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## ESTABLISHING APPROPRIATE PARAMETERS FOR ROOTING OF MICROPROPAGATED PEAR ROOTSTOCK OHF X 333 (*PYRUS COMMUNIS* L.)

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### Abstract

The study was carried out in period September 2016 – April 2017, in laboratory for in vitro propagation of Fruit Growing Institute – Plovdiv. These rootstocks were obtained by hybridization of pear varieties of Old Home X Farmigdale in the American state of Oregon. Micropropagated plants *in vitro* were rooted on B (Dimanov, 1987) nutrient media in two different forms (solid and liquid with perlite) with different concentration of plants grow up regulators- auxins (IAA and IBA). The results show that the micropropagated plants of this rootstock are highly dependent from the nutrient media composition and concentration of grow up regulators for rooting. Plants with roots was produced 15 – 20 days after cultivated on nutrient media for rooting, but results were reported 30 days after cultivation date. According from forms (solid and liquid) and plants regulators –auxins (IBA and IAA), percent of the rooted plants it is different.

**Keywords:** pear rootstocks, rooting, micropropagated, *in vitro*.

### Introduction

The pear is valuable fruit crop in our and international market. In the last years the cultivated areas with this culture are significantly reduced. One of the real reasons are Fire blight caused by the enterobacterium *Erwinia amylovora* and incompatible of some pears cultivars with quince (*Cidonia oblonga*) rootstock. By biotechnological methods can be produce a pear rootstocks, resistant to *Erwinia amylovora*, nematodes and *Phytophthora cactorum*. Tolerant of drought, heavy and calcareous soils and low temperatures and suitable with all pear kinds. These rootstocks were obtained by hybridization of pear varieties of Old Home x Farmigdale in American state Oregon. Some clones of OH x F are difficult to propagate, that is why they are propagated in vitro. Also they are free from viruses, with guaranteed varietal authenticity.

### Material and methods

The attempts are conducted in laboratory for in vitro propagation of the Fruit-Growing Institute-Plovdiv in period September 2016-April 2017. The scope of this study is to explore the micropropagated rootstocks, for risogenesis on different variants B (solid and liquid with perlite) nutrient media. The Perlite was accepted as a component of the examined nutritive media that means easier manipulation with micro-cuttings from the practical point of view. The chemical composition of the nutrient media is combination of few basic ones medias for tissue cultures: macronutrients in ½ MS (Murashige and Skoog 1962), micronutrients in H (Heller, 1953), is removed AlCl<sub>3</sub> and vitamins in MW (Morel and Wetmore 1951). For rooting were used two plant growth regulators (IBA and IAA) in different concentration (0,5; 1,0; 1,5) mg/l. Like a carbohydrate source used 20g/l sucrose. The pH was adjusted to 5,7 ± 0,1 for all media prior to autoclaving at 121°C for 20 min and 1 atm. In solid nutrient media included 6g/l agar-agar. The plants are placed in a growth chamber at 22 ± 2 °C and photoperiod 16/8 light/dark. The study was carried out in glass jars with a volume of 600 ml (solid media) and 150 ml (liquid with perlite) (Fig.5). Each container contained 100 ml, respectively 40 ml of culture medium, on which 10 shoot tips with a length of 15 mm and 6 unfolded leaves were set.

Table 1. Rhizogenesis nutrient media with different PGRs concentration and forms

| Nutrient media | PGR | Concentration (mg/l) |
|----------------|-----|----------------------|
| B` solid       | IAA | 0,50                 |
| B` liquid      | IAA | 0,50                 |
| B1 solid       | IAA | 1,00                 |
| B1 liquid      | IAA | 1,00                 |
| B2 solid       | IAA | 1,50                 |
| B2 liquid      | IAA | 1,50                 |
| B3 solid       | IBA | 0,50                 |
| B3 liquid      | IBA | 0,50                 |
| B4 solid       | IBA | 1,00                 |
| B4 liquid      | IBA | 1,00                 |
| B5 solid       | IBA | 1,50                 |
| B5 liquid      | IBA | 1,50                 |

The results were reported 30 days after cultivation date. After propagated, the next step is rooting of the plants. Initially, the culture media B (Dimanov, 1987) was customized for Solanaceae sp, but in our case, depending from the forms (solid or liquid with perlite) and from PGRs concentration (Table 1) this media is suitable for OHFx333 rootstock.

### Results and discussion

Rhizogenesis induction in vitro and the rate at which the progress goes, it is essential for overall development of plants. The source nutrient media, showed that the rooting of the micro - propagated plants are heavily dependent from PGRs and the shape of the nutrient medium (liquid with perlite and solid); (Fig. 6). The results presents in Fig.1 shows that the most favorable for the rooting were obtained on solid culture media as opposed to liquid. The highest percentage of rooting we received for the plants cultivated on B` solid nutrient media ≈97% and B2 solid nutrient media ≈ 93%. Both of them are with same PGR (IAA), but in different concentration B` 0, 5 mg/l IAA, and B2 - 1, 5 mg/l IAA. In the other variants of media the percentage of rooted plants is smaller (Fig.1).

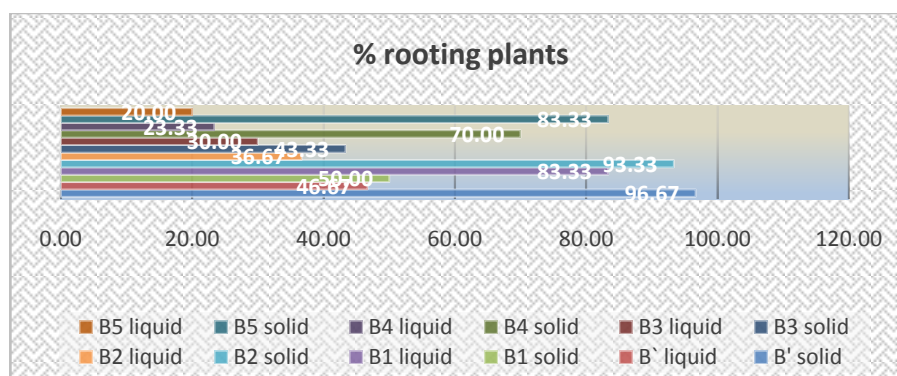


Figure 1. Rooting plants on different B media (%)

Taking into account the results, plants with average number of roots we have received again on B` solid and B2 solid media. The smallest average number of roots were obtained on B5 liquid media, with 1,5 mg/l IBA. (Fig.2) The next indicator, average length of the roots is showed on Fig.3. The induction of rhizogenesis and root system development to different degrees is reported for all media variants after 30 days of cultivation. Greatest average length of the roots is for plants rooted on B1 liquid media. Similar results were obtained on nutrient media B2 solid. The forms and PGR concentration are the differences between both of mediums. B1 liquid with perlite– 1, 00 mg/l IAA and B2 solid- 1, 5 mg/l IAA. Rooted plants with shortest roots were obtained on B4 liquid with perlite and B5 liquid with perlite media.

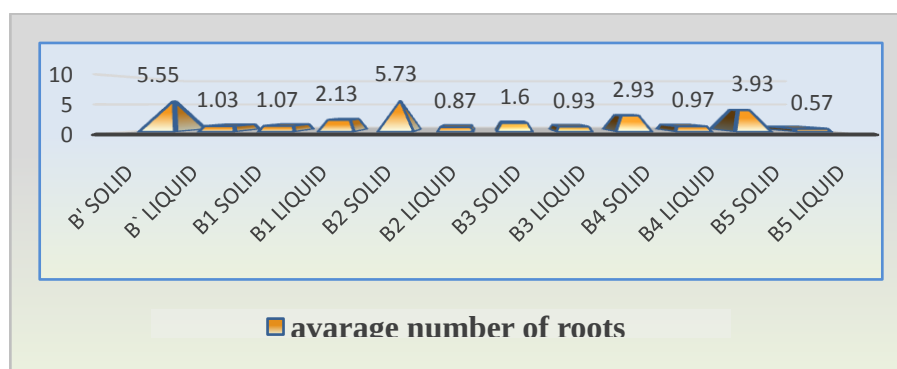


Figure 2. Average number of roots cultivated on variants B media, differences between solid and liquid with perlite and kind of PGRs and their concentration

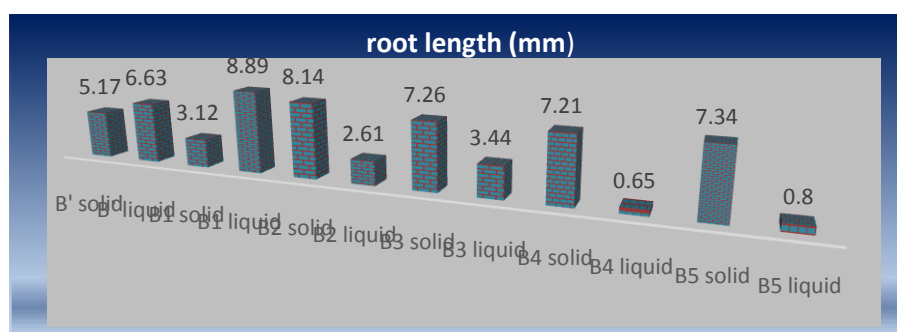


Figure 3. Average root length cultivated on variants B media, differences between solid and liquid with perlite and kind of PGRs and their concentration

The average length of roots is smaller than 1mm. After multiplication, the plants were set on rooting culture medium, with shoot length 15mm. For 30 days, shoots had grown up with different height. Fig.5. In attempt, highest shoot growth was measured on B4 solid and B5 solid. The both of them include the same composition. The only difference is concentration of PGR IBA. B4 solid – 1, 00 mg/l IBA and B5 solid – 1, 5 mg/l IBA. Rootstock with lowest shoots were obtained in B' liquid with perlite culture media.

In the first time, the medium B has been developed for tobacco rooting (Dimanov, 1987), later is successfully used for growth and development of varieties of potatoes, (Dimitrova and Dimanov 2002), representatives of the family *Solanaceaea sp.* (Dimanov et al., 2013), chrysanthemum (Dimanov et al. 2001), and grape (Roychev et al. 2002). Other research showed a successfully plant rooting in vitro from OHF x333 rootstock on MS medium with ¼ MS macro-nutrient, 1.00mg/l IBA and ¼ MS macronutrient, 1, 00 mg/l IAA (Kornova and Popov. 2014), where rooting is more than 90%, independently from the PGRs. Liquid B media was successfully used for potatoes in vitro rooting (Dimitrova and Dimanov 2002), but is not applicable for studied plants. Our results showed that apart MS basic media, for the pear rootstock OHFx333 can be used the other with different chemical composition, like B media.

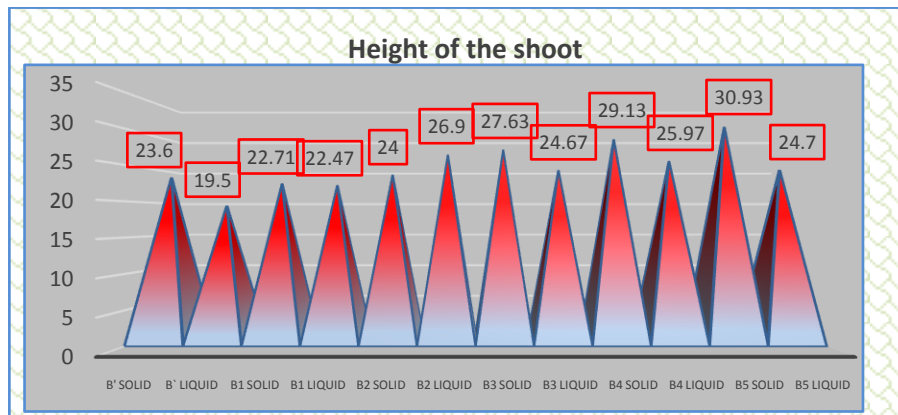


Figure 4. Average height of shoot (mm) on B nutrient media with differences PGR and forms (solid and liquid with perlite)



Fig.5. OHF x 333 rootstock in liquid with perlite and solid nutrient medium

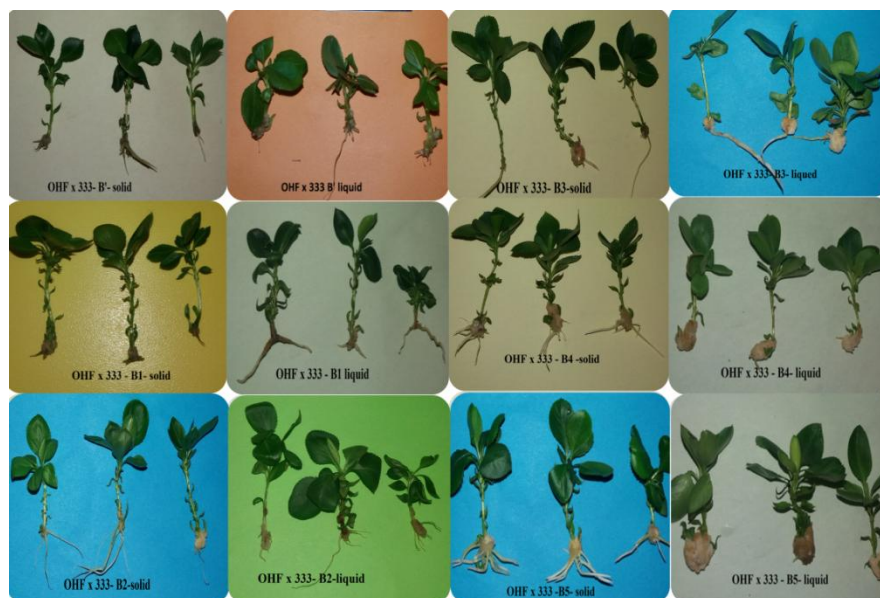


Figure 6. The rooting of the micro - propagated plants are heavily dependent from PGRs and the shape of the nutrient medium (liquid with perlite and solid)

### Conclusions

Successfully rooted micro-propagated plants of OHFx333 pear rootstock. Better results were achieved on solid nutrient medium than liquid. The highest percentage of rooting we received for

the plants cultivated on B` solid and B2 solid nutrient media. Greatest average length of the roots is for plants rooted on B1 liquid media. Highest shoot growth was received on B4 solid and B5 solid

#### References

1. Dimanov, D. (1987). Dissertation, Sofia, Bulgaria.
2. Dimanov, D., D. Dimitrova. and K. Varbanova. (2001). Influence of different nutritive regime over micropropagation of chrysanthemum in vitro. 129-134.
3. Dimanov, D., P. Kirovskyl and V.Roychev. (2001). Influence of different nutritive regime on *in vitro* cultivation of vine. In: Scientific works of Agricultural University- Plovdiv, XLVI (5): 251-255 (Bg).
4. Dimitrova, D., and Dimanov, D. (2002). *In vitro* micro-propagation of potatoes. Effect of differebt nutritive conditions, 141-146.
5. Dimanov, D., Masheva, V. and Dimitrova, D. (2013). Effect of nutritive media on *Solanaceae* sp. Reproduction in vitro, Bulgarian Journal of Agricultural Science, 19 (No 1) 2013, 139-142.
6. Kornova, K. and Popov, S. (2014). Micropropagation of pear rootstock OHF 333, Proceedings of Jubilee national scientific conference with international participation, House of science and technique - Plovdiv, pp. 111-116.
7. Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture, Plant Physiology, 15, 473-497
8. Heller, R., (1953). *Recherches sur la nutrition minérale des tissus végétaux cultivés in vitro* (Doctoral dissertation, Masson).
9. Morel, G. and Wetmore, R.H. (1951). Fern callus tissue culture. *Am. J. Bot.*, 38, pp. 141-143.

## THE IMPORTANCE OF PREDATORS IN CEREAL CROP AGROBIOCENOSIS IN LESKOVAC

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### Abstract

The monocultural way of grain crop production may cause the massive emergence and development of both harmful and useful organisms in agrobiocenosis. Predators as natural enemies give a significant contribution to the reduction of pest population. The aim of the research was to monitor the biodiversity of predators and establish the differences in their faunistic composition, with regard to the agrobiocenosis of both spring and winter crops. The following standard entomological methods were used throughout the research: visual inspection, examination of above-ground parts of a plant, the sweep net method and laboratory processing of gathered material. The research was carried out from 2011 to 2013 on the experimental plot of the Agricultural High School in Leskovac. The research shows that the diversity maintenance and natural bioregulatory mechanisms are the basic rule in preservation of the grain agrobiocenosis against harmful insects. The predatory activities towards aphids were registered among the following five species of ladybirds belonging to the family of Coccinellidae: *Coccinella septempunctata* L., *Adalia bipunctata* L., *Hippodamia variegata* Goeze, *Propylea quatuordecimpunctata* L. and *Calvia quatuordecimpunctata* L. and 2 predators of family Chrysopidae: *Chrysopa perla* L. and *Chrysopa carnea* Steph. The research results showed similarity among organisms present in respective agrobiocenoses of spring and winter crops that were compared.

**Keywords:** bioregulatory mechanisms, Coccinellidae, Chrysopidae, spring crops, winter crops.

### Introduction

Cereal growing had already been taking place when our ancestors inhabited these areas. The leading cereal variety in Serbia is wheat, followed by barley. Long-term cereal production in Serbia enabled the creation of specific conditions in agrobiocenosis and reproduction of various beneficial and harmful insects. Those are primarily the insects that attack small grains and some polyphagous insect species (Čamprag, 2000). Mineral nutrition of high-yield plants (Savić et al., 2007) strengthens their protection mechanisms against various unfavourable ecological factors. Dense plantations and monoculture are the inevitability of contemporary crop production. Modern cereal growing and the use of agrochemicals often harm the natural balance by exterminating beneficial insects, predators and pests. Although the pesticide use in cereal production is not as widely spread as in the production of fruit, vegetables and vineyard crops, it still involves the use of soil insecticides before sowing, as well as the use of herbicides in early spring and insecticides against *Lema melanopus* L. in the dough developing stage of cereal growth (Spasov et al., 2014).

Aphids (Aphididae) are among the leading members of harmful cereal entomofauna, as they appear every year and cause harm usually after the ear formation phase (Petrović-Obradović, 2003). Predatory species that are natural part of cereal agrobiocenosis have a significant role in number reduction of aphids (Lazarevska, 1998). Out of all natural autochthonous aphids predators that have a significant bioregulatory effect on cereal agrobiocenosis in Leskovac, the most numerous ones are the ladybird populations (Coccinellidae) (Nikolić et al., 2016). Insects belonging to the family Chrysopidae (green lacewings) have a special place in integral protection. Members of this family have great consumptive power, high biological potential and a significant role in the biological battle all over the world (Krsteska et al., 2005).

Chemical protection measures against aphids are not extensively used in the production of small grains in Serbia. Therefore, cereal fields can be enormous ecological reservoirs for the preservation of beneficial insects and their natural populations. Due to all previously mentioned, grain crops provide ideal ecological conditions for the entomofauna preservation of the agroecosystem (Thalji et al., 2005).

The more complex and diverse agrobiocenosis is, the more resilient it is, thus having more efficient mechanisms for natural pest regulation (Čamprag, 2000). Specific conditions present in an agrobiocenosis lead to the emergence of numerous beneficial and harmful insects. This further leads to the basic goal of the research, aiming to monitor the biodiversity of predators and determine the differences in their respective faunistic composition in the agrobiocenosis of spring and winter grain crops.

### Material and methods

The research was carried out in the territory of Leskovac from 2011 to 2013. The research facilities were set up on the research plot of Agricultural High School in Leskovac. The research included:

- The micro-plot experiment with spring crops (set up on 19/04/2011) and the varieties of Vojvoda, Smaragd, Jadran, Slavuj, Rajac, Lovcen and Vranac.
- The micro-plot experiments with winter crops (set up on 18/11/2011 and 27/11/2012) and the varieties of Zemunska Rosa, Aleksandra, Srma, Janja, Zenit, Nirvana, Cipovka, 34/D-durum).

The following standard entomological methods were used for the emergence detection of beneficial and harmful organisms in natural agrobiocenosis:

- Visual method – used for monitoring the presence of beneficial and harmful insects. The examinations were carried out every 7-10 days during the entire research period, depending on the insect population dynamics and in accordance with the variability of meteorological conditions.
- Examination of above-ground plant organs – used for the estimation of aphids population number and the number of beneficial insects. The examinations were carried out after the ear formation phase.
- Hand reaping – used for monitoring the presence of beneficial insects (ladybirds and green lacewings) among densely planted crops.
- Laboratory treatment – the material gathered during the entire research period was subjected to taxidermy, labelling, determination and collection creation.

### Results and discussion

The research carried out from 2011 to 2013 provided a diversity review of the most prevalent aphids predators found in cereal crops. The awareness that natural bioregulatory mechanisms exist is the basic rule for the preservation of cereal crop agrobiocenosis from harmful insects. To preserve the complexity of bioregulatory mechanisms, it is necessary to conduct the appropriate monitoring procedures in the agrobiocenosis of cereal crops (Nikolić et al., 2012). Čamprag (2000) stated that the more complex and diverse the agrobiocenosis is, the more resilient it is; thus, its mechanisms for natural pest regulation are more efficient.

The presence of aphids (Aphididae) was determined during all the research years. Their number was the highest after the ear formation phase. In 2011 and 2013, the degree of the pest attack (Kolektiv autora, 1983) was labelled as a very weak infection (the emergence of a small colony). In 2012, the degree of the pest attack was labelled as a weak infection (a small number of small colonies present among plants). The meteorological conditions during the research period (high temperatures and dry weather) had the greatest influence on aphids development in 2012.

Predatory species that are naturally found in the agrobiocenosis of cereal crops play a significant role in the reduction process of aphids. The efficiency of natural predators, i.e. the quantity of aphids that predators can possibly eat, depends on the food availability from other sources (from nearby

plots). The unfavourable weather conditions and the lack of primary food sources may force ladybirds (or other predators) to feed from secondary food sources that are insufficient to provide for the species reproduction, but sufficient enough to enable the species survival (Lazarevska, 1998).



Picture 1. *Hippodamia variegata* Goeze on a leaf

During the research in Leskovac, the predatory activity against aphids was registered among the following five ladybird species belonging to the family Coccinellidae (Coleoptera): *Coccinella septempunctata* L., *Adalia bipunctata* L., *Hippodamia variegata* Goeze (Figure 1), *Propylea quatuordecimpunctata* L. and *Calvia quatuordecimpunctata* L. Two species of green lacewings belonging to the family Chrysopidae (Neuroptera), namely *Chrysopa perla* L. and *Chrysopa carnea* Steph. were also registered as predators (Picture 2).



Picture 2. *Chrysopa perla* L. on a ear

*C. septempunctata* had the greatest prevalence and activity during all three research years, whereas *P. quatuordecimpunctata* had the least. Classified by the research years, ladybirds displayed the

greatest predatory activity in 2012, when the greatest number of aphids was also recorded. The increased presence of weeds was registered in the peripheral parts of the research plots, where the increased number of aphids and ladybirds was also registered during all visual examinations. The most prevalent weeds were: *Chenopodium album* L. (Chenopodiaceae), *Atriplex patula* L. (Chenopodiaceae), *Convolvulus arvensis* L. (Convolvulaceae), *Sorghum halepense* L. (Poaceae), *Cichorium intybus* L. (Asteraceae) and *Polygonum persicaria* L. (Polygonaceae). The greatest number of aphids colonies was registered among weed species belonging to the family Chenopodiaceae (Picture 3).

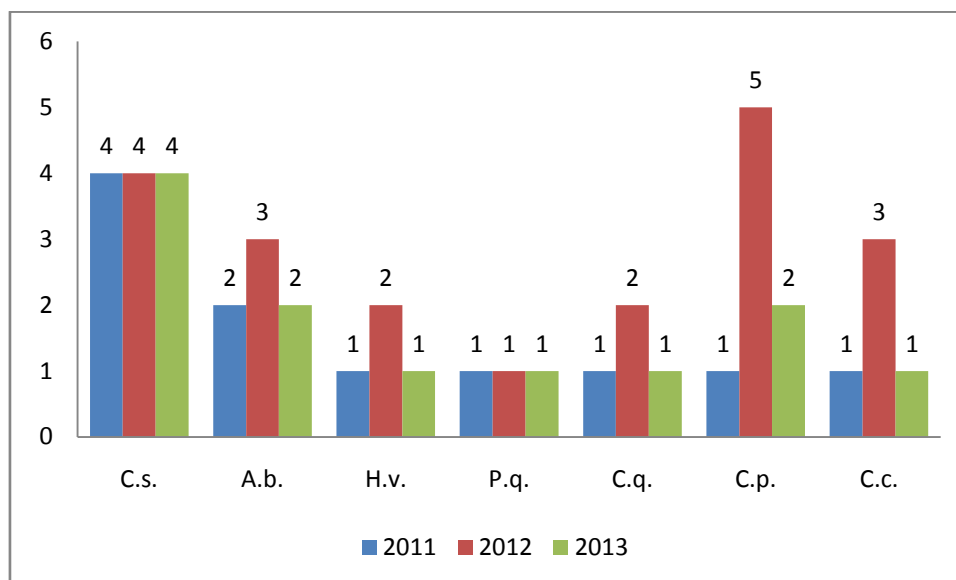


Picture 3. Aphids colonies and larvae *C. septempunctata* L. on a Chenopodiaceae

The research results showed the similarity among the registered predators present in the compared agrobiocenoses of spring and winter grain crops (Graph 1).

Throughout all three research years, the members of registered predatory species were found during four visual examinations. The greatest prevalence and activity during all three research years was exhibited by *C. septempunctata* and *C. perla*, which are cosmopolitan species and play a significant role in the biological battle all over the world (Krsteska et al., 2005).

During the research on wheat and weed vegetation belonging to the family Poaceae which was carried out in Strumica (Republic of Macedonia), Spasov et al. (2013) determined the presence of five predatory species: *C. septempunctata* L. (Coccinellidae, Coleoptera), *Adonia variegata* Gz. (Coccinellidae, Coleoptera), *A. bipunctata* L. (Coccinellidae, Coleoptera), *C. carnea* Steph. (Chrysopidae, Neuroptera) and *Scaeva pirasti* L. (Syrphidae, Diptera).



Graph 1. The prevalence of predators among cereal crops in Leskovac from 2011 to 2013

C.s. - *Coccinella septempunctata* L.

A.b. - *Adalia bipunctata* L.

H.v. - *Hippodamia variegata* Goeze

P.q. - *Propylea quatuordecimpunctata* L.

C.q. - *Calvia quatuordecimpunctata* L.

C.p. - *Chrysopa perla* L.

C.c. - *Chrysopa carnea* Steph.

Thalji et al. (2009) recorded the predatory activity of seven ladybird species during the research carried out in the surroundings of Novi Sad in 2008. Five out of seven ladybird species that they recorded were also found in our research: *C. septempunctata*, *A. bipunctata*, *H. variegata*, *P. quatuordecimpunctata* and *C. quatuordecimpunctata*. The remaining two ladybird species that were recorded by Thalji et al. but were not found in our research were *Hippodamia tredecimpunctata* L. and *Hippodamia undecimnotata* Schn. These authors claim that ladybirds are far more efficient in comparison to other predatory species, as both larvae and imagoes feed on aphids and thus act as active predators. Since the production of cereal crops does not use extensive chemical measures in the battle against aphids, cereal fields are an enormous ecological reservoir for the preservation of beneficial insects and their natural populations, leaf lice predators in particular. Our research on both spring and winter grain crops carried out from 2011 to 2013 reached the same conclusion.

## Conclusions

The basic rule for the preservation of cereal crop agrobiocenosis from harmful insects is diversity maintenance and the existence of natural bioregulatory mechanisms among harmful and beneficial insects. The presence of aphids (Aphididae) was registered in the cereal crop entomofauna after the ear formation phase throughout all the research years. The greatest number of aphids was recorded in 2012, when the meteorological conditions contributed the most to their development.

The research (2011-2013) carried out in Leskovac determined the predatory activity against aphids exhibited by five ladybird species: (Coccinellidae, Coleoptera): *C. septempunctata* L., *A. bipunctata* L., *H. variegata* Goeze, *P. quatuordecimpunctata* L. and *C. quatuordecimpunctata* L. Two species of green lacewings, namely (Chrysopidae, Neuroptera) *C. perla* L. and *C. carnea* Steph, also displayed predatory activity against aphids. *C. septempunctata* and *C. perla* displayed the greatest prevalence and activity during all three research years. Classified by the research years, the greatest predatory activity was registered in 2012, when aphids were also the most numerous.

