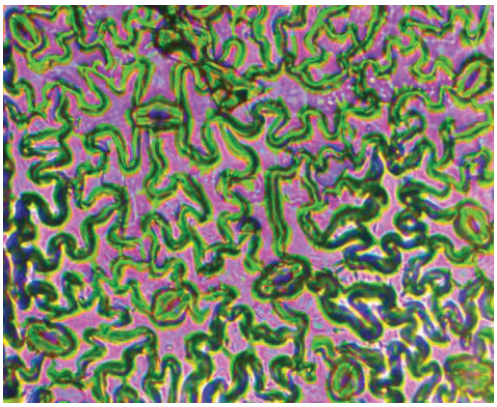

Figure 9. Analysis of lower epidermis (Ob. 20X)

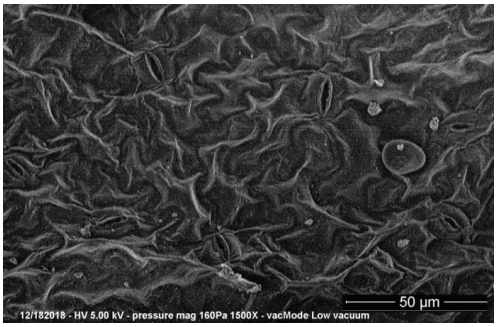

Figure 10. Micromorphological analysis of the lower epidermis (SEM 1500X)

In the fundamental parenchyma of the median rib there is a collateral vascular bundle, similar to the one in the rachis.

Both leaflets epidermis are unistratified with isodiametric cells and covered by a thin cuticle.

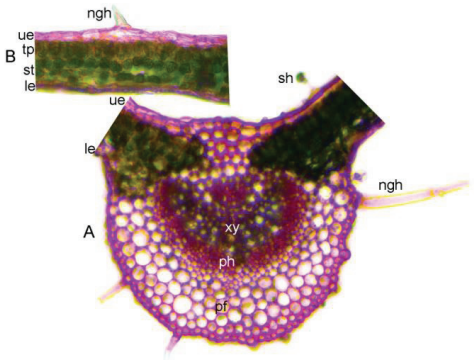

Figure 11. Cross section in the leaflet of *Vitex agnus-castus*: A. midrib; B. leaflets; ue – upper epidermis; le – lower epidermis; sh – secretory hairs; ngh – non glandular hairs; pf – fundamental parenchyma; ph – phloem; xy – xylem; tp – palisade tissue; st – spongy tissue

The upper epidermis has cells of about 11.80 µm larger than the lower epidermis which is about 7.12 µm.

In both epidermises there we observed non-glandular hairs, usually unicellular, with a conic-sharp tip.

The secretory hairs are particularly present in the lower epidermis along the ribs and this is also mentioned in some speciality papers (Mamoucha et al., 2016).

The mesophyll is bifacial type with palisade tissue toward upper epidermis and spongy tissue toward the lower epidermis (Figure 11 B).

Palisade tissue consists of a single layer of slightly elongated cells, rich in chloroplasts, without inter cellular spaces and with approx. 16.59 µm thickness.

The spongy tissue consists of two rows of isodiametric cells with a lower content in chloroplasts and intercellular spaces of approx. 29.65 µm thickness.

In the mesophyll there were observed the Calcium oxalate crystals and vascular bundles of collateral type were present.

In the studies of Yunus et al., published on 2008 year, there were not identified the secretory hairs on the epidermis of the leaflets and under the upper epidermis it was identified a layer of hypodermis.

CONCLUSIONS

After anatomically analysing the structure of young roots, stems and leaves of *Vitex agnus-castus* species were identified some characteristics:

The structure of the root is mainly secondary.

On the epidermis of the stem there were identified non-glandular hairs and rarely the secretory hairs.

In some stems there were observed the lenticels.

The leaflets are hypostomatic with anomocytic type of stomata.

In both epidermis of the leaflets there were present the non-glandular hairs but also secretory hairs, especially in the lower epidermis, along of the ribs.

The mesophyll of leaflet is bifacial type with only one cell layer of palisade parenchyma toward upper epidermis and two cells layer of spongy parenchyma toward the lower epidermis.

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