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### References

1. Kolektiv autora (1983): Priručnik izveštajne i prognozne službe zaštite poljoprivrednih kultura. Savez društva za zaštitu bilja Jugoslavije, Beograd, 682.
2. Krsteska, V., Ančev, E., Postolovski, M., Vuković, M. (2005): Vidovi od familijata Chysopidae – predatori na lisnite voški na tutunot. Tutun/Tobacco, Vol. 55, No 9-10, 204-210, Institut za tutun – Prilep, R. Makedonija.
3. Lazarevska, S. (1998): Entomocenozata na pšenica vo uslovi na različen priod vo zaštitata na kulturata. Doktorska disertacija, Univerzitet „Sv. Kiril i Metodij“ Zemjodelski fakultet Skopje, 307.
4. Nikolić, K., Tmušić N., Gudžić, S. (2012): Značaj monitoringa entomofaune žitnih kultura u zaštiti životne sredine. Zbornik radova 2, Peti međunarodni kongres “Ekologija, zdravlje, rad, sport”, Banja Luka, 06-09.09.2012., 177-181, Udruženje “Zdravlje za sve”, Banja Luka, Republika Srpska.
5. Nikolić, K., Tmušić, N., Gudžić, S. (2016): Bioregulatorska uloga bubamara u agrobiocenozi žitnih kultura. Zbornik radova, Osmi međunarodni kongres „Ekologija, zdravlje, rad, sport“, Banja Luka, 19-21.05.2016., 281-285, Udruženje „Zdravlje za sve“, Banja Luka, Republika Srpska.
6. Petrović-Obradović, O. (2003): Biljne vaši (Homoptera: Aphididae) Srbije. Poljoprivredni fakultet Univerziteta u Beogradu, 153.
7. Savić, N., Jelić, M., Knežević, J., Nikolić, K. (2007): Uticaj mineralne ishrane azotom na visinu biljke i dužinu klasa kod različitih sorti ozime pšenice. Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu, Specijalno izdanje: XIX naučno-stručni skup poljoprivrede i prehrambene industrije - Neum, 2006., godina LII, Volume LII, broj 58/2, Sarajevo, 37-42.
8. Spasov, D., Spasova, D., Atanasova, B., Serafimova, M. (2013) Aphids (Homoptera: Aphididae) and their predators, in wheat (*Triticum aestivum*) and in the weeds from Poaceae family in the Strumica region. Scientific works, 2 (1). pp. 187-191.
9. Thalji, R., Taldži, R., Štrbac, P. (2009): Dinamika populacije biljnih vašiju i njihovih predatora na ozimoj pšenici u okolini Novog Sada. Biljni lekar, god. XXXVII, br. 4, 373-381, Poljoprivredni fakultet Novi Sad, Departman za fitomedicinu i zaštitu životne sredine.
10. Čamprag, D. (2000): Integralna zaštita ratarskih kultura od štetočina. Desing studio Stanišić, Bačka Palanka, Poljoprivredni fakultet Institut za zaštitu bilja i životne sredine „Dr Pavle Vukasović“, Novi Sad, 215.

**NEW OPPORTUNITIES FOR CHEMICAL CONTROL OF *VENTURIA INAEQUALIS* AND  
*PODOSPHAERA LEUCOTRICH*A IN APPLE ORCHARDS IN MACEDONIA**

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**Abstract**

The possibility of simultaneous effective control of apple scab and apple powdery mildew, by using single fungicidal active substance, and to compare the obtained results with those from standard chemical control, were the main goal of these experiments. In this study two fungicides were included: Sercadis (a.m. fluxapyroxad) and Indar 5 EW (a.m. fenbuconazole). Experiment was conducted during the 2016 in region of Prespa and region of Tetovo, on two apple varieties, idared and golden delicious. In untreated variant in region of Tetovo, significantly high level of infection of apple scab (43%) and apple powdery mildew (18,2%) were recorded. In region of Prespa, the situation with the control variant was quite the opposite (15,2% apple scab and 36% apple powdery mildew infection). Regarding the efficacy of tested fungicides, in region of Tetovo, the standard fungicide Indar 5 EW (a.m. fenbuconazole) provided protection efficiency of 94,58% against apple scab and 98,18% against apple powdery mildew. In the same region, the efficacy in variants treated with fungicide Sercadis was quite similar (92,65% efficacy protect against apple scab and 98,68 against apple powdery mildew). In region of Prespa, the efficacy performance of standard fungicide against apple scab and powdery mildew (95,65% and 94,44% respectively) was almost equal with the performance of fungicide Sercadis (94,53% and 96,66%).

**Keywords:** apple scab, apple powdery mildew, Sercadis.

**Introduction**

Apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) are the economically most important apple diseases in all temperate climate production regions worldwide (Ivanovic, 1992). In the absence of proper and adequate control measures, both pathogenic fungi, can cause severe economically losses, which can reach up to 100%, especially in susceptible varieties (Ivanovic, 1992). Applications of fungicides throughout the growing season at weekly intervals, have been the foundation of apple scab management for the past 70 years (Kerik, 2015). In commercial plantations, from 10 up to 30 fungicidal treatments are performed throughout the vegetation (Soriano et al., 2009, Mac Hardy et al., 2001, Eccel et al., 2009). The excessive use of synthetic fungicides, raised a range of environmental issues, human health problems and increased the costs of production (Lespinasse et al., 2002). In this regard, apple producers continually seek to reduce the number of fungicide applications and improve the timing of applications by capitalizing on postinfection activity. As older broad-spectrum fungicides with postinfection activity were taken off from the market due to health concerns in the 1960s, only the “modern” single-site fungicides introduced in the later part of the twentieth and beginning of the twenty-first centuries could offer postinfection activity. Unfortunately, the introduction of new single-site fungicide, occurred in a slow successive manner and led to the unfortunate practice of overreliance and development of fungicide resistance in many of the fungicide classes effective for the management of apple scab and powdery mildew (Kerik, 2015). Numerous apple growers have lost or are in danger of losing several classes of highly effective fungicides for control of apple scab and powdery mildew, due to the development of

resistance (Turechek and Köller, 2004). For these reasons, the search for new active molecules, or the improvement of the existing, combination of old improved active molecules with new and similar, are a constant task of the manufacturers of fungicides (Kerik, 2015).

SDHI fungicides are derived from a diverse range of chemistry and, depending on the host and pathogen, have protectant, translaminar or systemic activity. They have been given the FRAC activity group code number 7 (NZPPS 2009) and comprise seven chemical groups including phenyl-benzamides and pyridinyl-ethyl-benzamides as well as furan-, oxathiin-, thiazole-, pyrazole- and pyridine-carboxamides (Beresford, 2011). SDHI fungicides specifically inhibit fungal respiration by blocking the ubiquinone-binding sites in the mitochondrial complex II (Avenot and Michailides 2010). Generation I of SDHIs, including carboxin and oxycarboxin, have been used internationally since the late 1960s and are highly effective against basidiomycete pathogens such as rusts or *Rhizoctonia* spp. The newer generation II of SDHIs, such as boscalid, fluxapyroxad, penthiopyrad, isopyrazam and fluopyram, have a spectrum of activity against a broader range of fungal pathogens of various crops (Avenot and Michailides 2010). Generation II of SDHIs are intended for use in integrated disease management programmes, or as mixing or alternation partners to prevent fungicide resistance. Fungicides from this class are effective against various diseases of cereals, grape, stonefruit, strawberry, kiwifruit, apple and cucurbit (Beresford, 2011).

The main goal of the experiment was to study the possibility for control of apple scab and powdery mildew, by use of innovative fungicide Sercadis (a.m. fluxapyroxad) and to compare the results with those from standard chemical control of this disease.

### Material and methods

The trial was conducted during the 2016 in two regions in the Republic of Macedonia: Prespa (variety Idared) and Tetovo (variety Golden delicious). In both tested regions, the experiment was consisted of 3 variants. Each variant was comprised of 10 apple trees. Two variants were treated with fungicides, while the third one served as control (Table 1). The treatments were performed with a pesticide sprayer with a volume of 10 L. During the trial, total of 3 treatments of apple trees were performed, with spraying interval of 7 days. Evaluation of the efficacy of the tested fungicides was performed 7 days after the last treatment. For that purpose, scale from 0 to 5 was used. The intensity of infection was calculated according to the formula of Townsend-Heuberger (1943), while the fungicide efficacy was evaluated by the formula of Abbott (1925).

Table 1. Variants in both tested regions (Prespa and Tetovo)

| No. | Active ingredient   | a.m. in formulation | Dosage   |
|-----|---------------------|---------------------|----------|
| 1.  | Fluxapyroxad        | 300 g/L             | 0,3 L/ha |
| 2.  | Fenbuconazole       | 50 g/L              | 1 L/ha   |
| 3.  | Control (untreated) |                     |          |

### Results and discussion

The obtained results regarding the intensity of infection and efficacy of the tested fungicides in the control of apple scab (*V. inaequalis*) and powdery mildew (*P. leucotricha*) are presented in Table 2 and Table 3.

The destructive potential of these two economically important diseases was confirmed in the control variants in two tested regions. In untreated variant in region of Tetovo, significantly high level of infection of apple scab (43%) and apple powdery mildew (18,2%) were recorded. In region of Prespa, the situation with the control variant was quite the opposite (15,2% apple scab and 36% apple powdery mildew infection).

Table 2. Intensity of infection and efficacy of tested fungicides in control of *Venturia inaequalis* in both tested regions

| Variant                 | Region of Prespa           |                            | Region of Tetovo           |                            |
|-------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                         | Intensity of infection (%) | Efficacy of fungicides (%) | Intensity of infection (%) | Efficacy of fungicides (%) |
| Fluxapyroxad (Sercadis) | 0,83                       | 94,53                      | 3,16                       | 92,65                      |
| Fenbuconazole (Indar)   | 0,66                       | 95,65                      | 2,33                       | 94,58                      |
| Control                 | 15,2                       | -                          | 43                         | -                          |

Table 3. Intensity of infection and efficacy of tested fungicides in control of *Podosphaera leucotricha* in both tested regions

| Variant                 | Region of Prespa           |                            | Region of Tetovo           |                            |
|-------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                         | Intensity of infection (%) | Efficacy of fungicides (%) | Intensity of infection (%) | Efficacy of fungicides (%) |
| Fluxapyroxad (Sercadis) | 1,2                        | 96,66                      | 0,24                       | 98,68                      |
| Fenbuconazole (Indar)   | 2                          | 94,44                      | 0,33                       | 98,18                      |
| Control                 | 36                         | -                          | 18,2                       | -                          |

Regarding the efficacy of tested fungicides, the results clearly showed that the novel fungicide Sercadis achieved extremely high efficacy in the control of the apple scab (94,53% and 92,65% respectively) and powdery mildew (96,66% and 98,68 respectively) in both tested regions. The standard fungicide Indar 5 EW (a.m. fenbuconazole) has also achieved high efficacy in the control of apple scab and powdery mildew, in both tested regions. This, in the region of Prespa, the efficacy performance of standard fungicide against apple scab and powdery mildew (95,65% and 94,44% respectively) was almost equal with the performance of fungicide Sercadis (94,53% and 96,66%). Similar results were obtained by other authors in the control of apple scab and apple powdery mildew (Cox, 2015; Beresford, 2011; Villani and Nance, 2017; Berrie and Xu, 2010). These authors, considered that SDHI fungicides, especially the II generation (such as boscalid, fluxapyroxad, penthiopyrad, isopyrazam and fluopyram), have a spectrum of activity against a broader range of fungal pathogens of various crops, and their efficiency in apple production is currently high (Avenot and Michailides, 2010). Generation II SDHIs are intended for use in integrated disease management programmes, or as mixing or alternation partners to prevent fungicide resistance. Fungicides from this class are effective against various diseases of cereals, grape, stonefruit, strawberry, kiwifruit, apple and cucurbit (Beresford, 2011).

### Conclusions

Apple scab and powdery mildew are diseases with devastating impact in apple production, which can result in decreased fruit quality and yield. Moreover, severe foliar infection can lead to premature defoliation and reduced tree vigor, which in turn may restrict or prevent formation of fruit buds for the next year. The obtained results in this study showed that two tested fungicides (Sercadis and Indar) achieved very high level of efficacy in the control of the two economically most important apple diseases (*Venturia inaequalis* and *Podosphaera leucotricha*). Still, fungicide applications require careful attention to timing, as preventing early infection is the most important step toward successfully controlling later infections. Moreover, good sanitation and cultural control practices can also minimize the need for fungicide sprays at some extent.

## References

1. Avenot HF, Michailides TJ 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Protection* 29: 643-651.
2. Beresford, R.M., 2011. Succinate dehydrogenase inhibitor (SDHI) fungicide resistance prevention strategy. New Zealand plant protection society (Inc). Plant & Food Research, Private Bag 92169, Auckland 1142, New Zealand.
3. Berrie, A.M., Xu, X.M., 2010. Managing apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) using Adem™. *International Journal of Pest Management*, 26.11.2010, p. 243-249.
4. Cox, K.D., 2015. Managing apple diseases with new fungicides. Dept. of Plant Pathology and Plant-Microbe Biology.  
<http://www.hort.cornell.edu/expo/proceedings/2016/TreeFruit.Managing%20Apple%20Diseases%20with%20New%20Fungicides.Cox.pdf>
5. Eccel, E., Rea, R., Caffara, A., Crisci, A. (2009). Risk of spring frost to apple production under future climate scenarios: the role of phenological acclimation. *Int J Biometeorol.* 53, pp. 273 – 286.
6. European commission, 2016. EU Pesticide database. [https://ec.europa.eu/food/plant\\_en](https://ec.europa.eu/food/plant_en)
7. Ivanovic, M., 1992. Mikoze biljaka. I izdanje. IP “Nauka”, Beograd.
8. Kerik D. C., 2015. Fungicide Resistance in *Venturia inaequalis*, the Causal Agent of Apple Scab, in the United States. *Fungicide Resistance in Plant Pathogens* pp. 433-447.
9. Lespinasse, Y., Pinet, C., Laurens, F., Durel, C.E., Parisi, L. (2002). European research for durable resistance to scab on apple: the D.A.R.E. project. *Acta Horticulturae (ISHS)*, 595, pp. 17 – 22.
10. Mac Hardy, W.E., Gadoury, D.M., Gessler, C. (2001). Parasitic and biological fitness of *Venturia inaequalis* relationship to disease management strategies. *Plant Dis.* 85, pp. 1036 – 1051.
11. Soriano, J.M., Joshi, S.G., van Kaauwen, M. (2009). Identification and mapping of the novel apple scab resistance gene *Vd3*. *Tree Genetics and Genomes*. 5, pp. 475 – 482.
12. Turechek, W.W., Köller, W., 2004. Managing Resistance of *Venturia inaequalis* to the Strobilurin Fungicides. *Plant Management Network*. Published 8 September 2004.
13. Urbanovich, O., Kazlovskaya, Z. (2008). Identification of scab resistance genes in apple trees by molecular markers. *Scientific works of the Lithuanian institute of horticulture and Lithuanian University agriculture*, 27 (2), pp. 347 – 357.
14. Villani, S.M., Nance, D.A., 2017. Evaluation of fungicide efficacy for the management of apple scab, powdery mildew, and cedar apple rust on 'Rome Beauty' apples in NC, 2016. *Plant disease Management report*. The American Phytopathological Society, March, 07.2017.

## BIOLOGICAL CONTROL OF *VENTURIA INAEQUALIS* – THE CAUSE OF APPLE SCAB IN APPLE

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### Abstract

The main goal of the experiment was to study the possibility for biological control of apple scab by use of innovative biofungicide Vacciplant (a.m. Laminarin) and to compare the results of biological control with those from standard chemical control of this disease. Experiment was conducted during the 2016 in region of Prespa and region of Tetovo, on two apple varieties, idared and golden delicious. In untreated variant in region of Tetovo, was observed very high level of infection (77.21% on the leaves and 24.35% on the fruits), which demonstrated the destructive potential of this apple disease in our country. In region of Prespa, significantly lower level of infection was observed in untreated variant (30% on the leaves and 9.5% on the fruits). Regarding the efficacy of tested fungicides, in region of Tetovo, standard fungicide Merpan (a.m. captan) used in chemical variant provided considerably lower degree of efficacy on leaves and fruits (71.38% and 60.86% respectively), compared with biofungicide used in biological variant (95.13% and 94.78% respectively). In region of Prespa, the efficacy performance of standard fungicides on the leaves and fruits (98.33% and 100% respectively) was almost equal with the performance of biofungicide (99.16% and 100%).

**Keywords:** apple scab, biological control, Vacciplant.

### Introduction

Apple scab, caused by *Venturia inaequalis* (Cooke) G. Wint., is the most important apple disease, which causes economic losses in apple production worldwide. Without the application of appropriate control measures, the damages can reach up to 100%, especially in susceptible varieties (Ivanovic, 1992). Besides, it leads to early defoliation (Urbanovich and Kazlovskaya, 2008). Its significance is indicated by the fact that up to 30 fungicide treatments are performed per season to control apple scab disease (Soriano et al., 2009; Mac Hardy et al., 2001; Eccel et al., 2009). Such a large number of chemical treatments raise a range of environmental issues, human health problems and cost increases (Lespinasse et al., 2002). Forecasting models which are based on weather data can reliably predict infection periods, but the success of the control strategies mainly depends on the availability of efficient curative fungicides. In the integrated production several chemical fungicides have been used until *V. inaequalis* developed resistances against these active ingredients (Jones, 1981; Kunz et al., 1997). Biological control of apple scab is has been investigated for over 50 years, but most of this researches are focused on overwintering stage of *Venturia inaequalis* (Kohl et al, 2015). Laminarin, the active ingredient of Vacciplant, is an oligosaccharide extracted from the seaweed *Laminaria digitata*. It is an elicitor which triggers plant natural resistance via the induction of plant defense mechanisms. Vacciplant included in protection programs against scab ensures similar protection to conventional programs and can results with the reduction of chemical fungicide treatments before harvesting allowing reducing the quantity of residues and the number of detected fungicide molecules in apple (Mery and Joubert, 2012).

The main goal of the experiment was to study the possibility for biological control of apple scab by use of innovative biofungicide Vacciplant (a.m. Laminarin) and to compare the results of biological control with those from standard chemical control of this disease.

### Material and methods

Experiment was conducted during the 2016 in two main regions for apple production in the Republic of Macedonia, Prespa and Tetovo, on two apple varieties, idared and golden delicious. In both tested regions, the experiment was comprised of 3 variants and in each variant 10 apple trees were included. Two variants were treated with fungicides, while the third one served as control (Table 1).

Table 1. Variants included in both tested regions (Prespa and Tetovo)

| No. | Active ingredient   | a.m. in formulation | Dosage   |
|-----|---------------------|---------------------|----------|
| 1.  | Laminarin           | 45 g/L              | 1 L/ha   |
| 2.  | Captan              | 80%                 | 2.5 L/ha |
| 3.  | Control (untreated) |                     |          |

The treatments were performed with a pesticide sprayer with a volume of 10 L. During the trial, total of 5 treatments of apple trees were performed, with spraying interval of 7-10 days. Evaluation of the efficacy of the tested fungicides was performed 7 days after the last treatment. For that purpose, scale from 0 to 5 was used. The intensity of infection was calculated according to the formula of Townsend-Heuberger (1943), while the fungicide efficacy was evaluated by the formula of Abbott (1925).

### Results and discussion

The obtained results regarding the intensity of infection and efficacy of tested fungicides in the control of apple scab (*V. inaequalis*) are presented in Table 2 and Table 3.

Table 2. Intensity of infection and efficacy of tested fungicides in control of *Venturia inaequalis* in region of Prespa

| Variant   | Intensity of infection on leaves (%) | Efficacy of fungicides on leaves (%) | Intensity of infection on fruits (%) | Efficacy of fungicides on fruits (%) |
|-----------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Laminarin | 0.25                                 | 99.16                                | 0                                    | 100                                  |
| Captan    | 0.5                                  | 98.33                                | 0                                    | 100                                  |
| Control   | 30                                   | -                                    | 9.5                                  | -                                    |

In untreated variant in region of Tetovo, very high level of infection was observed (77.21% on the leaves and 24.35% on the fruits), which demonstrated the destructive potential of this apple disease in our country. In region of Prespa, significantly lower level of infection was observed in untreated variant (30% on the leaves and 9.5% on the fruits).

Regarding the efficacy of tested fungicides, in region of Tetovo, standard fungicide Merpan (a.m. captan) used in chemical variant provided considerably lower degree of efficacy on leaves and fruits (71.38% and 60.86% respectively), compared with biofungicide used in biological variant (95.13% and 94.78% respectively). In region of Prespa, the efficacy performance of standard fungicides on the leaves and fruits (98.33% and 100% respectively) was almost equal with the performance of biofungicide (99.16% and 100%). Similar results regarding the efficacy of biofungicide Vacciplant in the control of apple scab, were obtained in other two studies. Thus, Mery and Joubert (2012) and Bernardon et al. (2013), reported that a.i. laminarin used to control secondary infections of apple scab (from July till harvest), ensured protection which was equivalent to conventional programmes for control of apple scab.

Table 2. Intensity of infection and efficacy of tested fungicides in control of *Venturia inaequalis* in region of Tetovo

| Variant   | Intensity of infection on leaves (%) | Efficacy of fungicides on leaves (%) | Intensity of infection on fruits (%) | Efficacy of fungicides on fruits (%) |
|-----------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Laminarin | 3.76                                 | 95.13                                | 1.27                                 | 94.78                                |
| Captan    | 22.09                                | 71.38                                | 9.53                                 | 60.86                                |
| Control   | 77.21                                | -                                    | 24.35                                | -                                    |

### Conclusions

Management of apple scab can be achieved by planting resistant cultivars, cultural practices and fungicide applications. Still, the management is mainly based on repeated fungicide applications in the season, that result in high costs in terms of money for the fungicide applications and in time dedicated to scab management. Because of increasing pressure on apple growers to reduce pesticide use and reduce production costs, while maintaining a high level of crop quality, it is crucial to simplify and optimize apple scab management. Moreover, development of resistance to synthetic fungicides complicates the situation further.

The development of novel antagonists for biological control of apple scab may offer alternative options for disease control. The results obtained in this study showed that the novel biofungicide Vacciplant (a.i. laminarin) achieved almost equal reduction of apple scab incidence and has a prospective use for control of *Venturia inaequalis* in apple production.

### References

- Bernardon, A M., Joubert, J-M, Horeau, A., 2013. Laminarin used against apple scab. Phytoma, march 2013, N° 662.
- Eccel, E., Rea, R., Caffara, A., Crisci, A. (2009). Risk of spring frost to apple production under future climate scenarios: the role of phenological acclimation. Int J Biometeorol. 53, pp. 273 – 286.
- Ivanovic, M., 1992. Mikoze biljaka. I izdanje. IP “Nauka”, Beograd.
- Jones, A.L. (1981) Fungicide resistance: Past experience with Benomyl and Dodine and future concerns with sterolinhibitors. Plant Disease, 65 (12): 990-992.
- Kohl, J., Scheer, C., Holb, I.J., Masny, S., Molhoek, W.M.L. (2015). Toward an Integrated use of Biological Control of *Cladosporium cladosporioides* H39 in Apple Scab (*Venturia inaequalis*) Management. Plant Dis. 99: 535-543.
- Kunz, S., Deising, H., and Mendgen, K. (1997) Acquisition of resistance to sterol demethylation inhibitors by populations of *Venturia inaequalis*. Phytopathology, 87(12), 1272-1278.
- Lespinasse, Y., Pinet, C., Laurens, F., Durel, C.E., Parisi, L. (2002). European research for durable resistance to scab on apple: the D.A.R.E. project. Acta Horticulturae (ISHS), 595: 17 – 22.
- MacHardy, W.E., Gadoury, D.M., Gessler, C. (2001). Parasitic and biological fitness of *Venturia inaequalis* relationship to disease management strategies. Plant Dis. 85: 1036 – 1051.
- Mery, A. B.; Joubert, J. M., 2012. Laminarin (Vacciplant®) against apple scab (*Venturia inaequalis*) and gloeosporium on apple (*Gloeosporium album* et *Perenans*). Association Française de Protection des Plantes (AFPP). 10e Conférence Internationale sur les Maladies des Plantes, Tours, France, 3, 4 & 5 Décembre, 2012 2012 pp. 630-639 ref.5
- Soriano, J.M., Joshi, S.G., van Kaauwen, M. (2009). Identification and mapping of the novel apple scab resistance gene *Vd3*. Tree Genetics and Genomes. 5, pp. 475 – 482.
- Urbanovich, O., Kazlovskaya, Z. (2008). Identification of scab resistance genes in apple trees by molecular markers. Scientific works of the Lithuanian institute of horticulture and Lithuanian University agriculture, 27 (2): 347 – 357.

## STUDY ON INFLUENCE OF SOME CONVENTIONAL AND BIOLOGICAL FERTILIZERS ON THE BIOCHEMICAL COMPOSITION OF FRESH AND DRIED FRUITS OF 'TEGERA' PLUM CULTIVAR

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### Abstract

The study presents the results of the effect of different fertilization types on the biochemical composition of fresh and dried plum fruit of 'Tegera' cultivar. The scientific experiment was carried out in 2016 at Research Institute of Mountain Stockbreeding and Agriculture - Troyan. Three fertilization types were applied in the experiment: biological, conventional and granulated chicken manure. Higher values for sugars, anthocyanins, tanning substances and pectin were reported as a result of fertilization in comparison with the control. The highest content of total polyphenols in fresh 210.00 mgGAE/100 and dried 390.00 mgGAE/100 fruit was found in the conventional fertilization. The greatest level of antioxidant activity of fresh fruit was found in the variant of chicken manure - 926.67  $\mu\text{molTE} / 100 \text{ g}$  and the conventional fertilization - 597.78  $\mu\text{molTE} / 100 \text{ g}$ , while it was 220.00  $\mu\text{molTE} / 100 \text{ g}$  in dried fruit variant with the biological fertilization.

**Keywords:** plums, antioxidant activity, total polyphenols.

### Introduction

Plum belongs to one of the most common groups of fruit trees (Blažek, 2007). It is differentiated as a perspective culture, traditional for Bulgarian agriculture. It takes the fourth place of distribution after apple, peach and pear. Fertilization is an important element in the soil fertility management in order to achieve good results, including fruit plum trees with strong growth, producing quality fruit. The fertilization process is one of the main agro-technical activities. It affects the vegetative and reproductive phenomena of trees. Both the insufficient soil stocking with basic nutrients as well as the high fertilization dose, lead to adverse effects on orchards (Arnhold et al. 1914, Thoden et al. 2011, Burmeister et al. 2015, Chauvin et al. 2015). The biochemical composition of fruit is directly dependent on the specific agrometeorological conditions of the area and plant cultivation technologies, such as soil treatment, fertilizing, pruning, etc. (Vitanova, 1990 Ruiz, 2006, Kader et al. 2004). Researches on the effect of fertilization on the biochemical composition of plum fruits were conducted by (Cuquel et al. 2011). They found that with fertilization with N and K in the amount of 40 and 110 kg / ha / year, fruits had the highest quality during storage.

In recent years, the so-called bio-fertilization, including composting, green fertilization, the use of fertilizers certified for biological application and others (Yadav et al., 2000). One of the main differences between conventional and organic agriculture is how the problem of soil fertility is addressed. Conventional agriculture relies on short-term solutions to improve plant nutrition, mainly by using mineral fertilizers. Organic agriculture uses environmentally-friendly preventive and/or long-term solutions to increase and preserve the organic matter in soil (Reganold et al. 1987, Stoykova, 2004, Stancheva, 2007).

The aim of present research is to study the biochemical composition of fresh and dried plums of 'Tegera' cultivar in different fertilizing variants.

### Material and methods

The experiment was carried out in 2016 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture - Troyan. The object of the study is fruits of plum cultivar of

'Tegera'. The planting distances are 4/2.5 m. Inter row spacing is covered with tall fescue and the intra row spacing is kept in black fallow. That culture is grown according to its agro-technical requirements. The following fertilization variants have been applied in the experiment:

- I variant - Bio-fertilization - including the following fertilizers: Agriful (soil) - 5 l/da, Tecamin Flower (foliar) - 0.3%, Teknokel Amino Ca (foliar) - 0.4%
- II variant - Conventional - Yara Mila Complex (soil) - 0.500 kg/tree, YaraVita Frutrel (leaf) - 0.500 ml/da, Yara Vita Universal Bio (leaf) - 0.500 ml/da
- III variant - Granulation of chicken manure - 0.500 kg/tree;
- IV variant - Control

Fertilization schedule:

- Agriful - applied five times from the beginning of vegetation over a period of 15-20 days;
- Tecamin Flower - imported twice. Applied before blossoming and during the formation of a fruit-set;
- Tecnokel Amino Ca - imported twice. Applied after blossoming and a month before harvesting;
- Yara Mila Complex - imported once in the intra row spacing;
- YaraVita Frutrel - four-fold application. First in the phase of winter buds, in phase of white button, during the formation of fruit-set and a month before the harvest;
- Yara Vita Universal Bio - three-fold application. Applied before and after blossoming and after harvest.
- Granulation of chicken manure - one application in the intra row spacing.

The biochemical composition of fruits is studied at the chemical laboratory of RIMSA - Troyan. The following indices are studied: dry matter according to Re (%), dry weight (%), sugars (%) (total, invert and sucrose) - according to Schoorl and Regenbogen method, acids (%) - by titration with 0.1n NaOH; ascorbic acid (mg %) - according to Fialkov method; anthocyanins (mg %) - according to Fuleki and Franciss method; pectin (%) - according to Melitz method, tanning substances (%) - according to Levental method. The total polyphenols (mgGAE / 100) and the antioxidant activity ( $\mu\text{molTE}$  / 100 g) of fruits were determined at the laboratory of Food Research and Development Institute in Plovdiv.

The fruit drying process was carried out at FRDI - Plovdiv, by means of a heat pump. Drying took place at temperatures up to 45 ° C, which preserved high quality and native properties of the product. The process runs in a closed cycle using the same air and eliminates the additional microbial visitation from outside air.

## Results and discussion

Fruits of 'Tegera' cultivar usually ripen in the second half of July, which characterizes it as an early ripening cultivar for Troyan region. The harvest in 2016 took place on 18 July.

The results of the biochemical analysis of plum fruits are presented in Table 1. The content of total polyphenols (mgGAE/100) and antioxidant activity ( $\mu\text{molTE}$  / 100 g) are presented in Figure 1 and Figure 2.

For fresh fruit, dry weight matter is 18% to 20%. As a result of the narrow boundaries between variants, the reported variance coefficient is low.

The amount of soluble dry matter is almost identical for all four variants ranging from 17% to 18% at a very low variation coefficient.

There is also a slight variation in the content of total sugars. It is 9.55% for the conventional fertilization (II variant), while the fertilization with the chicken manure shows 10.25%. Variation coefficient is low (2.99%). Almost identical results ranging from 10.31% to 11.78% were obtained by the analysis of seven plum hybrids and three control varieties (Milosevic and Milosevic 2012).

Table 1. Biochemical composition of fresh plums of 'Tegera' cultivar

| Indicators | Dry weight, % | Dry matter in Re, % | Total sugars, % | Invert sugar, % | Sucrose, % | Acids as malic, % | Ascorbic acids, mg % | Tannin substances, % | Anthocyanins, mg % | Pectin, % |
|------------|---------------|---------------------|-----------------|-----------------|------------|-------------------|----------------------|----------------------|--------------------|-----------|
| Variants   |               |                     |                 |                 |            |                   |                      |                      |                    |           |
| I          | 19.10         | 17.3                | 10.05           | 4.85            | 4.94       | 0.64              | 4.40                 | 0.270                | 19.19              | 0.87      |
| II         | 19.53         | 17.5                | 9.55            | 6.50            | 2.90       | 0.64              | 5.28                 | 0.270                | 16.61              | 0.71      |
| III        | 18.53         | 17.5                | 10.25           | 5.20            | 4.80       | 0.64              | 6.16                 | 0.208                | 10.00              | 0.45      |
| IV         | 18.70         | 17.5                | 10.05           | 4.05            | 5.70       | 0.64              | 5.28                 | 0.249                | 48.39              | 0.51      |
| CV %       | 2.35          | 0.57                | 2.99            | 19.82           | 25.97      | -                 | 13.61                | 11.73                | 72.23              | 30.25     |

A more significant difference is found in invert sugar values. The highest values are found in the conventional fertilization - 6.50% and the lowest in the control - 4.05%. The variation coefficient is high as a result of these differences. Concerning the amount of sucrose, a strong variation between the variants has been reported in its values. It is 2.90% for conventional fertilization, while the results of the control were twice higher - 5.70%. A high variation coefficient is reported. With respect to the organic acid indicator, it is 0.64%. The ascorbic acid indicator ranges from 4.40 mg /% in bio-fertilization to 6.16 mg /% in chicken manure at a low variation coefficient. The value of the result recorded in conventional fertilization corresponds to the value recorded for the control - 5.28 mg /%. There are no significant differences in the amounts of tanning substances. The results obtained are in the range of 0.208% for chicken manure to 0.270% for organic and conventional fertilization. Differences between variants have low variance coefficient (11.7%).

The lowest content of anthocyanins are recorded in the variant with chicken manure - 10.00 mg /% and the highest for the control - 48.39 mg /%. The variance coefficient is high for them (72.2%).

Pectin content is an indicator with significant differences between fertilizer variants. Its amount is high for bio fertilization - 0.87%, for conventional fertilization is less - 0.71% and it is the lowest for the other two variants (CV 30.2%). The highest values of total polyphenols in fruits were recorded in conventional fertilization - 210.00 mgGAE / 100, followed by control - 190.00 mgGAE / 100, organic fertilization - 127.00 mgGAE / 100 and chicken manure - 124.00 mgGAE / 100 (Figure 1). The results are of high variation coefficient, where the influence of fertilization on the values of the indicator can be taken into account. A similar amount of common polyphenols was analyzed by (Kaulmann et al. 2014) ranging from 5 to 209 mg GAE / 100 g FW. (Kim et al. 2003) also reported similar results where the total polyphenols in fresh fruit were in the range of 181.3 mgGAE / 100 g for 'Stanely' cultivar to 372.6 mg GAE / 100 g for 'Beltsville Elite' cultivar. In a study by (Chun et al. 2003), their amount also ranges from 138.1 mgGAE / 100 g for 'NY 9' (New York 9) cultivar to 684.5 mgGAE / 100 g for 'Beltsville Elite' cultivar.

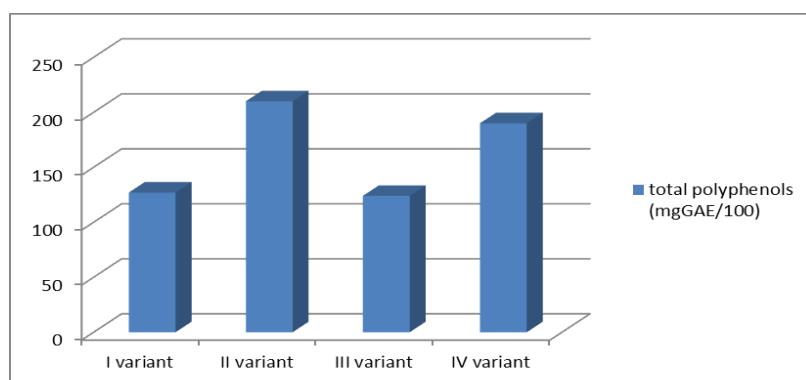


Figure 1. Influence of different fertilization variants on total polyphenols (mgGAE /100) and the antioxidant activity in fresh plums of 'Tegera' cultivar

There is a significant variation in the antioxidant activity in the fruits of the variants. It is highest for chicken manure - 926.67  $\mu\text{molTE}$  / 100 g and conventional fertilization - 597.78  $\mu\text{molTE}$  / 100 g. The lowest content was recorded in the control - 114.44  $\mu\text{molTE}$ /100 g (Figure 2). The results obtained have an extremely high variation coefficient (82.5%)

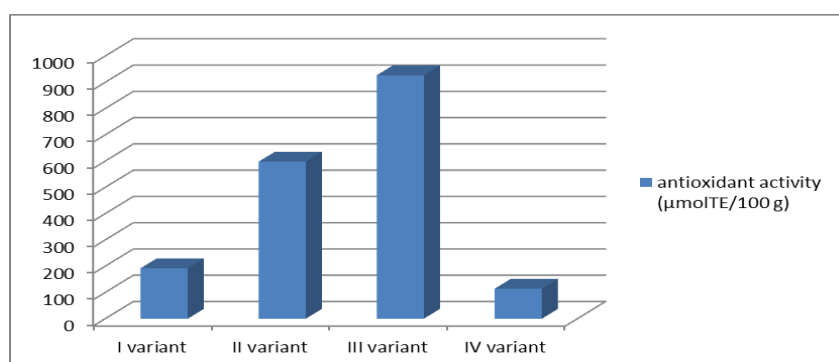


Figure 2. Effects of different fertilization variants on the antioxidant activity ( $\mu\text{molTE}$  / 100 g) in fresh plums of 'Tegera' cultivar

Table 2. Biochemical composition of dried plums of 'Tegera' cultivar

| Variants | Indicator | Dry weight, % | Total sugars, % | Invert sugar, % | Sucrose, % | Acids as malic, % | Ascorbic acid, mg % | Tannins, % | Anthocyanins, mg % | Pectin, % |
|----------|-----------|---------------|-----------------|-----------------|------------|-------------------|---------------------|------------|--------------------|-----------|
|          |           |               |                 |                 |            |                   |                     |            |                    |           |
| I        |           | 83.23         | 36.50           | 32.70           | 3.61       | 2.32              | 7.04                | 0.540      | 17.26              | 0.68      |
| II       |           | 82.85         | 42.70           | 34.40           | 7.88       | 2.19              | 8.80                | 0.457      | 20.32              | 0.41      |
| III      |           | 79.09         | 38.90           | 35.40           | 3.32       | 1.93              | 7.04                | 0.457      | 10.81              | 0.11      |
| IV       |           | 84.95         | 38.20           | 34.40           | 3.61       | 2.19              | 7.04                | 0.706      | 12.74              | 1.31      |
| CV %     |           | 1.17          | 6.70            | 3.27            | 47.50      | 7.58              | 11.76               | 21.73      | 28.21              | 81.45     |

Dry weight matter of dried fruit has significantly higher results than the fresh ones. The variations between the variants are narrow and as a result the variance coefficient is very low. The value for the control is higher - 84.95%. The lowest value was recorded in the third variant - 79.09% (Table 2). The highest content of total sugars is found in the conventional fertilization - 42.70% and lowest in bio-fertilization - 36.50%.

Almost similar results were obtained for invert sugar values at low CV% in the different fertilization variants. The highest content of sucrose was found in the conventional fertilizers - 7.88%. It is 2 times lower in the third variant than the conventional fertilizer application. A high variation coefficient (47.50%) has been reported.

The organic acid content is in the range of 1.93% for the chicken manure to 2.32% in the bio-fertilization variant with a low variation coefficient.

The ascorbic acid content is 8.80 mg /% for conventional fertilization and 7.04 mg /% for the other three variants. Tanning substances are in the range from 0.46% to 0.71% with a high variation factor. The highest content of anthocyanins is found in the second variant - 20.32 mg /%, as they decrease in the first variant to 17.26 mg /%. The lowest content is found in the third variant - 10.81 mg/% and for the control it is 12.74/%. The coefficient of variation is high for this indicator. Pectin results are different. The lowest content is reported for fruit fertilized with chicken manure and the highest for the control - 1.31%. Variation coefficient is high (81.4%). The highest values of total polyphenols are found in the conventional fertilization - 390.00 mgGAE / 100 (Figure 3), followed by bio-fertilization - 266.00 mgGAE/100, control - 264.00 mgGAE/100 and chicken manure - 260.00 mgGAE/100. In the study of (Cinquanta et al. 2002), the total content of polyphenols in dried plums ranges from 340 to 610 mg of GAE / 100 g of dry weight. The antioxidant activity is greatest in fruit with bio-fertilization - 220.00  $\mu\text{molTE}$  / 100 g and the control - 200.00  $\mu\text{molTE}$  / 100 g. It is the smallest for the chicken manure variant - 160.00  $\mu\text{molTE}$  / 100 g. (Figure 4). A low variation coefficient is reported. Identical result of 159.0 mM Trolox / g d.m. was analyzed by (Walkowiak-Tomczak et al. 2008) for plum brandy "Bluefre".

According to (Forni et al. 1992), the quality plum fruits must have an acidic coefficient between 12 and 24. The results obtained by the indicator are in the indicated range. The highest acidimetric coefficient for both types of fruit was recorded in the chicken manure variant, as for the fresh fruit it was 16.01 and for dried fruit - 20.15 (Table 3). The variation coefficient reported by the indicator for both fresh and dried fruit is low.

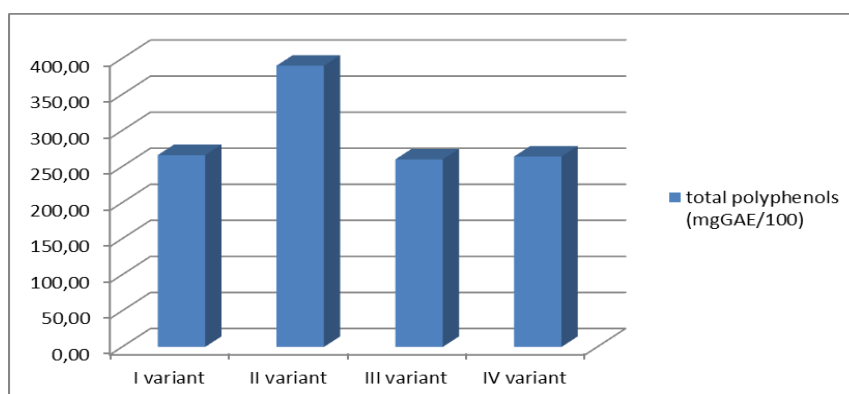


Figure 3. Influence of different fertilization variants on total polyphenols (mgGAE/100) in dried plums of 'Tegera' cultivar

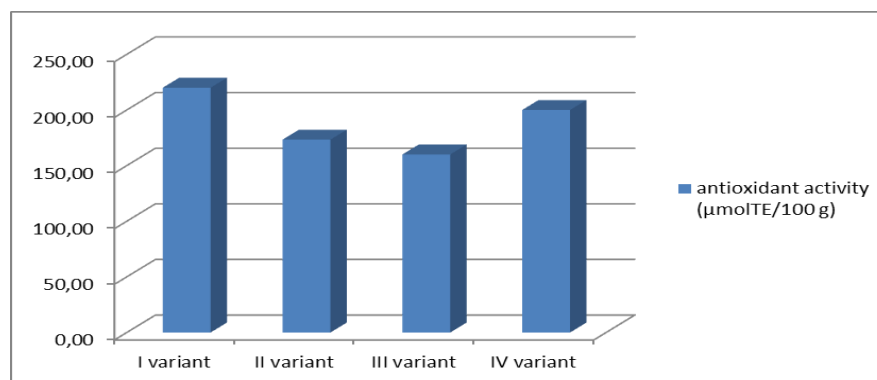


Figure 4. Effect of different fertilization variants on the antioxidant activity ( $\mu\text{molTE}/100\text{ g}$ ) in dried plums of 'Tegera' cultivar

Table 3. Acidimetric coefficient values for fresh and dried plums of 'Tegera' cultivar

| Variant | Acidimetric coefficient |              |
|---------|-------------------------|--------------|
|         | Fresh fruits            | Dried fruits |
| I       | 15,7                    | 15,73        |
| II      | 14,92                   | 19,5         |
| III     | 16,01                   | 20,15        |
| IV      | 15,7                    | 17,44        |
| CV %    | 2,98                    | 11,06        |

## Conclusions

A study has been conducted on the influence of certain types of fertilizers on the biochemical composition of fresh and dried 'Tegera' fruit. Their positive influence on the values of inverted sugar and sucrose, anthocyanins, tanning substances and pectin was found in some fertilization options. The highest content of total polyphenols in fresh (210.00 mgGAE/100) and dried (390.00 mgGAE / 100) fruits is found in conventional fertilization. The highest antioxidant activity of fruit is registered in the conventional fertilization and chicken manure, while for dried fruit it is in the variants with bio-fertilization and the control.

Acidimetric coefficient values are most distinct in the chicken manure variant.

## References

1. Arnhold, S., Lindner, S., Lee, B., Martin, E., Kettering, J., Nguyen, T T., Koellner, T. and Sik Ok Huwe, Y. B. (1914). Conventional and organic farming: soil erosion and conservation potential for row crop cultivation. *Geoderma* 219–220, 89–105.
2. Burmeister, J., Walter, R. and Fritz, M. (2015). Dungung mit Biogasgarresten –Auswirkungen auf Bodentiere. In: *Biogas Forum Bayern Nr. I - 27/2015*, Hrsg. ALB Bayerne.V., [http://www.biogasforumbayern.de/publikationen/Auswirkung\\_der\\_Dungung\\_mit\\_Biogasgarresten\\_auf\\_die\\_Bodentiere.pdf](http://www.biogasforumbayern.de/publikationen/Auswirkung_der_Dungung_mit_Biogasgarresten_auf_die_Bodentiere.pdf).
3. Blaz'ek, J. (2007). A survey of the genetic resources used in plum breeding. *Acta Horticulturae*, 734: 31–45.
4. Cinquanta, L., Di Matteo, M. and Esti, M. (2002). Physical pre-treatment of plums (*Prunus domestica*). Part 2. Effect on the quality characteristics of different prune cultivars. *Food chemistry*, 79: 233-238.
5. Chauvin, C., Dorel, M., Villenave, C., Roger-Estrade, J., Thuries, L. and Risede, J. M. (2015). Biochemical characteristics of cover crop litter affect the soil food web, organic matter

- decomposition, and regulation of plant-parasitic nematodes in a banana field soil. *Applied Soil Ecology*, 96: 131-140.
6. Chun, O.K., Kim, D.O., Moon, H.Y., Kang, H.G. and Lee, C.Y. (2003). Contribution of Individual Polyphenolics to Total Antioxidant Capacity of Plums. *Journal of Agricultural and Food Chemistry*, 51: 7240-7245.
  7. Forni, E., Erba, M. L., Maestrelli, A. and Polesello, A. (1992). Sorbitol and free sugar contents in plums. *Food Chem.*, 44: 269-275.
  8. Cuquel, F.L., Motta, A. C. V., Tutida, I. and May de Mio, L. L. (2011). Nitrogen and potassium fertilization affecting the plum postharvest quality, *Rev. Bras. Frutic.*, Jaboticabal - SP, Especial, E., Outubro: 328-336.
  9. Reganold, J., Elliott, L. and Unger, Y. (1987). Long-term effects of organic and conventional farming on soil erosion. *Nature.*, 330: 370-372
  10. Ruiz, R. (2006). Effects of different potassium fertilizers on yield, fruit quality and nutritional status of 'Fairlane' nectarine trees and on soil fertility. *Acta Horticulturae*, The Hague, 721: 185-190.
  11. Kader, A. A. and Rolle, R.S. (2004) .The role of post-harvest management in assuring the quality and safety of horticultural produce. *FAO Bulletin*, Washington, 152: 52.
  12. Kaulmann, A., Jonville, M. C., Schneider, Y. J, Hoffmann, L. and Bohn, T. (2014). Carotenoids, polyphenols and micronutrient profiles of Brassica oleraceae and plum varieties and their contribution to measures of total antioxidant capacity., *Food Chemistry* 155 Oxford: Elsevier Ltd: 240-250.
  13. Kim, D.O., Chun, O.K., Kim, Y.J., Moon, H.Y. and Lee, C.Y. (2003). Quantification of Polyphenolics and Their Antioxidant Capacity in Fresh Plums. *Journal of Agricultural and Food Chemistry*, 51: 6509-6515.
  14. Milošević, T. and Milošević, N. (2012). Main physical and chemical traits of fresh fruits of promising plum hybrids (*Prunus domestica* L.) from Cacak (Western Serbia), *Romanian Biotechnological Letters*, 17(3): 7358-7365.
  15. Stancheva, Y. (2007). Analysis of factors inducing instability in agricultural production in Bulgaria, [http://chm.moew.government.bg/SLM/files/1-Sust\\_agr.pdf](http://chm.moew.government.bg/SLM/files/1-Sust_agr.pdf)
  16. Stoykova, G. (2004). Organic agriculture in Bulgaria - origin, characteristics and advantages; – Scientific and Practical Conference with International Participation (Reports – vol. 2); Svishtov, 11-13.11.2004.
  17. Thoden, T. C., Korthals, G. W. and Termorshuizen, A. J. (2011). Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management. *Nematology*, 13(2): 133-153.
  18. Vitanova, I. M. (1990). Determination of needs for fertil-izer of plum trees. *Acta Horticulturae*, The Hague, 274: 501-508.
  19. Walkowiak-Tomczak, D., Reguia, J. and Jysiak, G. (2008). Physico-chemical properties and antioxidant activity of selected plum cultivars fruit, *Acta Sci. Pol., Technol. Aliment*, 7(4): 15-22
  20. Yadav, R. L., Dwivedi, B. S. and Pandey, P. S. (2000). Rice–wheat cropping system: assessment of sustainability under green manuring and chemical fertilizer inputs, *Field Crop Res.* 65: 15–30.

**MANAGEMENT AND CONTROL OF *AGRILUS AURICHALCEUS* REDT. (COLEOPTERA: BUPRESTIDAE) IN BIOLOGICAL RASPBERRY PRODUCTION**

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**Abstract**

The study was carried out in a raspberry plantation (2013-2015) in the experimental field of the Institute of Agriculture – Kyustendil. The purpose of this study is to investigate the effect of organic fertilizer application on the stock larvae of *Agrilus aurichalceus* and population management, using biological plant protection products. According to the results of this study, it has been proved that fertilizing with organic fertilizers has a reductive effect on the survival of the *A. aurichalceus* population and does not affect the population of the larval endoparasitoid *Tetrastichus heeringi* in the cultivars 'Willamette' and 'Lyulin'. The relationships between *A. aurichalceus* and *T. heeringi* in the conditions of organic raspberry production have been studied. The established degree of parasitism of the larvae by *T. heeringi* (32.7-68.4%) is capable of reducing and controlling the population of *A. aurichalceus* in the 'Willamette' without chemical control. Two treatments with bioinsecticides have been carried out for the protection of plants from cultivar 'Lyulin' due to a significantly lower degree of parasitism (18.1-50.0%). The most effective insecticides to the control of adults of *A. aurichalceus* have been identified: Pyrethrum FS EC-0.05% and NeemAzal®-T/S - 0.3 %. The adult control scheme involves two treatments with bioinsecticides of infested shrubs. The first treatment should be done during the buttoning, the second treatment during the mass flight of beetles (only at an established density of 3-4 beetles/m<sup>2</sup>).

**Keywords:** organic fertilizers, biological control, Rose stem girdler.

**Introduction**

Rose stem girdler (*Agrilus aurichalceus* Redtenbacher 1849) is one of the most important pests of raspberries in organic fruit production in all regions of Bulgaria. Its population density fluctuates strongly during vegetation under the influence of various factors: abiotic, biotic, agricultural, organisational and economic resources (Zapryanov 1980, Tsalbakov 1983, Karov et al. 2006, Tsoleva and Stoyanova 2007, Ivanov, 2009). Particular interest presents the parasitoids of this insect pest, as they are not yet well studied. In Bulgaria *Tetrastichus heeringi* Delucchi (Hymenoptera: Eulophidae) is reported to be a parasitoid on the larvae of *Agrilus cuprescens* Men of Staikov (1954), Nikolova (1968), Velcheva et al. (2008), Véték and Péntzes (2004, 2005). Studies on the biological features of *T. heeringi* in rose plantation were carried out by Zapryanov (1980) and Tsalbukov (1983). Parasitoid is designed to reduce the natural host population by about 50% and in some years above these values Véték, Thuróczy and Péntzes (2007).

The aim of the study is to investigate the density of the wintering population of larvae of *A. aurichalceus* under the conditions of organic raspberry production.

**Material and methods**

The investigations were carried out during the period 2013-2015 at the Institute of Agriculture in Kyustendil, Department of Berries in Kostinbrod. The organic raspberry plant was created in 2010 with the 'Willamette' and 'Lyulin' cultivars. The experience was set by block method in four variants and four replicates. The drip irrigation was applied at 80% evapotranspiration. Four types of liquid organic fertilizers were tested, applied three times by foliar treatment of the plants during the

buttoning, the beginning of flowering and the formation of the green joint; in the following variants: V0 (untreated); V1 (Humustim - 100 mL/da); V2 (Haemosim bio N5 – 5L/da + Haemofol H4 - 400 mL) and V3 (Biohumax® - 1000 mL/da). The between rows were maintained by mowing and mulching.

The experimental plants were grown, using authorized fertilizers and plant protection products according to Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. The degree of attack by insect pest on the stem was determined annually 2 times a month (May-October) on 100 shoots of each variant. The material for determining the wintering stock of harmful and beneficial species was collected during January and February by cutting of shoots different ages to the soil surface and processed in laboratory conditions.

Under field conditions during 2014-2015, a test of biological efficacy of pyrethrum and azadirachtin was carried out: pyrethrum in three concentrations (0.05, 0.06, 0.08%) and azadirachtin in 0.25; 0.30; 0.35%; in four replication. The data was converted into per cent mortality by using following formula given by Abbott (1925) and modified by Henderson and Tilton (1955). The statistical analysis of the results obtained was done by two-factor dispersion analysis (ANOVA).

### Results and discussion

The meteorological characteristics of the area during the 2013-2015 give the data in Table 1. They can be considered as one-sided with these characteristic areas but with their respective specific features.

Data analysis allows to note that the average daily temperature during the flowering of plants of remontant raspberry cultivars was optimal. The precipitation was below the normal, the air humidity was for the entire vegetation season. The flowering in the cultivar 'Willamette' was over 20 day, and in the cultivar 'Lyulin' over 60 days.

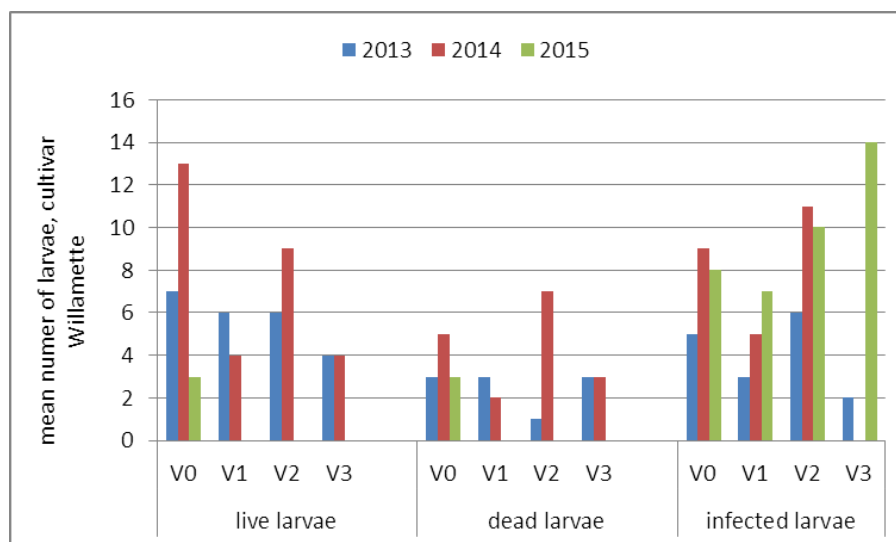


Figure 1. Over-wintering stock of *Agrilus aurichalceus* larvae in cultivar 'Willamette' in 2013-2015

The wintering stock of larvae was presented in Figure 1. The survival of the population ranged widely from 46.9% (2013) to 5.3% (2015). In terms of experience, the number of wintering larvae was highly variable and was influenced by the climatic conditions of the area. The number of wintering larvae was analyzed by fertilization variants, it was rather diverse, with variants (V1 and V2) being almost the same (12.2%) for 2013-2014. In the cultivar 'Lyulin' (Figure 2), during the study period, a pronounced tendency to decrease the population of the species by about twice from 57.9% to 32% was observed. It is interesting to note that during the three years of the study, the population of live larvae is highest in Humustim (V1) fertilization and with lower values in Biohumax® fertilization (V3).

The parasitism of larvae was highest (50%) in 2014, and twice lower in 2013 (18.1%).

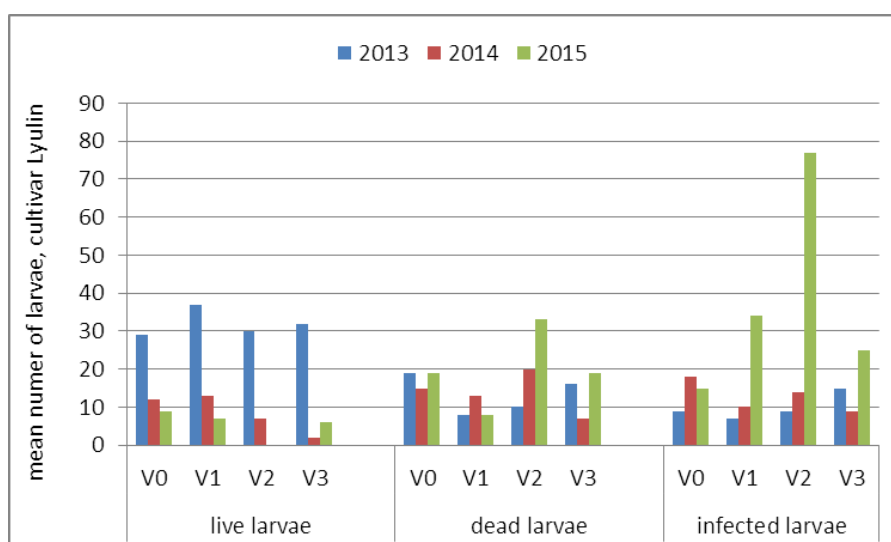


Figure 2. Over-wintering stock of *Agrilus aurichalceus* larvae in cultivar 'Lyulin' in 2013-2015

Tables 2 and 3 present the data from the statistical analysis of the results of observations on the wintering stock of live, dead and infected larvae of *A. aurichalceus* in the cultivars 'Willamette' and 'Lyulin' in 2013-2015. It is evident that the period of study (years) affected the stock of live larvae of *A. aurichalceus* in the cultivars 'Willamette' and 'Lyulin'. The differences between the fertilization variants during years of research have been statistically significant. There were no significant differences between the different variants for a given year in both cultivars. The mortality rate of the *A. aurichalceus* population is not directly dependent on fertilization.

Table 2. Two-factor dispersion analysis (ANOVA) of the effect of the fertilization variants on the over-wintering stock of *Agrilus aurichalceus* larvae in cultivar Willamette in 2013-2015

| live larvae                                                                                |          |    |          |          |                 |          |
|--------------------------------------------------------------------------------------------|----------|----|----------|----------|-----------------|----------|
| Source of Variation                                                                        | SS       | df | MS       | F        | P-value         | F crit   |
| <b>Fertilization</b>                                                                       | 44,66667 | 3  | 14,88889 | 3,748252 | 0,079128        | 4,757063 |
| <b>Years</b>                                                                               | 98,16667 | 2  | 49,08333 | 12,35664 | <b>0,007455</b> | 5,143253 |
| Error                                                                                      | 23,83333 | 6  | 3,972222 |          |                 |          |
| Total                                                                                      | 166,6667 | 11 |          |          |                 |          |
| dead larvae                                                                                |          |    |          |          |                 |          |
| Source of Variation                                                                        | SS       | df | MS       | F        | P-value         | F crit   |
| <b>Fertilization</b>                                                                       | 7        | 3  | 2,333333 | 0,8      | 0,537552        | 4,757063 |
| <b>Years</b>                                                                               | 24,5     | 2  | 12,25    | 4,2      | 0,072338        | 5,143253 |
| Error                                                                                      | 17,5     | 6  | 2,916667 |          |                 |          |
| Total                                                                                      | 49       | 11 |          |          |                 |          |
| parasitism of <i>Agrilus aurichalceus</i> larvae by parasitoid <i>Ttrastichus heeringi</i> |          |    |          |          |                 |          |
| Source of Variation                                                                        | SS       | df | MS       | F        | P-value         | F crit   |
| <b>Fertilization</b>                                                                       | 31,33333 | 3  | 10,44444 | 0,801706 | 0,536774        | 4,757063 |
| <b>Years</b>                                                                               | 67,16667 | 2  | 33,58333 | 2,577825 | 0,155585        | 5,143253 |
| Error                                                                                      | 78,16667 | 6  | 13,02778 |          |                 |          |
| Total                                                                                      | 176,6667 | 11 |          |          |                 |          |

The organic fertilizers have no effect on the infected larvae by *T. heeringi* in both cultivars. The reported low population of rose stem girdler in 2015 in cultivar 'Lyulin' is most likely due to the two-fold plant treatments carried out in 2014, resulting in the species population being reduced to less than 10%, and in the cultivar Willamette to the high degree of parasitism (68.4%) by the endoparasitoid *T. heeringi*.

According to the conducted studies on the relationships between *A. aurichalceus* and *T. heeringi* have shown that the degree of parasitism must be over 70% to be able to reduce the *Agrilus* population.

Table 3. Two-factor dispersion analysis (ANOVA) of the effect of the fertilization variants on the over-wintering stock of *Agrilus aurichalceus* larvae in cultivar Lyulin in 2013-2015

| live larvae                                                                                |          |    |          |          |                 |          |
|--------------------------------------------------------------------------------------------|----------|----|----------|----------|-----------------|----------|
| Source of Variation                                                                        | SS       | df | MS       | F        | P-value         | F crit   |
| <b>Fertilization</b>                                                                       | 84,66667 | 3  | 28,22222 | 2,247788 | 0,183201        | 4,757063 |
| <b>Years</b>                                                                               | 1684,667 | 2  | 842,3333 | 67,0885  | <b>7,84E-05</b> | 5,143253 |
| Error                                                                                      | 75,33333 | 6  | 12,55556 |          |                 |          |
| Total                                                                                      | 1844,667 | 11 |          |          |                 |          |
| dead larvae                                                                                |          |    |          |          |                 |          |
| Source of Variation                                                                        | SS       | df | MS       | F        | P-value         | F crit   |
| <b>Fertilization</b>                                                                       | 213,5833 | 3  | 71,19444 | 1,601875 | 0,284932        | 4,757063 |
| <b>Years</b>                                                                               | 104,6667 | 2  | 52,33333 | 1,1775   | 0,370352        | 5,143253 |
| Error                                                                                      | 266,6667 | 6  | 44,44444 |          |                 |          |
| Total                                                                                      | 584,9167 | 11 |          |          |                 |          |
| parasitism of <i>Agrilus aurichalceus</i> larvae by parasitoid <i>Ttrastichus heeringi</i> |          |    |          |          |                 |          |
| Source of Variation                                                                        | SS       | df | MS       | F        | P-value         | F crit   |
| <b>Fertilization</b>                                                                       | 31,33333 | 3  | 10,44444 | 0,801706 | 0,536774        | 4,757063 |
| <b>Years</b>                                                                               | 67,16667 | 2  | 33,58333 | 2,577825 | 0,155585        | 5,143253 |
| Error                                                                                      | 78,16667 | 6  | 13,02778 |          |                 |          |
| Total                                                                                      | 176,6667 | 11 |          |          |                 |          |

These results required two bioinsecticide treatments (Tsolova and Stoyanova, 2007). The results of the application of Pyrethrum FS EC® and NeemAzal-T/S against adults showed that the effect of three single application doses (0.05%, 0.06% and 0.08%) was unsatisfactory. The efficacy of Pyrethrum FS EC® in the second treatment was highest at a concentration of 0.05% and during the two years of the experimental period. Similar results were obtained at a concentration of 0.06%, but the differences were negligible. The single treatment with Neemazal-T/S 0.3% against rose stem girdler also showed unsatisfactory results from 58.8 (2014) to 69.1% (2015) and double (90.1-91.8%). Double treatment with Pyrethrum FS EC® at a concentration of 0.05% and Neemazal-T/S -0.3% showed efficacy 90% over the years of the study. The results of the obtained data allow the two products to be used for pest control in integrated and organic raspberry production (Table 4).

## Conclusions

The duration of fertilization with organic fertilizers under the conditions of organic production of raspberries has a reductive effect on the survival of *A. aurichalceus*. The tested fertilization variants do not have a direct influence on the mortality of *A. aurichalceus* and the infestation of the larvae by *T. heeringi* in the cultivars Willamette and 'Lyulin'. The endoparasitoid *T. heeringi* has been shown to exhibit cultivar selectivity. The cultivar Willamette was characterized by a high degree of parasitism as the 'Lyulin' by low. It was found that the rose stem girdler can be successfully controlled with two

treatments (pre-blossoming and post-blossoming) with Pyrethrum FS EC® - 0.05% and Pyrethrum FS EC® 0.3%. The application of single post-blossoming treatment with botanical insecticides does not produce good results.

Table 4. Efficacy of bioinsecticides against *Agrilus aurichalceus* adults under field conditions in 2014-2015

| Variants         | Conc.<br>% | Number of treatments   |                 |               |                        |                 |               |
|------------------|------------|------------------------|-----------------|---------------|------------------------|-----------------|---------------|
|                  |            | Single treatment       |                 | Efficacy<br>% | Double treatment       |                 | Efficacy<br>% |
|                  |            | Damaged shoots, number |                 |               | Damaged shoots, number |                 |               |
|                  |            | before treatment       | after treatment |               | before treatment       | after treatment |               |
| 2014             |            |                        |                 |               |                        |                 |               |
| Untreated        | 0.00       | 21.2                   | 9.2             | -             | 11.4                   | 3.9             | -             |
| Pyrethrum FS EC® | 0.05       | 16.4                   | 6.3             | 61.6          | 14.7                   | 0.4             | 90.5          |
|                  | 0.06       | 13.3                   | 5.2             | 60.9          | 10.4                   | 0.6             | 90.9          |
|                  | 0.08       | 10.6                   | 4.2             | 60.1          | 5.7                    | 1.3             | 87.4          |
| Untreated        | 0.00       | 10.6                   | 4.7             | .-            | 7.1                    | 2.0             | -             |
| Neemazal-T/S     | 0.25       | 9.4                    | 3.9             | 58.5          | 8.4                    | 1.5             | 85.2          |
|                  | 0.30       | 10.2                   | 4.2             | 58.8          | 5.1                    | 0.3             | 93.7          |
|                  | 0.50       | 9.6                    | 4.1             | 63.2          | 6.6                    | 1.4             | 90.1          |
| 2015             |            |                        |                 |               |                        |                 |               |
| Untreated        | 0.00       | 13.1                   | 8.5             | -             | 9.1                    | 4.4             | -             |
| Pyrethrum FS EC® | 0.05       | 14.4                   | 9.9             | 61.8          | 7.6                    | 0.5             | 91.2          |
|                  | 0.06       | 10.3                   | 8.8             | 64.4          | 10.8                   | 0.9             | 90.1          |
|                  | 0.08       | 9.9                    | 6.4             | 59.9          | 12.5                   | 1.2             | 90.4          |
| Untreated        | 0.00       | 12.3                   | 6.8             | -             | 10.2                   | 5.5             | .-            |
| Neemazal-T/S     | 0.25       | 11.1                   | 5.6             | 63.1          | 10.3                   | 1.9             | 86.9          |
|                  | 0.30       | 9.9                    | 3.4             | 69.1          | 5.3                    | 0.4             | 91.8          |
|                  | 0.50       | 7.7                    | 5.5             | 58.8          | 7.6                    | 1.2             | 89.6          |

## References

1. Calbukov, P. (1983). Vzájomnosheniia mezhdú agrilusa po maslodajната roza i tetrastihus heeringi. Rastitelna zashtita, 7: 2-3.
2. Colova, E., Stoianova, N. (2007). Biologichni osobenosti na *Ttrastichus heeringi* Del. (Hymenoptera:Eulophidae) i roliata mu za regulirane na plynostta na *Agrilus ribesi* Schaefer (Coleoptera: Buprestidae). Rastenievydni nauki, 44: 295-298.
3. Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91
4. Henderson, C.F. and Tilton, E. W. 1955. Tests with acaricides against the brow wheat mite, J. Econ. Entomol. 48:157-161.
5. Ivanov, A. (2009). Biologichno proizvodstvo na plodove ot niakoi ovoshtni vidove. Sofiia, Dionis, 255.
6. Karov, S., Mitov, P., Andreev, R., Karov, S. (2006). Biologichno proizvodstvo na malini. Asociaciia za biologichno zemedelie "Ekofarm", Plovdiv. 30.
7. Velcheva, N., G. Baeva, N. Bakyrzhieva, E. Colova, M. Ivanova, M.Cenova, 2008. Rykovodstvo za integrirano upravlenie na vreditelite pri loza i iagodoplodni kulturi. MZH, NSRZ, 1-96.
8. Véték, G., Péntes, B. (2004). A málnavessző-szúnyog (*Resseliella theobaldi* Barnes) és a málnakarcsúdíszbogár (*Agrilus aurichalceus* Redt.) előfordulása különböző művelésmódú málnaültetvényekben. 50. Növényvédelmi Tudományos Napok, 2004. február 24–25., Budapest. Előadások összefoglalói, 67.

9. Véték, G., Péntzes, B. (2005). New data about the damage and life cycle of rose stem girdler (*Agrilus cuprescens* Ménéttriés). *5th International Conference of PhD Students*, 14–20. August, 2005, Miskolc, Hungary. Agriculture, 243–247.
10. Véték, G., Thuróczy, Cs. and Péntzes, B. (2007). Some important notes on the parasitoids of raspberry cane midge, *Resseliella theobaldi*, and rose stem girdler, *Agrilus cuprescens*. *6th Workshop on Integrated Soft Fruit Production*, 24–27 September 2007, East Malling, UK. Book of Abstracts, p. 13.
11. Zaprianov, A. (1980). Изучение особенности биологии и роль личиночного паразита *Ttrastichus heeringi* Del (Hymenoptera: Eulophidae) в снижении численности агрилусь масличной розы-*Agrilus cuprescens* Men (Coleoptera: Buprestidae) В: Изучении хищников и паразитов членостоногих. Доклады международного симпозиума, НАПС, 25-27.

**RESEARCH ON FINDINGS ABOUT CULTIVATION AND USE OF ANNUAL AND BIENNIAL  
FLOWERING PLANT SPECIES IN THE REGION OF ŠIBENIK AND KNIN – THE REPUBLIC OF  
CROATIA**

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**Abstract**

Most plant species that are cultivated in climatic conditions prevailing in the Republic of Croatia as annual or biennial flowering plants originate from warm and temperate climate zones of the planet. The plant groups in question are primarily used in privately-owned gardens and on various landscapes. They can be planted in raised flower beds, as well as used for landscape edging, rock gardens, decorative pots and they are also used as cut flowers. They are cost effective and provide the premises with a decorative value added. The objective of the survey research was to verify the findings about cultivation and use of annual and biennial flowering plants that are frequently used in gardens and landscaping in Šibenik and Knin. The survey research was conducted in May 2016 on a sample comprising of 30 respondents (for annual species) and 29 different respondents (for biennial species) from the region of the cities Šibenik and Knin and their outskirts. The respondents assessed the decorative features of six selected species per individual flower group and the method of their maintenance with an average grade (4). The participants in the survey showed a higher level of knowledge on annual flower species (67.7%-100%) compared with the knowledge on biennial flower species (57.9%-75.9%). The persons questioned correctly assessed the use of various species according to their function in the premises. Most respondents used decorative pumpkins (*Cucurbita pepo*) amongst annual flower plants for decoration of premises, whilst amongst biennial plants they primarily opted for daisies (*Bellis perennis*). The data provided can be used for the promotion of cultivation and use of flowering plants in practice.

**Keywords:** Gardens, landscape, knowledge.

**Introduction**

Annual and biennial flowering plants are an integral feature of all parks, gardens, yards, balconies and terraces. Their biological lifecycle is restricted to one or two years. Their origins vary, yet they normally originate from areas with moderate or warmer climate (Vinceljak-Toplak, 1989).

The previously mentioned flower groups have been used in landscape architecture since ancient times, yet the preserved records about floral gardens, their arrangement and types within different plantings are scarce. It was only at the end of the 19th century, upon appearance of the civil society, that public parks were established (R. Visiani Park in Šibenik) (Piplović, 2003). During the first half of the 20th century, Mate Zorić, a gardener from Šibenik, cultivated flowers within his nursery to meet the requirements both of public spaces and the general public. Subsequently, primacy was taken over by the company named Zelenilo (Dorbić and Temim, 2016). A more committed landscape architecture with greenery and flowers in Knin commenced after the Second World War (Dorbić and Temim, 2015). In the area of Knin and Šibenik the following annual flowering plants are currently the most popular: begonias (*Begonia semperflorens*), petunias (*Petunia hybrida*), scarlet sage (*Salvia splendens*), marigolds (*Tagetes*), pumpkins (*Cucurbita pepo*) and sunflower (*Helianthus annuus*). Amongst the biennials it is important to highlight as follows: daisies (*Bellis perennis*), pinks (*Dianthus*

sp.), pansies (*Viola x vittrockiana*), bellflowers (fam. Campanulaceae), hollyhocks (*Alcea rosea*) and wallflowers (*Cheiranthus Cheiri*), amongst others. The popularity of seasonal flowers is in the fact that they are of modest needs, they prefer sunny habitats and plenty of water and they bloom lusciously throughout the summer months. They are widely used, since they can be planted in flower beds of different design, rock gardens, decorative flower pots, as well as used as cut flowers. In order to create an appealing floral composition, one needs to have an insight into the flowering time and duration, as well as the colour, the height, habitus, as well as biological and ecological traits of the specific species. Floral features are grouped in carefully selected venues in order to emphasise specific compositional traits. Flowering time and duration have an immense practical importance (Vujković and Došenović, 2014). Irrespective of the fact that currently, due to a lack of available time, reduced costs and ecological benefits, the use of autochthonous plants is given precedence (Židovec and Karlović, 2005.), annual and biennial flowering plants are still fashionable due to a wide variety of their flowers.

The objective of this paper is to analyse the perception of the general knowledge about annual and biennial flowering plants in the area of Knin and Šibenik through application of the survey method.

### Material and methods

In the drafting of this paper, various publications were used as secondary sources of information. Surveys conducted in the town of Knin and its surrounding area, as well as the area of the city of Šibenik, were used as primary sources of information. The goal of the survey was to ascertain general knowledge regarding annual and biannual flowering plant species.

The survey regarding annual flowering species was conducted during May 2016 among a sample of 30 subjects in the area of Knin and its surroundings, as well as Šibenik (17 female and 13 male subjects). Distribution of sample by age group was as follows: younger than 20 years of age – 5 subjects, between 20 and 30 – 11 subjects, 30-40 – 1 subject, 40-50 – 2 subjects, 50-65 – 3 subjects, and over 65 years of age – 8 subjects. Distribution of sample by level of education was: elementary school 13,3%, secondary professional qualification 63,3%, higher expertise 13,3% and higher professional qualification 10,0%.

For the purposes of this paper, a five-degree scale (measuring attitudes on decoration and maintenance of species) with values 1 to 5 was used. Data was processed using the criteria of central tendency, that is, based on the analysis of the arithmetic mean and standard deviation. Statistical analysis of data was done using software SPSS 16 for Windows interface.

### Results and discussion

The subjects evaluate and perceive floral characteristics and means of maintenance of the following species (Table 1.): begonia (*Begonia semperflorens*), decorative summer squash (*Cucurbita pepo*) with value of circa 4,0 and perceive petunia (*Petunia x Hybrida*), Mexican marigold (*Tagetes erecta*) and scarlet sage (*Salvia splendens*) as having somewhat higher value.

According to the survey and the photographs, the subjects recognize species in the following degrees (Table 2.): sufficient – begonia, good – petunia, scarlet sage, excellent – sunflower and Mexican marigold. As seen in chart 2, the subjects have used decorative squash, sunflowers and marigolds to a higher percentage, and have used begonia, petunia and scarlet sage in a lesser degree.

Table 1. Results of evaluation and maintenance of certain flowering species

| Ordinal No. | Questions                                                                                                                            | Arithmetic Mean | Standard Deviation |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------|-----------------|--------------------|
| 1.          | Evaluate the decorative characteristics of the begonia ( <i>Begonia semperflorens</i> ). (1 – non-decorative, 5 – very decorative)   | 3,87            | ,900               |
| 2.          | Evaluate the maintenance of begonias (1 – very difficult, 5 – easy)                                                                  | 4,03            | ,964               |
| 3.          | Evaluate the decorative characteristics of the summer squash ( <i>Cucurbita pepo</i> ). (1 – non-decorative, 5 – very decorative)    | 3,97            | 1,129              |
| 4.          | Evaluate the maintenance of the summer squash (1 – very difficult, 5 – easy)                                                         | 3,93            | 1,015              |
| 5.          | Evaluate the decorative characteristics of the petunia ( <i>Petunia x hybrida</i> ). (1 – non-decorative, 5 – very decorative)       | 4,47            | ,900               |
| 6.          | Evaluate the maintenance of the petunia (1 – very difficult, 5 – easy)                                                               | 4,03            | ,850               |
| 7.          | Evaluate the decorative characteristics of the sunflower ( <i>Helianthus annuus</i> ). (1 – non-decorative, 5 – very decorative)     | 3,83            | 1,147              |
| 8.          | Evaluate the maintenance of the sunflower (1 – very difficult, 5 – easy)                                                             | 3,80            | 1,064              |
| 9.          | Evaluate the decorative characteristics of the Mexican marigold ( <i>Tagetes erecta</i> ). (1 – non-decorative, 5 – very decorative) | 4,30            | 1,022              |
| 10.         | Evaluate the maintenance of the Mexican marigold (1 – very difficult, 5 – easy)                                                      | 4,27            | ,691               |
| 11.         | Evaluate the decorative characteristics of the scarlet sage ( <i>Salvia splendens</i> ). (1 – non-decorative, 5 – very decorative)   | 4,17            | ,834               |
| 12.         | Evaluate the maintenance of the scarlet sage (1 – very difficult, 5 – easy)                                                          | 3,39            | 1,015              |

Table 2. Results of recognition and usage of certain flowering species

| Ordinal No. | Questions                                                       | Yes % | NO % |
|-------------|-----------------------------------------------------------------|-------|------|
| 1.          | Do you recognize the begonia ( <i>Begonia semperflorens</i> )   | 66,7  | 33,3 |
| 2.          | Have you used begonias?                                         | 53,3  | 46,7 |
| 3.          | Do you recognize the summer squash ( <i>Cucurbita pepo</i> )    | 100   | /    |
| 4.          | Have you used summer squash?                                    | 83,3  | 16,7 |
| 5.          | Do you recognize the petunia ( <i>Petunia x hybrida</i> )       | 73,3  | 26,7 |
| 6.          | Have you used petunias?                                         | 50,0  | 50,0 |
| 7.          | Do you recognize the sunflower ( <i>Helianthus annuus</i> )     | 100   |      |
| 8.          | Have you used sunflowers?                                       | 56,7  | 43,3 |
| 9.          | Do you recognize the Mexican marigold ( <i>Tagetes erecta</i> ) | 90,0  | 10,0 |
| 10.         | Have you used Mexican marigold?                                 | 70,0  | 30,0 |
| 11.         | Do you recognize the scarlet sage ( <i>Salvia splendens</i> )   | 70,0  | 30,0 |
| 12.         | Have you used scarlet sage?                                     | 30,0  | 70,0 |

As seen in Table 3., the subjects have used the following for balconies and terraces: *Begonia semperflorens* (60%), *Petunia x hybrida* (43%), *Tagetes erecta* (23,3%) and *Salvia splendens* (63,3%). In gardens, the subjects mostly used: *Cucurbita pepo*, *Helianthus annuus* (over 90%), *Tagetes erecta* and *Salvia splendens* (20%). In parks, the subjects perceive: *Petunia x Hybrida* (43,3%) and *Tagetes erecta* (47,7%).

It is evident from the Table 4. that the best values were assigned to the decorative characteristics of: daisy (*Bellis perennis* L.) graded 4,34 and Sweet William (*Dianthus barbatus*) graded 4,34. The

subjects perceive Sweet William (4,21) as easiest and common wallflower (3,66) as hardest to maintain.

Table 3. Results for manners of usage of certain flowering species

| Ordinal No. | Questions                                                                                                                               | A %  | B %  | C%   |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------|------|------|------|
| 1.          | Evaluate way of begonias ( <i>Begonia semperflorens</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces  | 16,7 | 23,3 | 60,0 |
| 2.          | Evaluate way of summer squash ( <i>Cucurbita pepo</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces    | 90,0 | /    | 10,0 |
| 3.          | Evaluate way of petunias ( <i>Petunia x hybrida</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces      | 13,3 | 43,3 | 43,3 |
| 4.          | Evaluate way of sunflowers ( <i>Helianthus annuus</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces    | 93,3 | 3,3  | 3,3  |
| 5.          | Evaluate way of Mexican marigold ( <i>Tagetes erecta</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces | 30,0 | 46,7 | 23,3 |
| 6.          | Evaluate way of scarlet sage ( <i>Salvia splendens</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces   | 20,0 | 16,7 | 63,3 |

#### Results of the survey research for the biennial flowers species

Surveys were conducted in the town of Knin and its surrounding area, as well as the area of the city of Šibenik. The goal of the survey was to ascertain general knowledge regarding biannual flowering plant species.

According to the survey and the photographs, the subjects recognize species in the following degrees (Table 5.): The highest number of subjects (79,3%) recognizes common hollyhock (79.3%), daisy (75,9%), Sweet William (75,9%) and pansy (75,9%). Only 51% of the subjects recognizes Canterbury bells. As seen in the chart, more subjects used common hollyhock (48,3%), Sweet William (44,8%) and pansy (34,5%) than daisy (24,1%), common wallflower (13,8%) and Canterbury bells (10,3%).

As seen in table 6., the subjects value the following species highest for balconies and terraces: *Dianthus barbatus* (69,0%); for gardens: *Campanula medium* (58,6%), *Altea rosea* (48,3%) and *Cheiranthus cheiri* (37,9%), *Viola x wittrockiana* (31,0%), *Dianthus barbatus* (17,2%), *Bellis perennis* L. (17,2%); for parks: *Bellis perennis* L. (75,9%), *Viola x wittrockiana* (69,0%) and *Cheiranthus cheiri* (55,2%).

Table 4. Results of evaluation and maintenance of certain flowering species

| Ordinal No. | Questions                                                                                                                              | Arithmetic Mean | Standard deviation |
|-------------|----------------------------------------------------------------------------------------------------------------------------------------|-----------------|--------------------|
| 1.          | Evaluate the decorative characteristics of the daisy ( <i>Bellis perennis</i> L.) (1 – non-decorative, 5 – very decorative)            | 4,34            | ,857               |
| 2.          | Evaluate the maintenance of the daisy (1 – very difficult, 5 – easy)                                                                   | 3,97            | ,906               |
| 3.          | Evaluate the decorative characteristics of the Sweet William ( <i>Dianthus barbatus</i> ). (1 – non-decorative, 5 – very decorative)   | 4,34            | 1,078              |
| 4.          | Evaluate the maintenance of the Sweet William (1 – very difficult, 5 – easy)                                                           | 4,21            | ,978               |
| 5.          | Evaluate the decorative characteristics of the pansy ( <i>Viola x wittrockiana</i> ). (1 – non-decorative, 5 – very decorative)        | 4,07            | ,884               |
| 6.          | Evaluate the maintenance of the pansy (1 – very difficult, 5 – easy)                                                                   | 4,00            | ,802               |
| 7.          | Evaluate the decorative characteristics of the Canterbury bells ( <i>Campanula medium</i> ). (1 – non-decorative, 5 – very decorative) | 3,83            | 1,104              |
| 8.          | Evaluate the maintenance of the Canterbury bells (1 – very difficult, 5 – easy)                                                        | 3,59            | 1,053              |
| 9.          | Evaluate the decorative characteristics of the Wall flower ( <i>Cheiranthus cheiri</i> ). (1 – non-decorative, 5 – very decorative)    | 4,14            | ,953               |
| 10.         | Evaluate the maintenance of the Wall flower (1 – very difficult, 5 – easy)                                                             | 3,66            | ,936               |
| 11.         | Evaluate the decorative characteristics of the Common hollyhock sage ( <i>Alcea rosea</i> ). (1 – non-decorative, 5 – very decorative) | 4,24            | ,689               |
| 12.         | Evaluate the maintenance of the Common hollyhock (1 – very difficult, 5 – easy)                                                        | 4,03            | ,944               |

Table 5. Recognition and usage of decorative species

| Ordinal No. | Questions                                                         | Yes % | NO % |
|-------------|-------------------------------------------------------------------|-------|------|
| 1.          | Do you recognize the daisy ( <i>Bellis perennis</i> )?            | 75,9  | 24,1 |
| 2.          | Have you used daisy?                                              | 24,1  | 75,9 |
| 3.          | Do you recognize the Sweet William ( <i>Dianthus barbatus</i> )   | 75,9  | 24,1 |
| 4.          | Have you used Sweet William?                                      | 44,8  | 55,2 |
| 5.          | Do you recognize the pansy ( <i>Viola x wittrockiana</i> )        | 75,9  | 24,1 |
| 6.          | Have you used pansy?                                              | 34,5  | 65,5 |
| 7.          | Do you recognize the canterbury bells ( <i>Campanula medium</i> ) | 51,7  | 48,3 |
| 8.          | Have you used Canterbury bells?                                   | 10,3  | 89,7 |
| 9.          | Do you recognize the wall flower ( <i>Cheiranthus cheiri</i> )    | 69,0  | 31,0 |
| 10.         | Have you used wall flower?                                        | 13,8  | 86,2 |
| 11.         | Do you recognize the common hollyhock ( <i>Alcea rosea</i> )      | 79,3  | 20,7 |
| 12.         | Have you used common hollyhock?                                   | 48,3  | 51,7 |

## Conclusion

A survey has revealed the following facts. The subjects evaluated the decorative characteristics of the all annuals and biennials flower species and means of maintenance with a grade of „very good“,

while their knowledge of the species is based on a percentage of: *Cucurbita pepo* (100%), *Tagetes erecta* (90%), *Dianthus barbatus* (75,9%), *Begonia semperflorens* (67,7%), *Campanula medium* (51,7) etc. They would use in: garden, parks balconies / terraces. The subject are used in different percentages annuals and biennials flower species (*Cucurbita pepo* (83,3%), *Tagetes erecta* (70%), *Helianthus annuus* (56,7%), *Viola x wittrockiana* (34,5%) etc.

Table 6. Evaluation of manners of usage of certain flowering species

| Ordinal No. | Questions                                                                                                                                 | A %  | B %  | C%   |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------|------|------|------|
| 1.          | Evaluate way of daisy ( <i>Bellis perennis</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces             | 17,2 | 75,9 | 6,9  |
| 2.          | Evaluate way of Sweet William ( <i>Dianthus barbatus</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces   | 17,2 | 13,8 | 69,0 |
| 3.          | Evaluate way of pansy ( <i>Viola x wittrockiana</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces        | 31,0 | 69,0 | 0    |
| 4.          | Evaluate way of Canterbury bells ( <i>Campanula medium</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces | 58,6 | 31,0 | 10,3 |
| 5.          | Evaluate way of wall flower ( <i>Cheiranthus cheiri</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces    | 37,9 | 55,2 | 6,9  |
| 6.          | Evaluate way of common hollyhock ( <i>Alcea rosea</i> ) application in landscape.<br>A) gardens<br>B) parks<br>C) balcony / terraces      | 48,3 | 31,0 | 20,7 |

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## References

1. Dorbić, B. i Temim, E. (2015). Povijesni pregled razvoja vrtlarstva i krajobraznog uređenja Šibenika i okolice u razdoblju 1945.-1985. godine. *Annals for Istrian and Mediterranean studies-Series Historia et Sociologia*, 25 (3): 637-650.
2. Dorbić, B. i Temim, E. (2016). Povijesni pregled razvoja vrtlarstva i krajobraznog uređenja Šibenika i okolice u razdoblju 1880.-1945. godine. *Annals for Istrian and Mediterranean studies-Series Historia et Sociologia*, 26 (2): 227-246.
3. Maegdefrau, K. (1997). Udžbenik botanike za visoke škole: sistematika, evolucija i geobotanika. Školska knjiga, Zagreb.
4. McDonald, E. (2003). 400 vrtnih biljaka za uređenje okućnice. Dušević & Kršovnik: 100.-150.
5. Piplović, S. (2003). Cvijeće na prostorima Dalmacije u XIX. stoljeću. *Agronomski glasnik* 3-5: 85-88.
6. Vinceljak-Toplak, M. (1989). Cvjećarstvo-Jednogodišnje i dvogodišnje cvjetne vrste. Opći i specijalni dio-Skripta. Zavod za ukrasno bilje, krajobraznu arhitekturu i vrtnu umjetnost. Zagreb.
7. Vujković, Lj. i Došenović, Lj. (2014). Dizajn vrta. Šumarski fakultet Univerziteta u Banja Luci: 118-119.
8. Židovec, V. i Karlović, K. (2005). Primjena autohtonog bilja u uređenju gradskog prostora. *Agronomski glasnik*, 67 (2-4): 152.

## CHALLENGES FOR THE VEGETABLE SUBSECTOR DEVELOPMENT IN THE REPUBLIC OF MACEDONIA

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### Abstract

The Republic of Macedonia has approximately 260 ha of agricultural cultivable area under glasshouses, but the exploitation of the facilities ranges between 75-80%, of their total capacity, leaving around 20-25 % of the facilities not being used. The area cultivated under plastic tunnels is not precisely known, but it is estimated between 4000 and 6000 ha dedicated for cultivation of tomatoes, cucumbers, peppers and cabbage. The open field production is gradually decreasing especially for production of tomatoes, cucumber and peppers due to lower productivity and increased problems with diseases. For other vegetables (melons, potatoes and onions) open production and strong seasonal aspects are dominating. The ex-Yugoslavia, considered as one market, traditionally has been and is still the largest consumer of the Macedonian fresh product. The position of Macedonian vegetable exporters in these markets is under threat for several reasons: market requirements shifting towards EU standards, related to the arrival of modern retail operations. The large producers are using local or international help which is very costly. There are no available financial instruments that stimulate the farmers to invest in new technologies and production, and the limited profits, mainly that the primary producers are making. High cost of input (energy) for production (energy is app.70% of the cost of the product) and the weak organization and cooperation of the stakeholders (vertical cooperation, horizontal cooperation and small sized production) are also significant challenges that should be dealt with in future. However, the biggest problem that causes weakness in this subsector is the limited Governmental and local investment in research. Due to that, there are no local producers of quality seeding material that causes higher cost of inputs and uncertainty of the quality of materials.

**Keywords:** vegetable production, markets, research and development.

### Introduction

The vegetable production used to take considerable portion of the overall agricultural production in the time of Ex-Yugoslavia. After the breakup of closed markets, vegetable producers in Macedonia, as well as the researchers and policy makers, could not identify appropriate development policies. It resulted in even worse fragmentation of agricultural parcels, decrease in quantity and quality of vegetable products, deterioration of greenhouse establishments and losing of regional markets. During the last 25 years several World Bank, EU, USAID and other donor-funded projects have been carried out to support the vegetable sector, but lacking sustainable development strategy the funds were inefficiently consumed. This also applies to the government direct payments that are approaching the amount of 100.000.000 EUR annually and still do not yield the expected results. In this report we have tried to identify the key issues that limit the vegetable sector development that would further be challenges for all stakeholders in the subsector. From perspective of research and development several issues that merit appropriate attention have been underlined.

### Material and methods

To prepare this analyses of the vegetable sector constrains a comprehensive desk research was carried-out on existing data and information obtained from the Office of Statistics, the Ministry of

Agriculture Forestry and Water Economy, reports of consulting companies from that have participated in different assessment regarding vegetable production, processing and trading. The findings from previous research at the Faculty of Agricultural Sciences and Food, as well as the Institute of Agriculture at UKIM University have also been taken into consideration.

### Results and discussion

The Republic of Macedonia is recognized in the region by a specific fresh taste of vegetables. Large scale vegetable production took over industrial crops (cotton, poppy, tobacco) in early 70s with the brake-trough of the first greenhouses. As presented in Table 1, the participation of vegetable outcome is constantly between 30% and 37% of the overall agricultural output. The agricultural production, on other hand, participates with 10% in the GDP. (Statistical Yearbook, 2017)

Table 1. Participation of vegetable production in overall agricultural output [000 EUR]

|                          | 2011      | 2012      | 2013      | 2014      | 2015      |
|--------------------------|-----------|-----------|-----------|-----------|-----------|
| Agricultural output      | 1 137 853 | 1 088 975 | 1 223 040 | 1 281 853 | 1 348 138 |
| Crop output              | 871 804   | 818 195   | 928 455   | 982 975   | 1 048 167 |
| Vegetables (-potatoes)   | 425 170   | 370 504   | 415 040   | 451 837   | 504 239   |
| % in agricultural output | 37.37     | 34.02     | 33.94     | 35.25     | 37.40     |
| % in of crop output      | 48.77     | 45.28     | 44.70     | 45.97     | 48.11     |

Source: Statistical State Office, 2017

#### Open field production

Open field production is at largest absorbed by the processing industry. The acreage is relatively steady (Table 2) with slight tendency of increase over the last 10 years. Exemptions are melons and water melons that are planted on 1000ha smaller area from the one in 2006 and cabbages that take 50% larger acreage and have tendency to expand.

Table 2. Area of open field production of main vegetable crops 2007-2016 [ha]

|         | 2007  | 2008  | 2009  | 2010  | 2011  | 2012  | 2013  | 2014  | 2015  | 2016  |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Potato  | 13799 | 13554 | 13527 | 13037 | 13454 | 13204 | 13477 | 13178 | 13360 | 13279 |
| Onion   | 3131  | 3182  | 3361  | 3554  | 3488  | 3527  | 3499  | 3588  | 3606  | 3584  |
| Beans   | 4864  | 4789  | 4925  | 4612  | 4557  | 4726  | 4608  | 4834  | 4783  | 4887  |
| Cabbage | 3236  | 3362  | 3638  | 3706  | 3766  | 3734  | 4491  | 4367  | 4815  | 4597  |
| Tomato  | 5368  | 5319  | 5731  | 5665  | 5632  | 5614  | 5478  | 5746  | 5657  | 5609  |
| Pepper  | 8331  | 8199  | 8438  | 8474  | 8465  | 8626  | 8511  | 8528  | 8622  | 8766  |
| Lences  | 1013  | 1003  | 1060  | 936   | 947   | 936   | 82    | 80    | 84    | 84    |
| Garlic  | 87    | 85    | 69    | 90    | 68    | 74    | 928   | 956   | 980   | 978   |
| Mellons | 6152  | 6211  | 5977  | 5732  | 5800  | 5691  | 5598  | 5740  | 5562  | 5506  |

Source: MAFWE – period of 2007-2016

Processors usually complain about the raw-material quality and quantity as very often vegetable varieties that are grown in open-field are intended for fresh-consumption aiming for higher prices. Therefore they lack satisfactory dry matter content and other necessary parameters for processing. A solution to this problem might be establishment of functional co-operatives that can apply single chosen variety on larger parcels. This integration of the parcel lots can also enable mechanization of different agricultural practices, including harvesting that would consequently reduce the costs of production.

Organic cultivation is another interesting development direction for enrichment of the open field production. For the time being mostly pumpkins are organically grown vegetables, although there is

space and facilities for cultivation of more sensitive cash-crops if proper services and training would be provided to the growers (Agic at al., 2012).

#### Reproductive material and plant breeding

The production of vegetable seeds in the Republic of Macedonia (up until 30 years ago) had its own tradition. Taking the favorable conditions in certain agro-climate regions in consideration, seed was produced from more garden sorts with high quality attributes (purity, germination, viability etc.), while speaking in terms of quantity it was sufficient to cover the country's own needs, and to be able to export to several Ex-Yugoslavia countries, and some European countries as well. For example, for the Netherlands, onion, carrot, tomato and watermelon seeds have been produced. From nearly 200 tons seed material from various vegetable crops in the 70's (Figure 1), today Macedonia has virtually no seed production. It is completely dependent from import of several vegetable crops, mainly from the Netherlands and Israel.

Taking into consideration the climate conditions in Macedonia, it can certainly be organized in the first two agro-climatic regions: the Sub-Mediterranean with the following locations: Gevgelija, Dojran, Valandovo and Strumica and the Continental mixed with Sub-Mediterranean region with locations: Tikvesh, Veles, Shtip, Kochani, Skopje and Kumanovo.

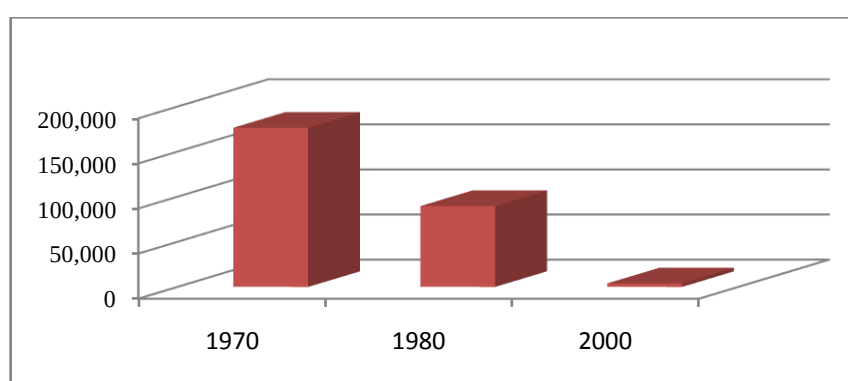


Figure 1. Historical trend of vegetables seed production quantities in the Republic of Macedonia [kg]

Seed production of vegetable crops can be easily organized in agro-climatic areas characterized with a lengthy vegetation period of 200 days registering above 10°C and a yearly sum of temperatures 4500°C. That is the most important and an irreplaceable factor for producing high quality seeds.

Table 3. Projection of seed production for domestic market

| Crop                         | Seed yield [kg-ha] | Open field cultivation [ha] | Seed rate for one ha [kg] | Total seed requirements [kg] | Wholesale price per kg seed [EUR] | Income [EUR] |
|------------------------------|--------------------|-----------------------------|---------------------------|------------------------------|-----------------------------------|--------------|
| Tomato                       | 100                | 5600                        | 0.2                       | 1120                         | 50                                | 56 000       |
| Pepper                       | 200                | 8800                        | 1.2                       | 10560                        | 50                                | 528 000      |
| Cabbage                      | 1000               | 4600                        | 0.5                       | 2300                         | 100                               | 230 000      |
| Onion                        | 1000               | 3600                        | 4.5                       | 16200                        | 150                               | 2 430 000    |
| Leek                         | 900                | 1200                        | 3                         | 3600                         | 12.5                              | 45 000       |
| Water melon                  | 100                | 3000                        | 1.2                       | 3600                         | 20                                | 72 000       |
| Melon                        | 150                | 2000                        | 2                         | 4000                         | 20                                | 80 000       |
| Carrot                       | 900                | 800                         | 7                         | 5600                         | 25                                | 140 000      |
| Snap beans                   | 1100               | 1000                        | 120                       | 120000                       | 12.5                              | 1 500 000    |
| Peas                         | 2200               | 1300                        | 200                       | 260000                       | 12.5                              | 325 0000     |
| Total predicted income [EUR] |                    |                             |                           | 8 331 000                    |                                   |              |

As presented in the Table 3, Macedonian economy can save around 10 million EUR with domestic production of vegetable seeds. The idea is not compete with the greenhouse hybrids, but to work on breeding programs and further propagation of local vegetable landraces that are well adopted and accepted in the region (Popsimonova et al., 2017).

The Republic of Macedonia does not have specialized center for production of seedlings. Currently, most of the individual farmers produce the seedlings by themselves, often in outdated way for example in warm beds, while the greenhouse producers use modern methods of container production. Very small percent of greenhouse producers obtain the transplants from specialized centers for transplant production from the neighboring countries. Based on the production area under plastic houses and greenhouses the need for vegetable transplants on annual bases in Republic of Macedonia can be estimated on 130 million units (Ristevska, 2012). The number would significantly increase if the production on open field is taken into account. The data shows the necessity of organized transplant production which will also include production of grafted seedlings.

### Greenhouses

There are approximately 260 ha of agricultural cultivable area under glasshouses, but the exploitation of the capacities ranges between 70-75%. Most of the greenhouse complexes are blocks of 6-24 ha units in extent. As presented in the Figure 2, the production area of vegetables, especially tomato, in glasshouses is drastically reduced in the last ten years. The area under plastic-houses and tunnels vary between 4000-6000ha.

The start of cultivation depends on the possibility of heating the greenhouses. The few glasshouse operations that have the financial means to purchase sufficient crude oil and start nurseries (first tomato, later the more cold-sensitive cucumber) already in November, transplant the seedlings in December and harvest the crop in February and March when very high prices can be obtained. Most glasshouse operators, who cannot afford the heating expenses (up to 0,39 Euro/kg.), start later to save on fuel consumption but fetch lower prices for the crop (Epicenter, 2012).

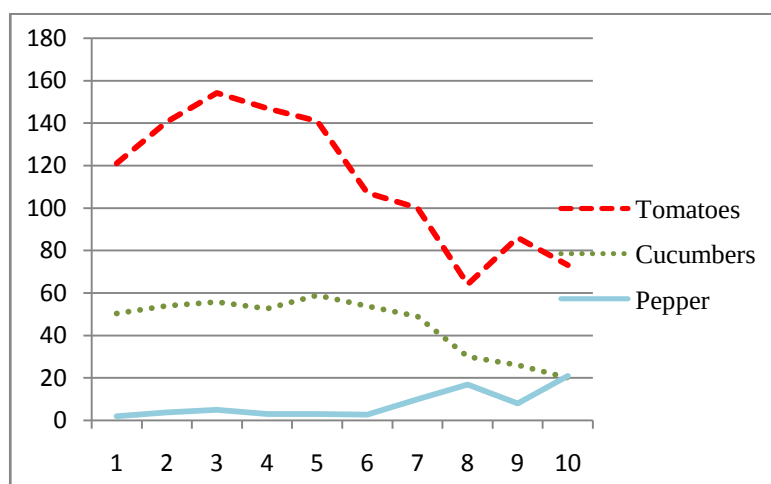


Figure 2 Decreasing trend of greenhouse area in the period 2007-2017 [ha]

Next, production in the plastic tunnels starts in February/March with harvest in May/June, depending on whether or not, the tunnels are heated. In case of heating this is done subsoil by passing hot water through plastic tubes that are laid below the ground. Most foil tunnels, however, are not heated and thus come later into production. Then the open field season starts in April, often using seedlings grown in protected nurseries and continues until September. In the hot summer months, protected cultivation cannot compete with open field cultivation. The protected cultivation starts again in September for a second crop (only tomato and cucumber-often gherkins) as practiced by an increasing number of growers, where heating is available.

Due to the climatic diversity, the production of fresh vegetables is pretty regional and almost 80% is an output of the Southeastern Macedonian region (Diagram 1). Changed Mediterranean climate that dominates in the encircled region meets the temperature requirements of most vegetables. Therefore it would be viable solution updated technologies to be introduced (such as soil-less cultivation) along with reconstruction of the heating and ventilation equipment in order year-round production to be obtained.

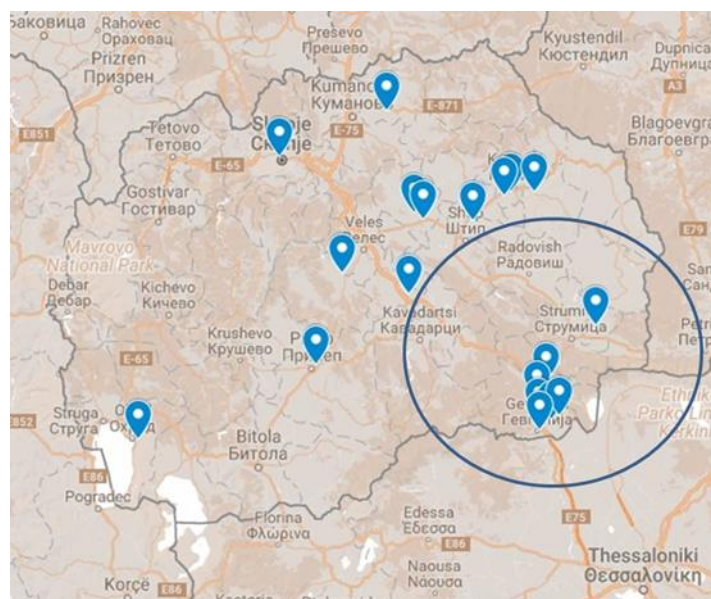


Diagram 1. Distribution of glass-houses in the Republic of Macedonia

In the rest of the country advanced technologies can be only applied in mid or long term perspective, whereas successive planting of different cold resistant crops (possibly flowers) or varieties in adequate climatic regions can be readily adopted followed by minor interventions in production technology and greenhouse structures in order to conserve and maintain energy throughout the season. In that way, the cropping season can be prolonged for few weeks.

## Conclusions

Macedonian vegetable production faces a big challenge – to move forward from the position that has been established few decades ago in completely different economic, social, and technological environment. The solutions proposed in this paper refer to open field production, protected crops and plant breeding programs. These are only few steps that need to be taken towards more productive vegetable sub-sector and they have to be supported by sound research and extension services. Due to unfortunate policy decisions and luck of research projects in the past the agricultural institutes that used to be engaged in seed production, plant breeding and technology application are now focused on teaching only. So, the very first step to improvement should be made in the research field.

## References

1. Agic Rukie, Gordana Popsimonova, Afrodita Ibusoska, Zvezda Bogevska, Margarita Davitkovska, Biljana Ristovska, Igor Iljovski (2012). Perspective of the Organic Production in Macedonia Acta Horticulturae 960, 263-268.
2. EPI CENTAR. (2013). Cost-benefit analysis of the use of different energies in greenhouses/glasshouses. Study developed for the USAID AgBiz Programme in Macedonia.
3. Ministry of Agriculture Forestry and Water Economy of the Republic of Macedonia (2006-2016). Annual Reports for Vegetable Production – personal communications.

4. Popsimonova, G., R. Agic, Z. Bogevska, M.Davitkovska, G.Georgievski.(2017. Perspective tomato landraces for fresh consumption in the Republic of Macedonia, presented on VII SEE Symposium on vegetables and potatoes, ISHS, June 20-23, Maribor, Slovenia.
5. Popsimonova G., Georgievski G., Dimitrovski D., Agić R. (2016). The greenhouse vegetables sector development in the Republic of Macedonia. *Acta Hortic.* 1142, 455-460.
6. Ristovska Biljana. (2012). Grafting-perspective way for vegetable transplants production, Master thesis Faculty of Agricultural Sciences and Food, UKIM
7. State Statistical Office of the Republic of Macedonia. (2017) Statistical Yearbook for 2017.

## BIOLOGICAL CONTROL POTENTIAL OF AN APHIDOPHAGOUS PREDATORS, CANNIBALISM AND INTERSPECIFIC COMPETITION

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### Abstract

Aphidophagous predators have a great importance in biocenosis as bio-regulators of aphids. Specific relationships between predators, interspecific competition and cannibalism, have an important role in biological control. The focus of this study was on biological control potential of predators, interactions between larvae of *Coccinella septempunctata*, *Chrysopa carnea*, *Sphaerophoria scripta*, *Aphidoletes aphidimyza* and incidence of cannibalism. Observations were made in Prilep region (Macedonia) 2012-2015 and there was applied method of survey of 20 randomly selected tobacco stalks infested with aphids. Predatory larvae were reared with *Myzus persicae* in petri dishes under laboratory conditions. All experiments, prey consumption, interaction between predators and cannibalism were conducted in the laboratory. *C. septempunctata*, *C. carnea*, *S. scripta* and *A. aphidimyza* are primarily predators and they occupy the third trophic level of food chain (tobacco-*M. persicae* -predator). The growth of population of predators continuously follows the growth of aphid population on tobacco. During investigations, 5861 predators were determined in 2012 and 3788 in 2013. According investigations during 2013 they are voracious predators of aphids. In laboratory conditions, adults of *C. septempunctata* consume in average 468 and larvae 350 aphids, *C. carnea* larvae 458 aphids, *S. scripta* 333 aphids and *A. aphidimyza* 200 aphids. Interspecific competition and cannibalism are important factors in rearing conditions of predators. The outcome of interactions between predators 2014/2015 mainly depends on the body size of the competitors. In general large individuals behaved as predator while small individuals became prey. Eggs and L1 were extremely vulnerable in regard to larger larvae. Cannibalism in *C. septempunctata* and *C. carnea* occur when eggs or young instar larvae are kept together with larger larvae, in the absence of aphids. Predators are often used as biological control agents in Integral Pest Management, so mass rearing techniques must be developed which guarantee higher survival rates of predators.

**Keywords:** biological control, aphids, specific relationships.

### Introduction

Constant progress in the field of biological control has raised the need to: create specific norms to allow its further development, produce biological control items, and make its use feasible by growers. Interest in the use of biological control techniques has grown worldwide as an alternative to chemical control, especially due to the adverse effects of chemicals on the environment and human health (Nogueira et al., 2016). Predation rate and numerical response are basic to any investigation of predator-prey relationships and key components in the selection of predators for biological control (Madahi et al., 2015). Predators and parasites keep the biocenosis balance and are able to reduce the calamity of pests on cultivated and weed plants. Aphidophagous predators have a great importance in biocenosis as bio-regulators of aphids (Vasilev, 1971; Kaitazov, et al., 1982; Vukovic, 1986; Vukovic, 1990; Harizanov and Babrikova, 1990; Simova-Tosic et al., 1989; Vujic and Radenkovic, 1995; Lazarevska, 1998; Janusevska, 2001; Krsteska, 2007). *C. septempunctata* has a broad ecological range. It is a polyphagous species; it mainly preys on aphids and other similar insects. The lady for whom it was named was "the Virgin Mary" and seven spots symbolize her seven

joys and seven sorrows (Lazarevska, 1998). The main predators of ladybirds are usually birds, but they are also the prey of frogs, wasps, spiders, and dragonflies. Attractive red colour warns would-be predators, that the ladybird is distasteful or toxic. *C. septempunctata* is predator of *M. persicae* on tobacco (Krsteska et al., 2004). The common green lacewing, *C. carnea*, is found in many parts of America, Europe and Asia. The adults feed on nectar, pollen and aphid honeydew, the larvae are active predators and feed on aphids, other small insects and also on caterpillars. *C. carnea* is predator of *M. persicae* on tobacco (Krsteska et al., 2005). Lacewings can be attracted by using certain companion plants and tolerating beneficial weeds. *C. carnea* adults consume pollen and nectar of Umbeliferrae species (Szentkirályi, 2001).

*S. scripta* is obligate aphidophagous species and has a worldwide distribution. It is useful pollinator in its adult stage and presents a natural enemy of plant pests, especially of aphids in its larval stage. *S. scripta* is most widely distributed in almost all tobacco growing regions in R. Macedonia (Prilep, Bitola, Krusevo, Kavadarci, Valandovo, Strumica, Radovis, Stip, Veles, Sv. Nikole). It is characteristic for all types of open habitats (Krsteska, 2008). Predatory midge *A. aphidimyza* is general aphid predator, attacking many different species of aphids on different host plants. Larvae are voracious native predators of over 60 species of aphids. Aphidoletes are used to control aphids indoors in commercial greenhouses and interior plants as well as outdoors in orchards, shade trees, roses and home gardens. *A. aphidimyza* played an important bio-regulatory role in the control of *M. persicae* on tobacco (Krsteska et al., 2003). Interspecific interactions among natural enemies also are named intraguild predation (IGP). Interactions between coexisting species of predators that share the same aphid prey resource in a patchy habitat often result in intraguild predation (Polis et al., 1989; Rosenheim et al., 1995, cit. Agarwala, 2003). In IGP the outcome of an encounter between two guild partners depends on their following characteristics: relative body size, mobility, vigour aggressiveness, defensive strategies and degree of feeding specificity (Szentkirályi 2001). In specific relationships between aphidophagous predators, cannibalism has an important role in biological struggle.

The focus of this study was on biological control potential of predators, interactions between *C. septempunctata*, *C. carnea*, *S. scripta* and *A. aphidimyza* (as the most present predators on tobacco) and factors that can induce cannibalism.

### Material and methods

Observations were made on tobacco in region of Prilep during 2012-2015. To investigate population dynamics of predators, in 2012/2013 there was applied method of survey of 20 randomly selected tobacco stalks infested with aphids, in 10 days interval during vegetation. All experiments: the measurement of prey consumption (biological control potential of predators), interaction between predators, cannibalistic behavior of predators were conducted in the laboratory. The eggs, larvae, are placed individually in petri dishes and were reared on tobacco leaves infested with *M. persicae* in laboratory conditions. Once hatched the larvae are subject to establishing the number of larval stages, their voracity and their morphological changes. To determine the number of leaf aphids needed for the development of the predator, we fed the newly hatched larvae with leaf aphids in the same uniform age structure as food of predator in these experiments. Every 24 h the number of consumed aphids was recorded, the remaining aphids were removed, and new aphids were introduced into the petri dish. The larvae feeding were prolonged until they converted into pupae. We calculated "meals" of leaf aphids, in new hatched adults of *C. septempunctata*.

In 2014/2015 we study interaction between predators and cannibalistic behavior of predators in laboratory conditions. Larvae of the five species, that are in different stages of development were kept together (opposing one with another predator) to investigate interaction between predators. For measurement of interaction of predators, first step is gentle separation of predators, opposing one with another predator, out of the colonies of plant aphids, and on tobacco leaves in Petrie containers infested with aphids. To investigate the incidence of cannibalism in bigger petri dishes,

larvae of each species were kept together in plenty of food and in another experiment in absence of aphids.

### Results and discussion

During investigation (2012-2015) of fauna species of the Aphididae family, it appeared that tobacco crop was attacked only by *M. persicae*. *C. septempunctata*, *C. carnea*, *S. scripta* and *A. aphidimyza* are primarily predators and they occupy the third trophic level of food chain (tobacco-*M. persicae*-predator).

*C. septempunctata* is one of the commonest ladybird predators of *M. persicae* in tobacco habitats. It occurs with the biggest quantitative representation of 5285 individuals. The seven-spot ladybird living almost anywhere there are aphids and during our investigations it reaches its maximum in mid-August. Adult are oval, red, with seven black spots on elytra. The head is black with a white or pale spot on either side of the head. Females lay eggs vertically in groups, near their prey. Eggs are oval, yellowish-orange. Larva is gray-black and on first, fourth and seventh abdominal segment has a bright orange stain. Pupae are black with orange patterns, unprotected by a cocoon. Both the adults and the larvae are voracious predators of aphids. Mandibles are used for chewing the aphids or another pray. During 2013 in laboratory conditions, adults of *C. septempunctata* consume in average 468 aphids. The larvae pass through four instars and consume in average 350 aphids.

Todoroski and Maceljiski, 1983, study that larva consume 361 aphids and imago 756 aphids. According Mahyoub, 2013, the average number of aphids consumed by each of the four instar larva are 35, 63, 96, and 290, respectively, under laboratory conditions. Sattar et al., 2008, reported that single larva during 1st, 2nd, 3rd and 4th instars consumed 21.9, 55.9, 107.4 and 227.3 aphids respectively. When aphid colonies are small and prey is scarce cannibalism of eggs, larvae and pupae may occur, especially when the age structure among the larvae population was heterogeneous.

Cannibalism is adaptive in that it improves the chances of survival of this species. Adult exude repulsive smell as reflex-bleed from the tibio-femoral joints of its legs when it was disturbed, as defense mechanism against predators. A threatened adult of *C. septempunctata* may play dead to protect itself. Stinking smell is found also among larva of *C. septempunctata*.

Larvae are mobile, and they may wander for pray. When acting as a predator, *C. septempunctata* used mandibles to tear large irregular holes in aphids. Adult coccinellidae were very aggressive and even in the presence of aphids attacked all larval stage of *C. carnea* (Szentkirályi, 2001).

A new method for *C. septempunctata* egg manipulation was developed in Egypt, which consists of a plastic cylindrical puts inside the rearing cages to lay eggs in it and after laying eggs-masses transferred to a separated machine to separate the egg-masses and to be ready to stick on the card to release (Mahyoub, 2013). *C. carnea* is dominant species of lacewing as bio-regulators of *M. persicae* on tobacco. Monitoring the dynamics of growth of populations revealed that it reaching its maximum in mid-August. Adults are green, with long antennae and membranous, pale green wings and golden eyes. In the evenings and at night they are attracted by lights and we often catch adults with light trap. In field we found laid eggs in groups underneath tobacco leaves near potential prey. Egg is oval and secured to the plant by long slender stalks as protection of cannibalism. The larvae are voracious predators of aphids and actively search for prey. The larvae are brown are known as "aphid lions". Larva grasps their prey with mandibles and suck out the liquidated body fluids of an aphid. During the larval stage it molt three times. Larvae can consume large numbers of aphids. In laboratory conditions, larvae consume in average 458 aphids. The mature larva secrete silk and build round white cocoon in concealed positions on tobacco plants and pupate.

Lacewings use various defensive methods for protection against natural enemies. Larva is mobile, when it was attacked by another prey, larva quickly increase the distance from the aggressor. Adults exude some strong, stink smell when were attacked (Szentkirályi, 2001).

Larvae of *C. carnea* are highly aggressive in confined petri dishes. In confrontation with another predator when *C. carnea* was predator, puncture holes from the mandible insertion could be

observed in the body of the prey. Same aged or older and stronger larvae of *C. carnea* killed the larvae of *C. septempunctata* in the absence of other aphid prey (Szentkirályi, 2001).

When food is scarce larvae of *C. carnea* turn cannibal. Cannibalism can ensure the survival of population. Usually cannibalism in all experiment occurs when eggs or young instar larvae are kept together with larger larvae. The extent of cannibalism even in the same size larvae or same instar larvae is high. In manipulation, *C. carnea* must be distributed as eggs, to avoid cannibalism.

*S. scripta* is important natural regulator of aphid population on tobacco. The adult is black, with yellow strips. Sexual dimorphism is present. The female has long body, broadened somewhere near the middle and ending conically. The male has a long, slim, cylindrical body, and the last two bands are often blurred. The male's eyes are touching. The female's eyes are not touching. After mating, the females lay eggs singly on the underside of tobacco leaves, among the aphid colonies. Egg is off-white, small rounded at both ends, with surface patterning. The larvae are green with a wide pale white dorsal band on either side of the heart line. During their growth, pass through three stages. Larva does not leave black excrement frequently, but only before pupation. Pupa is formed from the last larval skin and its color and pattern resemble the 3rd stage. Larvae kill a great number of aphids. They have strong mouth hooks, suitable for catching the prey, sharp mouthparts, strong pharynx and head muscles which help them to stab and suck the prey. In laboratory conditions, larvae consume in average 333 aphids. Larvae greed increases during the second, and particularly during the third larval stage. When hungry, the larva sucks the first aphids completely and as it becomes satiated, it doesn't eat it thoroughly, but goes to find another aphid. Cannibalistic tendencies in *S. scripta* are very low and we have not established a mutual attack.

The larvae with confrontation with another predator move very fast, always touching the substrate with their heads and excreting oral slime to defend themselves against the opponent. When *S. scripta* larvae were superior in confrontations, the remaining body parts of the prey showed a rather large circular hole as a result from repeated mouth-hook insertions. There is a slime secretions on the whole body surface of prey.

*A. aphidimyza* is recognized as being an important naturally occurring control agent of aphids on tobacco. Adults are black, small, delicate midges (flies) with long legs and antennae. Adult midges are very efficient at locating aphid colonies. Females deposit tiny shiny orange eggs singly or in small groups among aphid colonies. Small, bright orange, slug-like larvae inject a toxin into aphids' leg joints to paralyze them and then suck out the aphid body contents through a hole bitten in the thorax. Larvae can consume aphids much larger than themselves and may kill many more aphids than they eat when aphid populations are high. In laboratory larvae of *A. aphidimyza* consume in average 200 aphids of *M. persicae*. Cannibalistic tendencies in Aphidoletes are very low and we have not established a mutual attack. Interactions between *A. aphidimyza* and *C. septempunctata*, *C. carnea*, *S. scripta* were asymmetric and *A. aphidimyza* larvae were always victims.

During investigations, 5861 predators were determined in 2012 and 3788 in 2013. The growth of population of predators 2012/2013 continuously follows the growth of aphid population on tobacco (Figure 1 and 2). Cannibalism is important factor in rearing conditions of predators. According our investigations, in plentiful populations of aphids, cannibalism among predators is rare feature. Hindayana, 2001, stated that in the absence of aphids, eggs of Syrphidae and Aphidoletes were heavily cannibalized by the conspecific larvae, especially by older larvae. In the absence of aphids, L1 was vulnerable to older larvae too. In general, the cannibalism on L1 (in both species) decreased significantly in the presence of aphids. According our investigations cannibalism among older larvae or larvae with equal size did not occur, neither in the absence nor in the presence of aphids. The larvae of Syrphidae and Aphidoletes would rather starve to death instead to eat the conspecific larvae. Cannibalism is common in *C. septempunctata*, especially when the aphid population is small or in the absence of prey (aphids) and when the age structure among the larvae is heterogeneous.

On the other hand, cannibalism in *C. carnea* frequently occurred and was high even when food was abundant and population density low (Ridgway et al., 1970; Phoofolo and Obrycki, 1998, cit. Hindayana, 2001).

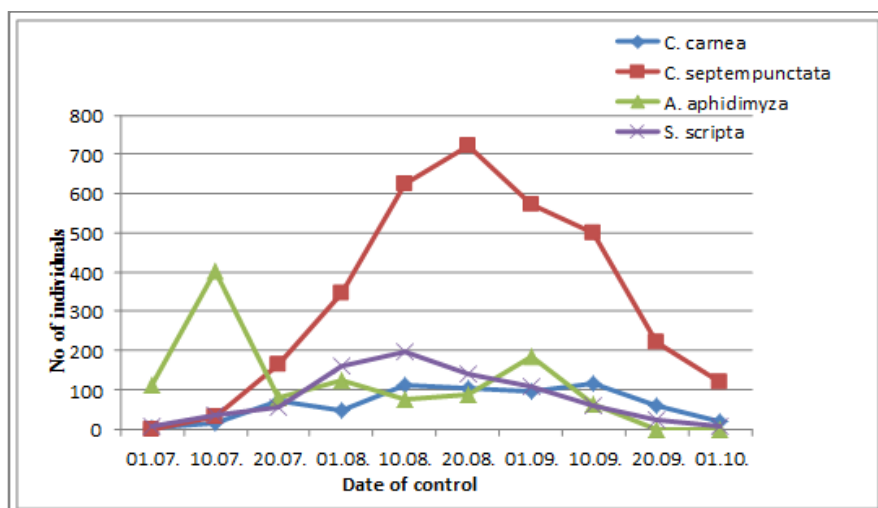


Figure 1. Population dynamics of aphidophagous hoverflies on tobacco in 2012

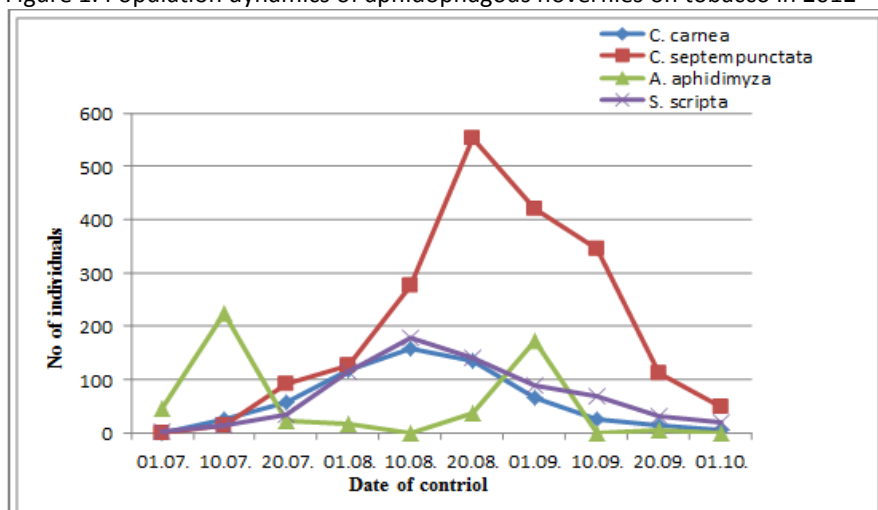


Figure 2. Population dynamics of aphidophagous hoverflies on tobacco in 2013

According our investigations, cannibalism in *C. carnea* occurs when eggs or young instar larvae are kept together with larger larvae. It was still high, even in the same instar larvae. Almost in all habitats with leaf aphids all variety of natural enemies of aphids is also present. Predators whose use similar, often limited resources, are potential competitors. *S. scripta*, *C. septempunctata*, *C. carnea* and *A. aphidimyza* share the same foraging habitat. The outcome of interactions between predators mainly depends on the body size of the competitors. In general large individuals behaved as predator while small individuals became prey. Eggs and first instar larvae were extremely vulnerable in regard to larger larvae. Mobility of predators also affects the occurrence of interspecific interaction. All instars of the rather small, immobile and defenseless *A. aphidimyza* larvae were always prey to *S. scripta*, *C. carnea* and *C. septempunctata* larvae. Eggs and young instars of *S. scripta* were very sensitive to predatory behavior by *C. septempunctata* and *C. carnea*, and their larvae consume prey in all experiments without aphids. In L1, *S. scripta* did not show defense reactions or counterattack behavior in confrontations, unlike older larval stages (L2 and L3). In those cases where *S. scripta* larvae behaved as predator, L2 was the first developmental stage that was able to kill larvae of *C. septempunctata*, *C. carnea* and *A. aphidimyza*. However in competition, large proportion of *C. septempunctata* and *C. carnea* larvae survived an aggression, i.e., were not consumed by *S. scripta*. Larvae of both predators were killed depending on the relative body size of prey (*C. carnea* or *C. septempunctata*) and predator (*S. scripta*).

In experiments with *C. septempunctata* and *C. carnea*, the presence of aphids significantly decreased specific interaction between predators. *C. carnea* possess powerful mandibles and it is able to pierce the other predators. Spikes on the dorsal integument of *C. septempunctata* larvae prevent successful attacks of predaceous larvae. In *C. septempunctata* and *C. carnea* defense mechanisms were less pronounced in stages eggs and L1. Confrontations between predators of similar size often resulted in symmetric interactions where neither species consistently took on the role of the predator. In this experiment the large individuals behaved as predator while small individuals became prey. Interspecific competition is an adaptation due to the short-term nature of the colonies of the plant aphids. In laboratory experiments, both cannibalism and IGP decreased with increasing aphid density.

### Conclusions

Aphidophagous predators compete for the same kind of victim or plant aphids in natural angorecosystems. *S. scripta*, *C. septempunctata*, *C. carnea* and *A. aphidimyza* are predators of *M. persicae* on tobacco. Under favourable conditions (temperature and food resource availability) there they may develop a large population. The growth of population of predators approximates roughly the period of mass reproduction of aphid population on tobacco. During the diet, predators often encounter various types of leaf aphid predators, from other families. In these situations, the same types of insects in the food chain can be predators and victims. The size of the predators determines the outcome of the interaction. Cannibalism in lacewings and ladybirds generally occurs when food resources are rare. Predators can be used in biological control of insect pests of crops. Interspecific competition and cannibalism are important factors in conditions of breeding and biological struggle.

### References

1. Agarwala, B.K. and Dixon, A.F.G. (1992). Laboratory study of cannibalism and interspecific predation in ladybirds. *Ecological Entomology*, 17, 303–309.
2. Agarwala, B.K., Bardhanroy, P., Yasuda, H. and Takizawa, T. (2003). Effects of conspecific and heterospecific competitors on feeding and oviposition of a predatory ladybird: a laboratory study. *The Netherlands Entomological Society Entomologia Experimentalis et applicata* 106: 219–226, *Annual Review of Ecology and Systematics*.
3. Fox, L.R. (1975). Cannibalism in natural populations. *Annu. Rev. Ecol. Syst.*, 6, 87–106.
4. Harizanov, A. and Babrikova, T. (1990). *Biologicna borba sresu nepriatelite po rasteniata*, Izdatelstvo "Zema", Sofia.
5. Hindayana, D. (2001). *Ressourcennutzung von Episyrphus balteatus DeGeer (Diptera: Syrphidae) und Intraguild predation*, Universität Hannover, Doktors der Gartenbauwissenschaften, Dissertation.
6. Janucevska, V. (2001). *Predatori i paraziti na lisnata voska Myzus persicae Sulz. na tutunot*. Magisterski trud. Zemjodelski fakultet Skopje.
7. Kaitazov, A., Cankov, T., Videnova, E. and Nackova, B. (1982). *Narocnik za biologicna borba s nepriatelite po rasteniata*. Zenizdat-Sofia.
8. Krsteska, V., Anceev, E. and Postolovski, M. (2003). *Aphidoletes aphidimyza (Cecidomyiidae) - bio regulator of tobacco aphids*, *Tutun/Tobacco*, 53 (9-10): 307-313.
9. Krsteska, V., Anceev, E. and Postolovski, M. (2004). *Ladybeetles (Coccinellidae, Coleoptera) predators of Myzus persicae Sulz. on tobacco*. *Tutun/Tobacco*, 54 (7-8): 164-173.
10. Krsteska, V., Anceev, E., Postolovski, M. and Vukovic, M. (2005). *Species of the Chrysopidae family – predators of tobacco aphids*, *Tutun/Tobacco*, 55 (9-10): 204-210.
11. Krsteska V. (2007). *Afidofagni osoliki muvi (Diptera, Syrphidae) na tutunot vo Prilepsko*. Doktorska disertacija. Zemjodelski fakultet- Skopje.
12. Krsteska, V. (2008). *Faunistic analysis of Sphaerophoria scripta L.*, *Tutun/Tobacco*, 58 (5-6): 120-130.

13. Лазаревска, С. (1998). Ентомоценоза на пченица во услови на различен приод во заштитата на растенијата Докторска дисертација, Земјоделски факултет, Скопје.
14. Madahi, K., Sahragard, A. and Hosseini, R. (2015). Predation rate and numerical response of *Aphidoletes aphidimyza* feeding on different densities of *Aphis craccivora*, *Journal Biocontrol Science and Technology*, Vol. 25, Issue 1.
15. Mahyoub, J.A., Mangoud, A. A. H., Ghamdi, K.M.A.L. and Ghramh, H.A.Al. (2013). Method for Mass Production the Seven Spotted Lady Beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae) and Suitable Manipulation of Egg Picking Technique, *Egypt. Acad. J. Biolog. Sci.*, 6 (3): 31 -38.
16. Nogueira, S.L.A., Peres, M.C.Y.P., Moraes, G.J., Scardini, M.P. J., Prado, S., and Vasconcelos, M. R. (2016). Estações quarentenárias e aspectos legais do uso de agentes de controle biológico no Brasil *Pesq. agropec. bras.* vol.51 no.5.
17. Sattar, M., Hamed M. and Nadeem S. (2008). Biology of *Coccinella septempunctata* Linn. (Coleoptera : Coccinellidae) and its predatory potential on cotton aphids, *Aphis gossypii* glover (Hemiptera : Aphididae), *Pakistan journal of zoology* 40 (4):239-242.
18. Simova-Tosic, D., Vukovic, M. and Antic, M. (1989). Prilog proucavanju bubamara predatora lisnih vasi (Coccinellidae, Coleoptera). *Zastita bilja*, Vol. 40 (1), br. 187, Beograd.
19. Simova-Tosic, D., Vukovic, M. and Gajic, M. (1989). Prilog proucavanju osolikh muva (Diptera, Syrphidae) predatora lisnih vasi. *Zastita bilja*, Vol. 40 (2), br.188, Beograd.
20. Szentkirályi, F. (2001). *Lacewings in the Crop Environment*, Cambridge University Press Chapter 5 Ecology and habitat relationship, 82-115.
21. Todoroski, B. and Maceljiski, M. (1983). *Myzus (Myzodes) persicae*, Priracnik izvestajne i prognozne sluzbe zastite poljoprivrednih kultura, Kolektiv autora, Beograd.
22. Vasilev, Lj. (1971). Bioloski razvoj i predatorsko vlijanie na *Coccinella septempunctata* L. vrz reduciranjeto na lisnata voska *Myzus (Myzodes) persicae* Sulz. na tutunot . *Tutun*, 21, (3-4): 81-102.
23. Vukovic, M. (1986). Predatori biljnih vasi sa posebnim osvrtom na familiju Chrysopidae, Magisterski rad, Poljoprivredni fakultet, Zemun.
24. Vukovic, M. (1990). *Biologija afidofagne musice Aphidoletes aphidimyza Rond. (Cecidomyiidae)*", Doktorska disertacija, Poljoprivredni fakultet, Novi Sad.
25. Vujic, A. (1987). *Sirfide (Diptera:Syrphidae) Vrsackih planina*. Magistarski rad, Univerzitet u Novom Sadu, PMF, 1-211.

## THE INFLUENCE OF THE ANTHROPOGENIC FACTOR ON THE BIODIVERSITY OF CODLING MOTH NATURAL ENEMIES

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### Abstract

The human role in bioecological examinations of codling moths is of crucial importance for the integral protection of pome fruit and the production of healthy and safe food. The codling moth (*Cydia pomonella* L.) is one of the most significant apple pests in terms of causing the diminished yield and poorer fruit quality in apples. As such, the codling moth requires daily monitoring by agricultural producers. The aim of this research was defined by the need for establishing the presence of codling moth natural enemies and the estimation of their codependence in apple plantations with various methods of protection. The examinations were carried out on the territory of Southern Serbia from 2005 to 2008. During the research, the standard entomological methods, such as pheromone traps, branch beating and placing bands around tree trunks, were used to track the presence, number and population dynamics of the codling moth and its natural enemies. The research results revealed the dominant role that the anthropogenic factor has in many segments of the integral apple protection and the production of high-quality apple fruit. Faunistic researches are inevitable for the correct determination of human actions, due to the clear composition of autochthonous natural enemies of the codling moth. The natural enemies of the codling moth whose presence was determined during the research belong to the following orders: Hymenoptera, Diptera, Dermaptera, Neuroptera, Coleoptera and Heteroptera. The analysis that has examined the human influence on the autochthonous natural enemies of the codling moth also contributes to the preservation of biodiversity and agrobiocenosis, as well as to the production of high-quality, safe food.

**Keywords:** Human influence, faunistic researches, *Cydia pomonella* L., integral protection, Southern Serbia.

### Introduction

The attitude of man towards nature is important for the strategy of agricultural crop protection. People should pay attention not only to the battle against pests, but also on changing their attitude towards nature in terms of biodiversity preservation and enhancement of the role that useful entomofauna plays in agrobiocenosis. The more diverse and complex agrobiocenosis is, the more resilient it is, which leads to the more efficient mechanisms of natural pest control, better soil fertility and plant pollination. Only 1% of animals are harmful. All others are useful or potentially useful (Čamprag, 2000). The existence of every insect or any other living organism is justified and purposeful; however, their increased or decreased number is due to people's actions. The apple is the most profitable kind of fruit and its production requires lots of human labour. The most prevailing plantations in Serbia are the traditional ones with outdated range of apple varieties. The leading apple variety is still Idared, with a tendency to become less common (Keserović et al., 2013). Favourable natural climatic and edaphic conditions, as well as human and scientific potentials in Serbia are far from being properly utilized. Provided that the modern technology of production is applied, the apple can bring the profit no other fruit variety can. (Veličković et al., 2009).

Intensification of fruit production leads to the emergence of great number of pests, which are most easily removed by using chemicals. However, long-term pesticide usage jeopardizes natural balance,

biodiversity and bioregulatory mechanisms. On the other hand, meteorological factors that are constantly changing in the direction of global warming have a specific influence on the development of apple production. Drought causes stressful reactions among fruit, which decreases their ability to resist pests. This makes apple irrigation mandatory and optimisation of irrigation deadlines and norms necessary (Milutinović et al., 2002) to preserve pest resilience of fruit. By using monoculture fruit production, man creates specific conditions in the agrobiocenosis of orchards. The codling moth (*C. pomonella* L.) is the predominant type of insect detected respectively in monoculture apple plantations, mixed plantations, neglected orchards and wild apple trees. Fighting for its survival, this pest feeds on the fruit that man is also interested in (Almaši et al., 2004). Man's role in biological and ecological examinations of the codling moth is of vital importance for the integral protection of apple-like fruit and the production of healthy food. The codling moth requires monitoring on a daily basis by agricultural producers, as it is one of the most significant apple pests in terms of causing reduced apple fruit yield and diminished apple fruit quality. The aim of the examination is determined by the necessity to detect the presence of codling moth's natural enemies and estimate their interdependence in apple plantations with different protection approaches. Apple protection should be based on the integrated principles, making natural insect growth regulators of the codling moth a priority when it comes to the examination of codling moths' biology and ecology. Monitoring the biology and ecology of harmful and useful organisms enables the adequate protective reaction, with appropriate protective measures at the right time, leading to the most effective pest reduction, while preserving useful organisms and protecting the environment at the same time. The controlled usage of chemicals preserves the natural population of useful insects and enables them to act as bioregulators in fruit plantations (Nikolić et al., 2012).

### **Material and methods**

The examinations were carried out on the territory of Southern Serbia from 2005 to 2008. The chosen examination areas were located at Beli Potok, Donje Stopanje, Leskovac, Stojkovce (all four belonging to Jablanica District), Nis (Nisava District), Prokuplje (Toplica District), Vladicin Han and Prekodolce (parts of Pcinja District). The examination and monitoring were carried out in each examination area, on one fruit plantation (monoculture or mixed) with chemical protection. Nearby the examination areas, there were apple fruit trees in neglected plantations or in yards that were not chemically protected. The examination areas were located at different altitudes, which is relevant for comparison of developmental phases of the codling moth and its natural enemies. Meteorological data (air temperature and precipitation) were collected from weather stations in Leskovac, Nis and Vranje.

During the examination, the visual method was used as the basis for monitoring the growth of pests and their natural enemies in each examination area. The branch beating method was used for the collection of entomological material from randomly chosen fruit trees in orchards. Pheromone traps were used for monitoring the number and flight dynamics of the codling moth. Insect-hunting belts made from corrugated cardboard were set on branches and tree trunks in order to collect larvae and pupae of *C. pomonella* and its natural enemies. The gathered entomological material was carried to the laboratory where it was subjected to the taxidermy, labeling, determination and collecting. Human activity has been analyzed throughout the examination – particularly the influence of producers on entomofauna in the monitored agrobiocenoses.

### **Results and discussion**

Southern Serbia has the humid continental climate with certain particularities. The valleys have a mild climate characteristic for the region of Zupa, whereas higher mountainous areas have the alpine and subalpine climate. Summer temperatures vary from moderate to very hot; winters are moderately cold, with spring and autumn in between. The districts of Jablanica, Pcinja, Nisava and Toplica are parts of Southern Serbia.

Agricultural production in Southern Serbia is fairly traditional, only partially influenced by new technologies, and with numerous small parts of heterogeneous arable land. The districts of Jablanica and Pcinja are the poorest areas in Serbia. The natural potential for the development of fruit production is satisfactory. However, the basic obstacle to the increase in fruit production is the anthropogenic factor (lack of manpower, unsatisfactory education of fruit producers, outdated types of plantations and an obsolete range of fruit varieties, an unfavorable age and educational structure of the workforce, poor traffic and hydro-technical infrastructure). A significant characteristic of the respective districts of Jablanica and Pcinja is well-preserved nature and rich biodiversity stemming from specific microclimatic conditions and geodiversity.

The examination period (2005-2008) was very dry and extremely hot. The year of 2007 was one of the hottest years ever recorded in Serbia. As a result of high temperatures, fruit plantations succumbed to the loss of freshness and premature fruit drop (in Prokuplje, Nis and Beli Potok). Fruit with decreased hardness and freshness is more likely to be attacked by codling moth caterpillars. Repeated mechanical apple fruit damage caused by hail was also recorded in 2006 and 2007 (in Prokuplje, Prekodolce and Leskovac). Mechanically damaged apple fruit is more prone to the attacks of codling moth caterpillars.

The examination results have shown that the anthropogenic factor has a dominant role in many segments of integral apple protection and production of high-quality apple fruit. The number of treatments carried out against *C. pomonella* in the examination areas between 2006 and 2008 varies from 2 (Leskovac in 2006/2007; Vladicin Han in 2008 and Prekodolce in 2007) to 12 (carried out in Beli Potok in 2008). The choice of chemicals is of crucial importance for efficient chemical protection. Keserović et al. (2013) pointed out that integral apple production cannot survive without the use of agrochemicals; it is, therefore, necessary to improve the efficiency of their application. Almaši et al. (2004) also discussed the possibility of controlled and selective use of chemicals. Thus, according to Nikolić et al. (2012), natural population of useful insects is preserved, alongside their undisturbed bioregulatory function in fruit plantations. According to Ognjanov et al. (2007), the concept itself of integral and biological apple production represents the unique and most important path fruit production should take in preparation for the integration into the European Union.

Faunistic research is inevitable for the correct conceptualization of human activity, due to a clear and easily visible species composition of *codling moths' autochthonous natural enemies*. Lazarevska et al. (2005) claim that the species composition of natural enemies depends on natural geographical and microclimatic conditions. Lacey and Unruh (2005) emphasize the key role that natural enemies of the codling moth play in the integral protection system against *C. pomonella*. Monteiro et al. (2008) point out to numerous predators and pests that have insufficient control over the codling moth in commercial plantations.

The research carried out on the territory of Southern Serbia determined the presence of the following natural enemies of the codling moth: Hymenoptera, Diptera, Dermaptera, Neuroptera, Coleoptera and Heteroptera.

***Trichomma enecator*** Rossi. (Hymenoptera: Ichneumonidae)  
***Pristomerus vulnerator*** Panz. (Hymenoptera: Ichneumonidae)  
***Ascogaster quadridentata*** Wesm. (Hymenoptera: Braconidae, Cheloniinae)  
***Chelonus annulipes*** Wesm. (Hymenoptera: Braconidae, Cheloniinae)  
***Itoplectis maculator*** F. (Hymenoptera: Ichneumonidae, Pimplinae)  
***Itoplectis alternans*** Grav. (Hymenoptera: Ichneumonidae, Pimplinae)  
***Phaeogenes invisor*** Thumb. (Hymenoptera: Ichneumonidae)  
***Agathis laticarpa*** Telenga (Hymenoptera: Braconidae, Agathinae)  
***Apanteles*** sp. (Hymenoptera: Braconidae)  
***Campoletis chloridae*** Uchida (Hymenoptera: Ichneumonidae)  
***Scambus nigricans*** Thoms (Hymenoptera: Ichneumonidae)  
***Temelucha discoidales*** Szépligeti (Hymenoptera: Ichneumonidae)

*Bracon sphaerocephalus* Szepi. (Hymenoptera: Braconidae)  
*Eulophus pennicornis* Ness. (Hymenoptera: Eulophidae, Eulophinae)  
*Torymus bedeguaris* L. (Hymenoptera: Torymidae, Toryminae)  
*Perilampus intermedius* Boucek (Hymenoptera: Perilampidae, Perilampinae)  
*Dibrachys affinis* Masi (Hymenoptera: Pteromalidae, Pteromalinae)  
*Habrocytus chrysosus* Walkien. (Hymenoptera: Pteromalidae)  
*Trichogramma* sp. (Hymenoptera: Trichogrammatidae) (сл. 1.)

*Actia pilipennis* Fall. (Diptera: Tachinidae)

*Forficula auricularia* L. (Dermaptera: Forficulidae)  
*Forficula smyrnensis* (Dermaptera: Forficulidae)

*Chrysopa carnea* Steph. (Neuroptera: Chrysopidae)  
*Chrysopa perla* L. (Neuroptera: Chrysopidae) (сл. 2.)  
*Dichochrysa ventralis* Curtis (Neuroptera: Chrysopidae)

*Coccinella septempunctata* L. (Coleoptera: Coccinellidae)  
*Coccinula quatuordecimpustulata* L. (Coleoptera: Coccinellidae)  
*Adalia bipunctata* f. *Tybica* (Coleoptera: Coccinellidae)  
*Adalia bipunctata* f. *Sexpustulata* (Coleoptera: Coccinellidae)  
*Adalia decempunctata* L. (Coleoptera: Coccinellidae)  
*Chilocorus renipustulatus* (Scriba) (Coleoptera: Coccinellidae)  
*Hippodamia variegata* Goeze (Coleoptera: Coccinellidae)  
*Oenopia conglobata* L. (Coleoptera: Coccinellidae)  
*Platynaspis luteorubra* Goeze. (Coleoptera: Coccinellidae)  
*Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae)  
*Psyllobora vigintiduopunctata* L. (Coleoptera: Coccinellidae)  
*Scymnus frontalis* Fabr. (Coleoptera: Coccinellidae)  
*Deraeocoris ruber* L. (Heteroptera: Miridae)

The research shows that the parasites of the codling moth (*C. pomonella*) have been present in very small numbers during all the years of the research and in all the research facilities. The prevalence of the parasite species is greater in the chemically protected plantations in comparison to those plantations that are not chemically protected. This leads to the conclusion that chemical protection does not have an important influence on faunistic composition of the parasites of the codling moth (Nikolić, 2015).

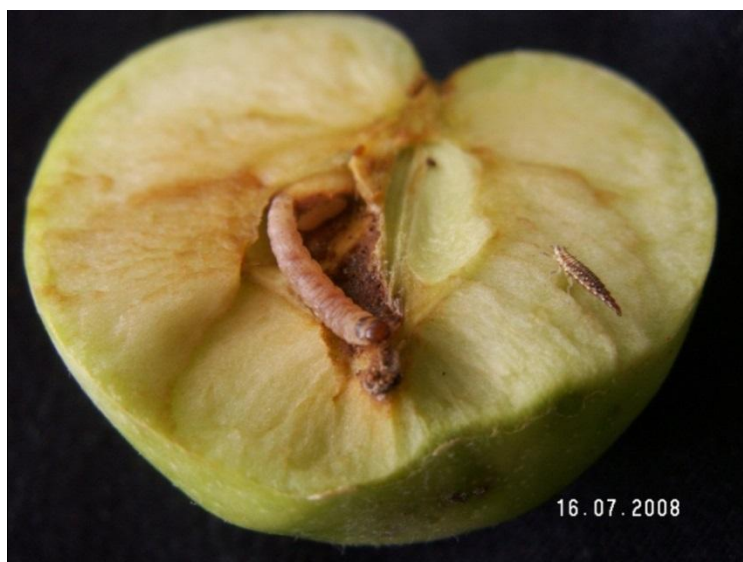
There are certain agrotechnical measures applied by man that have an influence on the increased resilience of the apple fruit against the codling moth, which results in the apple fruit that is stronger and less susceptible to the attacks of codling moth caterpillars. These are preventive measures aiming to solve the problem of wormy apples caused by the codling moth in a long run. Thus, the choice of the spot intended for apple growing is a very significant factor. In Beli Potok, the apple plantation is nearby the artificial reservoir that is part of Porecje – Vucje Ltd. Irrigation was carried out on several occasions during all the research years, which led to apple production of greater quality with minimal damage (3-5%).

Land cultivation in autumn is very important as a measure for destruction of codling moth caterpillars that hide in fallen fruit or in the land nearby. Pruning is important for fertility regulation, aeration and an increased exposure of a tree crown to sunlight; thus, leading to more regular formation of apple fruit. Apples that do not touch each other excessively have better form; they are more easily chemically protected and less susceptible to the attacks of codling moth caterpillars. Potassium fertilization increases plant resilience and hardens the apple skin; thus, making it more

difficult for codling moth caterpillars to bite through the apple skin and make tunnels inside an apple. Small portions of land nearby apple plantations that are left without agrotechnical and chemical measures (refugia) should enable the development of codling moths' natural enemies. According to Thalji (2010), abandoned orchards and individual trees in yards have entomofauna that differs from the one found in plantations that are well taken care of; therefore, they have a significant place in preserving useful entomofauna and retaining biodiversity of natural ecosystems. The examination of apple sensibility and susceptibility to the codling moth, as well as the creation of apple varieties that are more resilient against *C. pomonella* should become more common. Apples that are more resilient to the attacks of the codling moth are those that have the thick and hairy apple skin, hard apple flesh and the solid core. Kišpatic and Maceljiski (1989) state that fruit resilience also depends on fruit juice content. The examinations of Tadić (1957) and Injac et al. (1992) show that the Jonathan apple variety is more resilient to *C. pomonella* in comparison to the Golden Delicious variety. Postolovski and Lazarevska (2014) indicate that the codling moth attack is weaker in short and thick plantations, among apple varieties with smooth bark, as caterpillars find it hard to pupate. The performed analysis of the influence man has on autochthonous natural enemies of the codling moth on the territory of Southern Serbia contributes to biodiversity preservation of fruit agrobiocenosis and to the production of high-quality and healthy food.



Picture 1. *C. pomonella* eggs attacked by the parasite *Trichogramma* sp.



Picture 2. *C. pomonella* and *Chrysopa* sp. larvae in an apple

## Conclusions

The codling moth is a pest that appears every year in all fruit plantations and causes significant damage. An inevitable task for every producer is to determine the presence of codling moths' natural enemies and their interdependency in plantations with different protection approaches. Thus, apple protection would be based on integral principles and natural regulators of the codling moth would be given the primary place in examination of their biology and ecology.

The anthropogenic factor has a dominant place in many segments of integral apple protection. Faunistic research is a necessity in appropriate conceptualization of man's work due to the transparent composition of autochthonous natural enemies of the codling moth. The registered species of codling moths' natural enemies on the territory of Southern Serbia in the period from 2005 to 2008 are as follows: Hymenoptera, Diptera, Dermaptera, Neuroptera, Coleoptera and Heteroptera. The parasites of *C. pomonella* were present in very small numbers in all the research facilities and in all the research years. Chemical protection did not have any significant influence on the faunistic composition of the parasites that attack the codling moth.

The performed analysis of the influence man has on autochthonous natural enemies of the codling moth enables biodiversity preservation of codling moths' natural enemies. The manner in which agrotechnical measures are applied in fruit plantations influences the quality of entomofauna, helps agrobiocenosis preservation and production of high-quality and healthy food.

## References

1. Almaši, R., Injac, M., Almaši, Š. (2004): Štetni i korisni organizmi jabučastih voćaka. Poljoprivredni fakultet, Novi Sad, 168.
2. Čamprag, D. (2000): Integralna zaštita ratarskih kultura od štetočina. Desing studio Stanišić, Bačka Palanka, Poljoprivredni fakultet Institut za zaštitu bilja i životne sredine „Dr Pavle Vukasović“, Novi Sad, 215.
3. Injac, M., Dulić, K., Živanović, M., Krnjajić, S. (1992): Delovanje virusa granuloze na jabukinog smotavca (*Cydia pomonella* L.). Pesticidi, Vol. 7, 2: 75-82, Beograd.
4. Keserović, Z., Magazin, N., Injac, M., Totis, F., Milić, B., Dorić, M., Petrović, J. (2013): Integralna proizvodnja jabuke. Poljoprivredni fakultet Novi Sad, 276.
5. Kišpatić, J., Maceljki, M. (1989): Zaštita voćaka (V dopunjeno izdanje). 415, Nakladni zavod Znanje, Zagreb.
6. Lacey, L. A., Unruh T. R. (2005): Biological control of codling moth (*Cydia pomonella*, Lepidoptera: Tortricidae) and its role in integrated pest management, with emphasis on entomopathogens. Vedula 12 (1): 33-60.
7. Lazarevska, S., Postolovski, M., Naceski, S., Tomeva, E. (2005b): *Laspeyresia pomonella* L. and natural enemies – parasitoides Hymenoptera. Agricultural University – Plovdiv, Scientific Works, vol. L, book 6, 187-192, Jubilee scientific conference "State-of-the-art and problems of agricultural science and education".
8. Milutinović, S., Aleksić, V., Miletić, R., Marić, M. (2002): Uticaj navodnjavanja na kvalitet i prinos jabuke. Jugoslovensko voćarstvo, vol. 36, br.137-138, 37/44, Jugoslovensko naučno voćarsko društvo, Čačak.
9. Monteiro L. B., Dor C., Franck P., Lavigne C., Sauphanor B. (2008): Pest management practices and environment factors affect natural regulation of the codling moth. Proceedings of the 7th International Conference on Integrated Fruit Production, Avignon (France), October 27-30, 2008: 248-251.
10. Nikolić, K., Selamovska, A., Nikolić, Z. Stojanović, M. (2012): Uticaj proratne faune i prirodnih neprijatelja jabukinog smotavca na kvalitet jabuke. Zbornik radova 2, Peti međunarodni kongres "Ekologija, zdravlje, rad, sport", Banja Luka, 06-09.09.2012., 182-186, Udruženje "Zdravlje za sve", Banja Luka, Republika Srpska.
11. Nikolić, K. (2015): Vlijanje na uslove na sredinata vrz razvojot na populacijata na jabolkoviot crv (*Laspeyresia pomonella* L., Lepidoptera: Tortricidae) i negovite prirodni neprijatelji vo Južna

- Srbija. Doktorska disertacija, Fakultet za zemjodelski nauki i hrana - Skoplje, Univerzitet „Sveti Kiril i Metodij“ u Skoplju, Republika Makedonija, 200.
12. Ognjanov, V., Gvozdenović, D., Keserović, Z., Cerović, S., Ninić - Todorović, J., Gološin, B., Paprić, Ć., Korać, N., Cindrić, P., Kuljančić, I. D., Balaž, J., Thalji, R., Bijelić, S., Magazin, N., Medić, M., Popović, T. (2007): Integralni i biološki koncept proizvodnje voća i grožđa. Savremena poljoprivreda, vol. 56, 6, 38-49, Novi Sad.
  13. Postolovski, M., Lazarevska, S. (2014): Zemjodelska entomologija. Univerzitet „Sv. Kiril i Metodij“, Fakultet za zemjodelski nauki i hrana, 578, Skopje.
  14. Tadić, M. (1957): Jabučni smotavac (*Carpocapsa pomonella* L.) – Biologija kao osnova za njegovo suzbijanje. Doktorska disertacija, 100, Univerzitet u Beogradu.
  15. Thalji, R. (2010): Biljne vaši i njihovi predatori. Biljni lekar, god. XXXVIII, 1, 15-27, Poljoprivredni fakultet, Departman za fitomedicinu i zaštite životne sredine, Novi Sad.
  16. Veličković, M., Oparnica, Č., Radivojević, D. (2009): Savremeni sistemi gajenja jabuke i kruške. Zbornik radova II Savetovanje „Inovacije u voćarstvu“, Unapređenje proizvodnje jabučastog voća, 11-12. februar 2009., Beograd, 57-69, Poljoprivredni fakultet, Beograd.

**COMBINATION OF ULTRASOUND AND OXALIC ACID TO CONTROL CHILLING INJURY IN  
CAPIA PEPPER (*Capsicum annuum* L. cv Yalova Yaglık)**

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**Abstract**

The chilling injury is caused important produce losses in peppers, after harvest. These losses are emerged both quantity and quality. The purpose of this study is to determine the effect of ultrasound (52 dB) and oxalic acid treatments on chilling injury (CI) and color preservation of Capia peppers at 4°C temperature. The peppers harvested at the green maturation stage divided into four group and the following treatments were done; 1. Control (C, dip to water at 25°C for 15 min), 2. Oxalic acid (OA, dip to the 3 µMol oxalic acid solution at 25°C for 15 min), 3. Ultrasound (US, dip to water bath in a 52 dB and at 25°C for 15 min), 4. US+OA (dip to the 3 µMol oxalic acid solution in a 52 dB water bath for 15 min). After treatments, the peppers were dried, placed in polystyrene foam dishes as three pepper per dish, and wrapped polyethylene stretch film. Then, the packaged peppers were stored in a cold room at 4±1°C temperature and 85-90% RH. The study was carried out in three replicates, and total soluble solids (TSS), color (L\*, a\*, b\*), weight loss, electrolyte leakage, visual quality scores and CI scores were determined during storage (at the beginning, 14th days, 14th+72h, 28th days, 28th+72h). According to the results, CI scores of samples in three treatments (OA, US, US+OA) were lower compared to control group. But, OA treatment was suppressed CI symptoms than the other treatments during all storage period. The color preservation of samples in OA, US, US+OA treatments were lower than control. In conclusion, it was concluded that, while the effect of US treatment on prevent CI symptoms is limited, the OA treatment is successful.

**Keywords:** Pepper, ultrasound, oxalic acid, chilling injury.

**Introduction**

Pepper has an important place among the fruit-consumed vegetable species. Peppers have different uses such as tomato paste, spices, dried, frozen, canned and sauce as much as they can be consumed freshly. For this reason, peppers are produced in significant amounts both in the world and in Turkey. 32 324 345 tonnes (FAO, 2014) in the world and 2 457 822 tonnes (TUIK, 2016) fresh peppers are produced in Turkey.

There are different types of peppers, including pointed, stuffed, spicy and chili peppers. Capia pepper is a type of pepper used for produce pepper-paste and it constitutes 40% of pepper production of our country. Since flesh of the pepper is thick and the amount of fat and dry matter is high, it can be used as either processed or freshly in both green and red mature period. The storage time of the pepper varies between 2-4 weeks depending on the varieties and can reach up to 4 weeks under optimum conditions. This time can be extended with some postharvest application such as low temperature, controlled atmosphere, storage in modified atmosphere (Halloran et al. 1995). On the other hand, the cold storage technique, which is required to reduce postharvest losses in all fresh fruits and vegetables, is also important for the pepper casing. However, chilling injury that occurring due to low temperature during long term storage in the case of subtropical and tropical species, limits the storage of the pepper at low temperature, so the storage temperature is partially kept high. Because peppers are among the susceptible species. Generally, chilling injury (CI) occurs at temperatures below 10°C. The symptoms of CI are speckle-shaped depressions on the surface of the fruit, decay in the later stages, discoloration of the stem and drying, and also seed browning. CI cause fruit to lose market value altogether (Erdoğan et al. 2015). Treatments such as UV-C

applications (Vicente et al. 2005), methyl jasmonate (Meir et al. 1996), hot water applications (González-Aguilar et al. 2000) are being used for preventing or delaying of CI. Recently, ultrasound applications have been used in addition to these applications (Yang et al. 2012).

Studies on post-harvest ultrasound applications in fresh fruit and vegetables are noteworthy. Studies have shown that ultrasound application reduces the softening of fruit flesh in plums (Chen and Zhu 2011; Bal, 2016) and peaches (Wang et al. 2006); it is effective in the preservation of some biochemical compounds in litchi (Chen et al. 2012) and tomato fruit (Pinheiro et al. 2016); it has been found that reduce of rotting in strawberries (Cao et al. 2010). In this study we aimed to determine the effects of ultrasound and oxalic acid applications on chilling injury and other quality parameters during low temperature storage of pepper harvested in green period.

### Material and methods

**Plant Material:** The capia pepper (*Capsicum annum* var. *conoides* cv. Yalova Yaglık) using in this study has been cultivated in a research and application greenhouse of Arslanbey Vocational School in Kocaeli University. Peppers were harvested during the green period, when they received their full size, but the redness did not begin. The harvested peppers were quickly moved to the laboratory and those that were not suitable for the experiment were removed.

**Ultrasound ve oxalic acid treatment:** Peppers were divided into 4 groups one group as control and the following applications being made.

1. Control (C, dip to water at 25°C for 15 min)
2. Oxalic acid (Oxalic acid (OA, dip to the 3 µMol oxalic acid solution at 25°C for 15 min),
3. Ultrasound (US, dip to water bath in a 52 dB and at 25°C for 15 min),
4. US+OA (dip to the 3 µMol oxalic acid solution in a 52 dB water bath for 15 min).

Following the applications, peppers are dried on drying papers and packed.

**Packaging and storage conditions:** After applications, the peppers are placed in foam dishes in sized 210 x 120 mm including three fruits in each, and covered with stretch film. The packaged products were stored for 28 days in a cold room containing 4±1°C temperature and 85-90% relative humidity. Measurements and observations were made at the beginning of storage and every 14 days during storage, as well as in peppers kept in room conditions (18-22°C) for 72 hours to determine the shelf life.

**Weight Loss:** Initially three pack of each treatment were separated for weight loss measurements and weighed at each analysis period. No weight loss measurements were made in +72 hours samples. The weight loss is calculated as a percentage according to initial value according to the following formula:

$$W.L. (\%) = (initial\ weight - weight\ at\ the\ time\ of\ analysis) \times 100 / initial\ weight$$

**Total soluble solids:** The juice obtained from the pepper fruits using micro-pressing was measured at 20°C using the Atago DR-A1 digital refractometer (Atago Co. Ltd., Japan) and was determined as (%).

**Color measurements:** For the color measurements, three sample packages were initially separated and measurements were made in the cold room. Measurements were made from stretch film, from three points of each fruit and as total nine reading. No color measurements were made in +72 hours samples. For this purpose, L\*, a\* and b\* values were measured with CR-400 colorimeter (Konica Minolta, Inc. Osaka, Japan) consisted with D65 lamp. Before measurements were made, the instrument was calibrated with a calibration plate. Accordingly, L\* (0 = black, 100 = white), a\* (+ red, - green), and b\* (+ yellow, - blue) indicate color. Using the measurements, hue angle values (h°) (Kasim and Kasim 2015) and the total redness index (TCI) (Obande, 2010) were calculated by the following equations.:

$$h^{\circ} = 180 + \tan^{-1}(b/a)$$

$$TCI = \frac{2000\ a}{\sqrt{L(a^2 + b^2)}}$$

The raw values obtained were compared with the initial color values, and given as the change ratio (%).

**Electrolyte Leakage:** Five 5 mm wide discs were taken from the pepper for electrolyte ion leakage measurements, washed twice with 50 mL distilled water and kept in 50 mL distilled water for 2 hours. After two hours, the electrical conductivity (EC) value in the solution was measured and the samples were frozen. Frozen samples were thawed and EC values were determined when the solution temperature reached 18°C; total electrolyte leakage was calculated as the ratio of these obtained values to the initial EC values and expressed as (%) (Kasim and Kasim 2014).

**Chilling injury (CI):** During each analysis period, the fruits were examined for CI symptoms such as shallow pitting, fruit stem shrinkage, end shrinkage and were evaluated by a 9-person jury on the scale of 0-4 (0: no sign of CI, 1: mild, 2: moderate, 3: severe 4: very severe). The assessments were made on fruit basis and averages were taken.

**Visual quality:** During each analysis period; The fruits were evaluated by a 9-person jury in terms of visual quality criteria such as brightness, firmness and color and evaluated according to the 5-1 scale (5: very good, 4: good, 3: moderate, 2: poor, 1: very poor). The assessments were made on fruit basis and averages were taken.

**Statistical analysis:** The experiment was conducted as a completely randomized design with factorial (applications x maintenance time) arrangement, and a minimum of-three replications. Variance analysis was performed using SPSS 16.0 statistical program and Duncan test was applied for comparison of the averages.

## Results and Discussion

### Weight Loss

As a result of the measurements, no statistically significant difference in weight loss between the applications was found. As a result of 28 days of storage, the maximum weight loss was found to be 11.16% in US treatment and the lowest weight loss was found in OA applications with 9.9% (Fig. 1). Plant tissues are in equilibrium with the environment, in the condition that at the same temperature conditions and with 99-99.5% relative humidity environment, there is no water loss (Burton, 1982). The protective layers such as waxy layer, cuticle layer, fur etc. into the skin and on surface of the skin of fruit and vegetables, serve to reduce water loss (Kasim and Kasim 2016). In the case of a loss that occurs in these layers, water loss, and therefore weight loss, increases. It is observed that the applications do not damage the skin and surface texture and therefore there is no significant difference between the applications in weight loss. It is also seen that ultrasound applications of peach are not effective on weight loss (Bal, 2013). However, US and US+OA treatments seem to have more weight loss than control and OA treatments. This result suggests that the application of ultrasound causes a slight change in the skin.

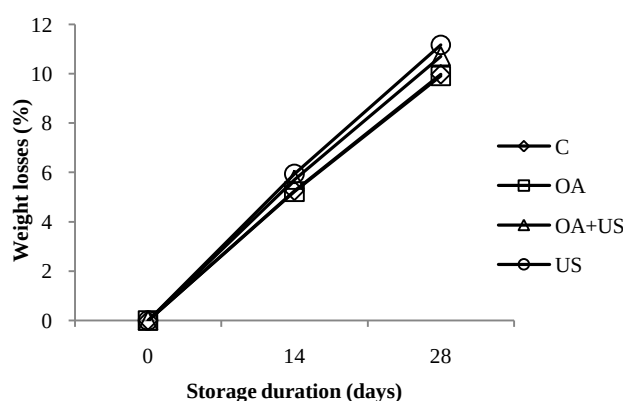


Figure 1. Effects of oxalic acid and ultrasound application on weight loss during pepper storage during storage

### Total soluble solids (TSS)

At the beginning, 4.7% TSS values show slight fluctuations during storage, but linear regression analysis shows a slight increase tendency except OA application (Fig 2). However, statistically significant changes in TSS values were not found. In the studies conducted by Cao et al. (2010) on strawberry and Yang et al. (2012) on peach, it is seen that the results of TSS are not significantly affected by ultrasound. However, it is noteworthy that the TSS in the OA application remains more stable. There was a slight increase in the amount of TSS in the samples kept at room temperature for 72 hours following storage. This is thought to be due to higher water loss at high temperatures.

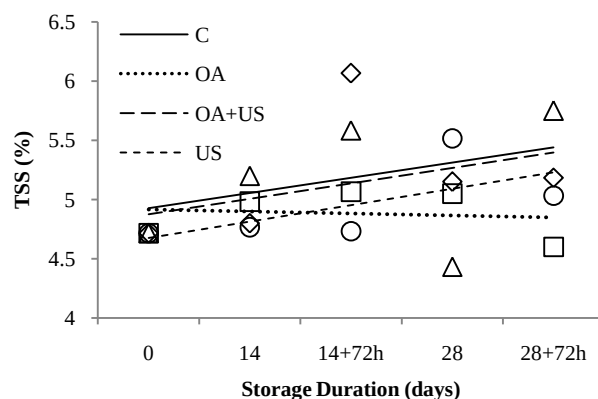


Figure 2. The effects of oxalic acid and ultrasound applications during storage on TSS of peppers

### Visual quality

As a result of the panel evaluation, the visual quality scores of control application was slightly lower, but the difference with other applications was not significant statistically. In all applications it did not fall below the three points that is marketable limit value at the end of the 28 days storage period (Fig 3).

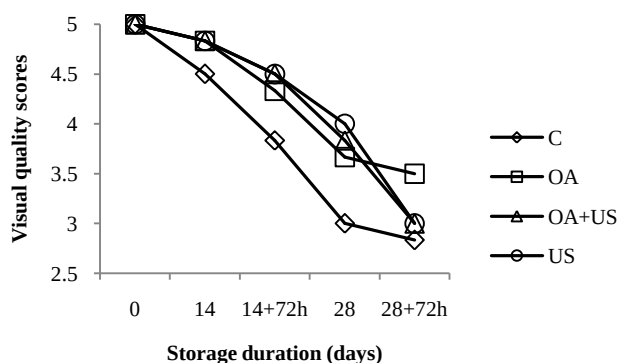


Figure 3. The effects of oxalic acid and ultrasound application on visual quality in pepper during storage

### Chilling injury and electrolyte leakage

It was determined that pitting on the surface was more severe in both US and OA + US applications. Therefore, it was seen that US treatments increased the chilling injury (Fig 4). It has been determined that only OA treatment reduces the severity of CI compared with both control and other treatments. The differences between the treatments are also found to be statistically significant.

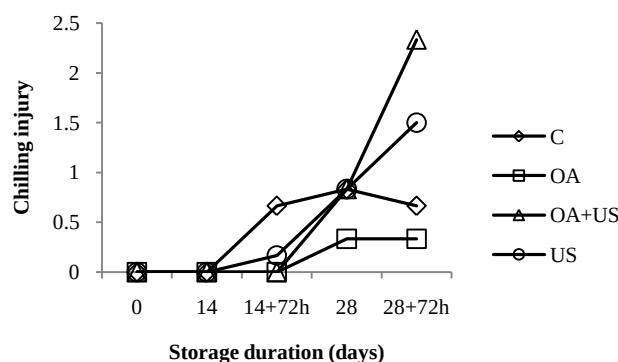


Figure 4. The effects of oxalic acid and ultrasound treatments on the severity of chilling injury of peppers during storage

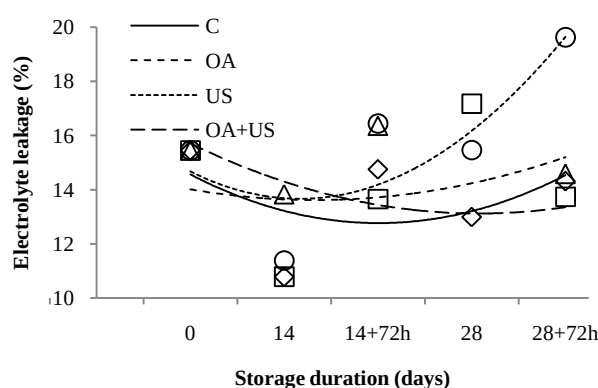


Figure 5. The effects of oxalic acid and ultrasound on electrolyte leakage of peppers during storage (symbols represent mean values, lines represent polynomial slope).

Similar results were obtained with measurements of electrolyte leakage that using to determine chilling injury symptoms on a cellular basis (Fig 5). As can be seen in Fig.5, it was found that the greatest increase was found in US treatment. It is not seen that a significant increasing of electrolyte leakage of samples in the other treatments.

The symptoms of CI were more evident of samples kept at room temperature for 72 hours than that of samples taken just after storage. CI scores of samples which is treated with OA+US and stored 28 days and after that hold in room temperature during 72 h (28+72h) were found to be the highest (2.33); is followed by US application with 1.5 points, and the CI scores of peppers treated with OA has the lowest. According to these results, US application has weakened skin texture, and US application together with OA seems to increased the damage even more. It is also noteworthy that the OA alone is delaying the chilling injury. Bal (2013) determined that the ultrasound treatment to peach is delayed of chilling injury but Yang et al. (2012) concluded that ultrasound application alone did not affect the chilling injury of peach.

#### *Changes of color*

As a result of examinations made at a \* value, it was found that there is a rapid change in control application (Fig 6). A change of 14.6% was occurred especially on the 14th day. This change means a decrease in green color. Even on the 28th day of storage in other applications, it has not been observed the changes like this. The differences between control and other applications is found to be significant, statistically.

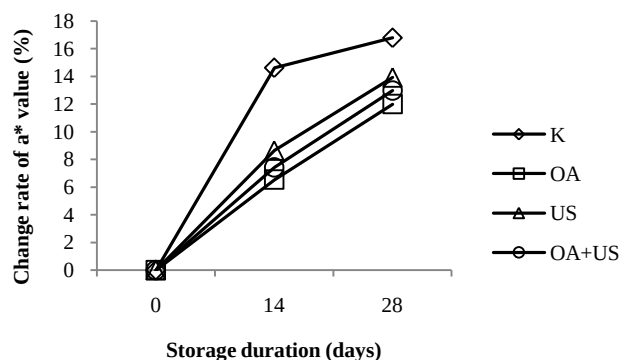


Figure 6. Effects of oxalic acid and ultrasound application on a\* value in pepper during storage

It has been seen that the changes of b\* values is also parallel to the a\* values (Fig 7). The color changes in control treatment was significantly higher than in other applications. This indicates that the green color of the control samples is rapidly disappearing and that the color of samples are started to yellow. This increase may be due to accelerated metabolism because of increased chilling injury in control samples. In addition, there is no significant change in the red color in the control values in terms of angle values and redness index data (Fig 8 ve 9). Therefore, it is thought that a rapid aging occurs in the samples due to the chilling injury. In addition, the application of ultrasound alone or in combination with oxalic acid improves both chilling and coloration in peppers (Fig 4 ve 9). On the other hand, the application of OA on its own seems to increase the quality of storage by delaying both coloring and chilling injury.

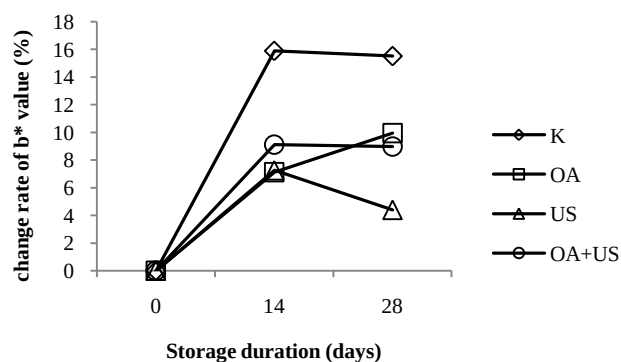


Figure 7. Effects of oxalic acid and ultrasound application on b\* value of pepper during storage

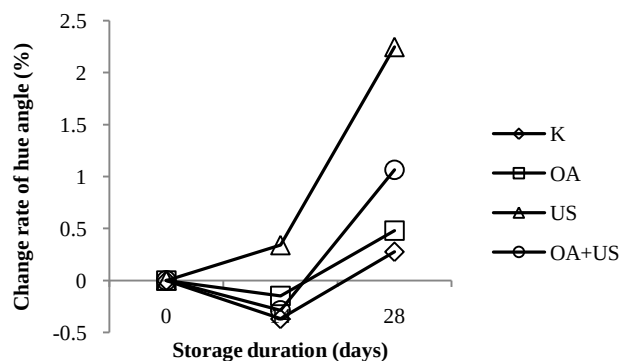


Figure 8. The effect of oxalic acid and ultrasound treatments on hue angle values of pepper during storage

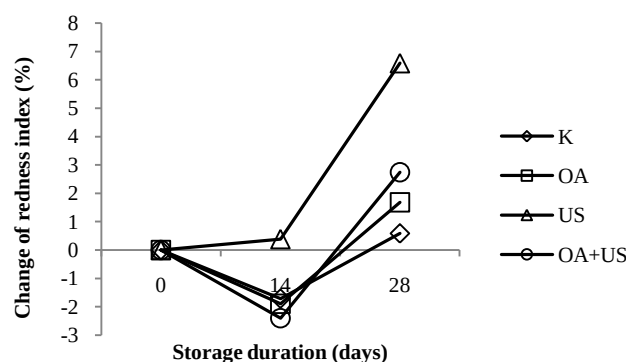


Figure 9. The effect of oxalic acid and ultrasound treatments on redness index of peppers during storage

### Conclusions

In this study, it was evaluated that the effect of ultrasound (52 dB) and oxalic acid treatments on chilling injury (CI) and color preservation of Capiá peppers at 4°C temperature. For this purpose, oxalic acid alone, ultrasound alone and oxalic acid and ultrasound together treated to Capiá pepper, and after application peppers were stored in a cold room at 4°C and 85-90% RH. As results of research, it was found that, when ultrasound alone or ultrasound and oxalic acid together applied to peppers, they accelerated senescence of peppers. Also chilling injury of samples in both US and US+OA is high due to damaging of tissue. However, oxalic acid treatment both delayed color formation and CI. Therefore it could be said that oxalic acid treatment a promising application for delaying CI symptoms of peppers at the lower temperature.

### References

- Bal, E. (2013). Effects of exogenous polyamine and ultrasound treatment to improve peach storability. *Chilean journal of agricultural research*, 73(4), 435-440.
- Bal, E. (2016). Effect of postharvest calcium chloride and ultrasound treatments on storage period and fruit quality of modified atmosphere packed fruit in plum cv. Santa Rosa. *Fruit Science*, 12-18.
- Burton, W.G. (1982). *Postharvest Physiology of Food Crops*. Longman House, Essex UK, London, 339s.
- Cao, S. Hu, Z. Pang, B. Wang, H. Xie, H. and Wu, F. (2010). Effect of ultrasound treatment on fruit decay and quality maintenance in strawberry after harvest. *Food Control*, 21(4), 529-532.
- Chen, Y. Jiang, Y. Yang, S. Yang, E. Yang, B. and Prasad, K. N. (2012). Effects of ultrasonic treatment on pericarp browning of postharvest litchi fruit. *Journal of Food Biochemistry*, 36(5), 613-620.
- Chen, Z. and Zhu, C. (2011). Combined effects of aqueous chlorine dioxide and ultrasonic treatments on postharvest storage quality of plum fruit (*Prunus salicina* L.). *Postharvest Biology and Technology*, 61(2), 117-123.
- Erdoğan, A. Ö. Kaynaş, K. and Kaya, S. (2015). Kırmızı biberde (*Capsicum annuum* L. cv. Kappa) bazı hasat sonrası uygulamaların depolama kalitesi üzerine etkileri (The effects of some postharvest applications on storage quality of red pepper (*Capsicum annuum* L. cv. Capiá)). *ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)*, 45-53.
- FAO. (2014). <http://www.fao.org/faostat/en>. date of access: 7 12, 2017.
- González-Aguilar, G. A. Gayosso, L. Cruz, R. Fortiz, J. Báez, R. and Wang, C. Y. (2000). Polyamines induced by hot water treatments reduce chilling injury and decay in pepper fruit. *Postharvest Biology and Technology*, 18(1), 19-26.
- Halloran, N. Yanmaz, R. Kasım, M. U. and Çağırın, R. (1995). Farklı ambalaj materyallerinin biberin (*Capsicum annuum* L. var. longum) soğukta muhafazasına etkisi. *Tarım Bilimleri Dergisi*, 1-6.

11. Kasım, R. and Kasım, M. U. (2014). Biochemical and color changes of fresh-cut melon (*Cucumis melo* L. cv.Galia) treated with UV-C. *Food Science and Technology (Campinas)*, 34(3), 547-551.
12. Kasım, M. and Kasım, R. (2015). Postharvest UV-B treatments increased fructose content of tomato. *Food Sci.Technol., Campinas.*, 35(4), 742-749.
13. Kasım, M. U. and Kasım, R. (2016). Taze Kesilmiş Baklada Yüksek Dozda Sitrik Asit Uygulamalarının Polifenol Enzim Aktivitesi ve Kalite Üzerine Etkisi. *Kahramanmaraş Sütçü İmam Üniversitesi Doğa Bilimleri Dergisi*, 19(3), 339-347.
14. Meir, S. Philosoph-Hadas, S. Lurie, S. Droby, S. Akerman, M. Zauberman, G. ... and Fuchs, Y. (1996). Reduction of chilling injury in stored avocado, grapefruit, and bell pepper by methyl jasmonate. *Canadian journal of botany*, 74(6), 870-874.
15. Obande, M. A. (2010). Effect of preharvest UV-treatment on shelf life of fruits and vegetables. Doktora Tezi. Loughborough University.
16. Pinheiro, J. C. Alegria, C. S. Abreu, M. M. Gonçalves, E. M. and Silva, C. L. (2016). Evaluation of Alternative Preservation Treatments (Water Heat Treatment, Ultrasounds, Thermo-sonication and UV-C Radiation) to Improve Safety and Quality of Whole Tomato. *Food and Bioprocess Technology*, 9(6), 924-935.
17. TUIK. (2016). <https://biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul>. access date: 7 12, 2017.
18. Vicente, A. R. Pineda, C. Lemoine, L. Civello, P. M. Martinez, G. A. and Chaves, A. R. (2005). UV-C treatments reduce decay, retain quality and alleviate chilling injury in pepper. *Postharvest Biology and Technology*, 35(1), 69-78.
19. Wang, J. Han, T. Li, L. P. Wang, K. S. and Sun, S. W. (2006). Effect of ultrasonic treatment on the peaches quality during storage. *Journal of Shihezi University (Natural Science)*, 24(6), 732-735.
20. Yang, Z. Cao, S. Zheng, Y. and Jiang, Y. (2012). Combined salicylic acid and ultrasound treatments for reducing the chilling injury on peach fruit. *Journal of agricultural and food chemistry*, 60(5), 1209-1212.

**ANTIBACTERIAL ACTIVITIES OF SOME WILD MUSHROOM EXTRACTS AGAINST  
*PSEUDOMONAS AERUGINOSA***

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**Abstract**

Mushrooms synthesize a multitude of low-molecular-weight secondary metabolites that have an important role as communication signals, to defend mushroom habitat or to inhibit the growth of competitors. Investigations have shown that some of these metabolites have potent antimicrobial activity and could be beneficial for humans. In this study, antimicrobial potential of the extracts from six wild mushrooms: *Amanita echinocephala*, *Russula medulata*, *Cerena unicolor*, *Hericium erinaceus*, *Ishnoderma benzoinum* and *Laetiporus sulphureus* was evaluated against Gram-negative bacterium *Pseudomonas aeruginosa*. The antimicrobial activities of the methanolic mushroom extracts were investigated by the microdilution method. All the extracts that demonstrated inhibitory activities were further tested for bactericidal activity and minimum bactericidal concentration (MBC) values were determined. Antimicrobial activity was observed in all species included in the study, while the activities depended on the type and concentration of extract. The tested microorganism was more sensitive to the examined extracts from the polypore fungi (*C. unicolor*, *H. erinaceus*, *I. benzoinum* and *L. sulphureus*). The highest antibacterial activity was obtained in the extracts from polypores *I. benzoinum* and *L. sulphureus* (MBC=15.625 mg/mL). This study demonstrated that the analysed wild macrofungi have the potential to accumulate bioactive metabolites that possess antimicrobial activity.

**Keywords:** Microdilution method, minimum bactericidal concentration (MBC).

**Introduction**

*Pseudomonas aeruginosa* is a common pathogen associated with a spectrum of infections in humans. The organism is intrinsically resistant to many antimicrobials and can develop resistance during anti-pseudomonal chemotherapy that causes infections with a high mortality rate (Poole, 2011). Interest in natural products as antimicrobial agents has waned in recent years, but the frequency of antibiotic resistance without new antibiotic classes on the horizon suggests the need of re-evaluation of natural products as a route to identify novel chemical skeletons with antibacterial activity (Moloney, 2016). Previous studies have indicated that macrofungi, as a specific response to the natural hostile environment, synthesize a multitude of metabolites with antimicrobial properties (Alves et al. 2012). Considering that humans and animals share common microbial pathogens with fungi, fungal defensive strategies against microorganisms could be of benefit for humans. Hence, various taxonomic mushroom groups have been investigated for their antimicrobial activities (Suay et al. 2000, Yamac and Bilgili 2006, Nikolovska Nedelkoska et al. 2013). Numerous studies have shown the pharmaceutical potential of Basidiomycetes, especially polypores, which are considered by many authors as major sources of bioactive natural products among species of the diverse fungal phylum Basidiomycota (Zjawiony, 2004). According to antimicrobial evaluation of 204 mushroom species, more than 75% of screened polypores exhibited strong antimicrobial potential (Suay et al. 2000). With an increasing number of bacteria developing resistance to commercial antibiotics, a rich

diversity of higher fungi, including polypores, provide a wide range of good candidates for critically needed new antibiotics. Therefore, the aim of this study was to evaluate the antimicrobial activity of the extracts from six wild mushrooms: *Amanita echinocephala*, *Russula medulata*, *Cerena unicolor*, *Hericium erinaceus*, *Ishnoderma benzoinum* and *Laetiporus sulphureus* against Gram-negative bacterium *Pseudomonas aeruginosa*.

## Material and methods

### Fruiting body selection

The fruiting bodies of the wild macromycetes *Amanita echinocephala*, *Russula medulata*, *Cerena unicolor*, *Hericium erinaceus*, *Ishnoderma benzoinum* and *Laetiporus sulphureus* were collected from different locations and habitats in Macedonia. Geographical location, natural habitat and collection number in the Macedonian Collection of Fungi (MCF) of the mushroom specimens are shown in Table 1. Taxonomic identification was made in the Mycological Laboratory at the Institute of Biology, Faculty of Natural Sciences and Mathematics in Skopje, by implementing standard methods of microscopic and chemical techniques, as well appropriate literature.

Table 1. Geographical location and natural habitat of the mushroom species studied for antimicrobial potential

| Species                 | Habitat                                                 | Geographical location    | Collection number |
|-------------------------|---------------------------------------------------------|--------------------------|-------------------|
| <i>A. echinocephala</i> | mycorrhizal (on ground in park)                         | Botanical garden, Skopje | MAK 10/13309      |
| <i>R. medulata</i>      | mycorrhizal (on ground in park)                         | Gazi Baba, Skopje        | MAK 10/13305      |
| <i>C. unicolor</i>      | saprotrophic (on living beech trunks in conifer forest) | Suva Gora Mt.            | MAK 11/13368      |
| <i>H. erinaceus</i>     | saprotrophic (on living oak trunks in deciduous forest) | Sk. Crna Gora Mt.        | MAK 11/13360      |
| <i>I. benzoinum</i>     | saprotrophic (on stump of pine trees)                   | Suva Gora Mt.            | MAK 11/13252      |
| <i>L. sulphureus</i>    | parasitic (on living black locust trunks)               | Kozle, Skopje            | MAK 11/13361      |

### Preparation of methanolic extracts of mushrooms

Samples were cleaned and subsequently air-dried in the oven at 40°C. Dried specimens were ground to fine powder and extracted by stirring with 80% (v/v) methanol in ultrasonic bath for 30 min at 4°C, and then centrifuged at 12000 rpm for 15 min. Supernatants were used for the evaluation of antimicrobial potential of the samples. The organic solvent in the extracts was evaporated to dryness under vacuum. The yields of methanolic extracts of the fruiting bodies are presented in Table 2. The tested extracts were dissolved in 10% (v/v) DMSO in sterile water. A solvent control test was performed to study the effect of DMSO on the growth of microorganisms. The test approved that DMSO had no inhibitory effect on the tested organisms.

Table 2. Yield of mushroom methanolic extracts

| Mushroom species        | Yield of extracts <sup>a</sup><br>(g/100 g of dry mushroom) |
|-------------------------|-------------------------------------------------------------|
| <i>A. echinocephala</i> | 33,082 ± 3,356                                              |
| <i>R. medulata</i>      | 4,167 ± 0,577                                               |
| <i>C. unicolor</i>      | 20,800 ± 1,131                                              |
| <i>H. erinaceus</i>     | 17,333 ± 0,764                                              |
| <i>I. benzoinum</i>     | 15,000 ± 1,323                                              |
| <i>L. sulphureus</i>    | 33,833 ± 4,254                                              |

<sup>a</sup>Each value is the mean of three replicate determinations ± standard deviation

*Test microorganism*

Antimicrobial activities of methanol extracts were tested against Gram-negative bacterium *Pseudomonas aeruginosa* ATCC 9027. The microorganism was provided from the collection held by the Microbiology Laboratory, Faculty of Natural Sciences and Mathematics in Skopje.

*Suspension preparation*

Microbial suspension was prepared by the direct colony method. The turbidity of initial suspension was adjusted by comparison with 0.5 McFarland's standard (Andrews, 2005). The initial suspension contained about  $10^8$  colony forming units (CFU)/mL. Additionally, 1:100 dilutions of initial suspension were prepared into sterile 0.9% saline.

*Microdilution method*

*In vitro* antibacterial activities of the mushroom extracts were assessed using the microdilution method with resazurin as an indicator of microbial growth (Sarker et al. 2007). The broth microdilution test is a standard reference method for quantitative assessment of an antimicrobial agent against a given bacterium. The antimicrobial assay was performed by using a sterile 96-well plate and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined (Nikolovska Nedelkoska et al. 2007). Each test plate included growth control and sterility control. MIC was defined as the lowest concentration of tested extracts that prevented a resazurin color change from blue to pink. All tests were performed in triplicate.

A sample from each well that tested positive for inhibitory activity was inoculated on fresh sterile Mueller-Hinton agar (MHA) plates and incubated additional 24 h at 37°C. Absence of colonies was regarded as positive for bactericidal activity, while growth of colonies was regarded as negative. MBC was defined as the lowest concentration of the mushroom extract that results in microbial death. All tests were performed in triplicate.

**Results and discussion**

The minimum bactericidal concentration (MBC) values were determined among the tested extracts and are presented in Table 3. The results showed that all selected mushroom species exhibited the bactericidal activity against tested bacterium, with MBC values ranging from 15.625 to 200 mg/mL. In this study the methanolic extracts from polypores *Ishnoderma benzoinum* and *Laetiporus sulphureus* demonstrated the strongest antimicrobial potential with the same MBC value of 15.625 mg/mL, followed by the extracts of *Hericium erinaceus* and *Cerena unicolor* (18.75 mg/mL and 25 mg/mL, respectively). Higher MBC values were obtained in the extracts from *Amanita echinocephala* and *Russula medulata* (50 mg/mL and 200 mg/mL, respectively), that corresponded to lower bactericidal potential against tested *Pseudomonas aeruginosa*.

Table 3. Minimum bactericidal concentration (MBC) of methanolic extracts from mushroom samples

| MBC values <sup>a</sup> |                    |                     |                     |                      |                    |
|-------------------------|--------------------|---------------------|---------------------|----------------------|--------------------|
| <i>A. echinocephala</i> | <i>C. unicolor</i> | <i>H. erinaceus</i> | <i>I. benzoinum</i> | <i>L. sulphureus</i> | <i>R. medulata</i> |
| 50                      | 25                 | 18.750              | 15.625              | 15.625               | 200                |

<sup>a</sup> Minimum bactericidal concentration (MBC) values given as mg/mL

The present study was a continuation of our previous study in which antibacterial activity of the same mushroom species have been tested against Gram-positive bacterium *Staphylococcus aureus* (Nikolovska Nedelkoska et al. 2017). According to obtained results the mushroom extracts included in the study showed bactericidal activities against Gram-positive *S. aureus* that were equal to (*Laetiporus sulphureus*) or stronger than the activities observed against Gram-negative *Pseudomonas aeruginosa*. The only exception was the extract from *Ishnoderma benzoinum*, which exhibited lower bactericidal activity against *S. aureus* (MBC=31.250 mg/mL). Those results are in accordance with earlier reported data which confirm that the Gram-positive bacteria are generally more sensitive to the antimicrobial effect of the macrofungi extracts compared to Gram-negative bacteria, but this

relationship does not hold for every mushroom species (Yamac and Bilgili 2006, Pala and Wani 2011, Alves et al. 2012).

In the current study the examined extracts from the polypore fungi *Ischnoderma benzoinum* and *Laetiporus sulphureus* demonstrated the most potent bactericidal activity against tested *Pseudomonas aeruginosa*. Several previous studies have shown the antimicrobial potential of these macrofungi species (Turkoglu et al. 2007, Teplyakova et al. 2012). Turkoglu et al. (2007) evaluated the antimicrobial effect of the ethanolic extract of *L. sulphureus* and observed strong inhibition of the growth of the Gram-positive bacteria tested. Teplyakova et al. (2012) investigated the antiviral activity of aqueous extracts from mycelium of 11 basidial fungi species and found that *I. benzoinum* is among the most perspective strains for producing antiviral medicines. In general, there is little information available on chemical characterisation of specific classes of antimicrobial compounds in tested polypores *I. benzoinum* and *L. sulphureus*. Based on the evidence reported in the literature, few antimicrobial secondary metabolites have been identified in mushroom extracts from *I. benzoinum* and *L. sulphureus*. For instance, an antibiotic, 1-hydroxy-2-nonyl-4-one, has been isolated from submerged cultures of several strains of *I. benzoinum* (Anke et al. 1982). Another example of antimicrobial secondary metabolites is a cyclodepsipeptide, beauvericin, produced by the polypore *L. sulphureus* (Zjawiony, 2004). All these observations were in accordance with the bactericidal activity reported here.

### Conclusions

The present study was undertaken to quantitatively assess the antimicrobial potential of methanolic extracts from fruiting bodies of six wild macromycetes (*Amanita echinocephala*, *Russula medulata*, *Cerena unicolor*, *Hericium erinaceus*, *Ischnoderma benzoinum* and *Laetiporus sulphureus*). Results from this study showed that all analyzed mushroom extracts exhibit bactericidal activity against *Pseudomonas aeruginosa*. Especially, extracts from the species *I. benzoinum* and *L. sulphureus* demonstrated most promising bactericidal activity against *P. aeruginosa* that may serve as potential candidates for the development of novel antibiotics. Concerning the development of natural antimicrobials, further work is needed toward the elucidation of the structure of the active compound and possible mechanism of action.

### References

1. Alves, M.J., Ferreira, I.C.F.R., Dias, J., Teixeira, V., Martins, A. and Pintado, M. (2012). A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta Medica*, 78 (16): 1707-1718.
2. Andrews, J. M. (2005). BSAC standardized disc susceptibility testing method (version 4). *J Antimicrob Chemother*, 56: 60-76.
3. Anke, T., Giannetti, B. and Steglich, W. (1982). Antibiotika aus Basidiomyceten, XV [1]. 1-Hydroxy-2-nonyl-4-on, ein antifungischer und cytotoxischer Metabolit aus *Ischnoderma benzoinum* (Wahl.) Karst. / Antibiotics from Basidiomycetes. XV [1]. 1-Hydroxy-2-nonyl-4-one, an Antifungal and Cytotoxic Metabolite from *Ischnoderma benzoinum* (Wahl.) Karst.. *Zeitschrift für Naturforschung C*, 37 (1-2): 1-4. doi:10.1515/znc-1982-1-201.
4. Moloney, M. G. (2016). Natural Products as a Source for Novel Antibiotics. *Trends in Pharmacological Sciences*, 37 (8): 689-701.
5. Nikolovska Nedelkoska, D., Atanasova-Pancevska, N., Karadelev, M. and Kungulovski, Dz. (2013). Screening of antibacterial and antifungal activities of selected Macedonian wild mushrooms. *Matica Srpska Journal for Natural Sciences*, 124: 333-340.
6. Nikolovska Nedelkoska, D., Atanasova-Pancevska, N., Karadelev, M. and Kungulovski, Dz. (2017). Bactericidal activities of selected macrofungi extracts against *Staphylococcus aureus*. *Matica Srpska Journal for Natural Sciences*, 133: 193-200.
7. Pala, S. A. and Wani, A. H. (2011). Mushrooms: The entities with multifarious medicinal properties. *Journal of pharmacy research*, 4 (12):4721-4726.

8. Poole, K. (2011). *Pseudomonas Aeruginosa*: Resistance to the Max. *Frontiers in Microbiology*, 2: 65.
9. Sarker, S. D., Nahar, L. and Kumarasamy, Y. (2007). Microtitre platebased antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods*, 42: 321–324.
10. Suay, I., Arenal, F., Asensio, F. J., Basilio, A., Cabello, M. A., Díez, M. T., García, J. B., del Val, A. G., Gorrochategui, J., Hernández, P., Peláez F. and Vicente, M. F. (2000). Screening of basidiomycetes for antimicrobial activities. *Antonie van Leeuwenhoek*, 78: 129-39.
11. Teplyakova, T. V., Psurtseva, N. V., Kosogova, T. A., Mazurkova, N. A., Khanin, V. A. and Vlasenko, V. A. (2012). Antiviral activity of Polyporoid mushrooms (higher Basidiomycetes) from Altai Mountains (Russia). *International Journal of Medicinal Mushrooms*, 14 (1): 37–45.
12. Turkoglu, A., Duru, M. E., Mercan, N., Kivrak, I. and Gezer, K. (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chem*, 101: 267–273.
13. Yamac, M. and Bilgili, F. (2006). Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushroom isolates. *Pharm Biol*, 44 (9): 660–667.
14. Zjawiony, J. K. (2004). Biologically active compounds from Aphyllophorales (polypore) fungi. *J Nat Prod*, 67 (2): 300-310.

## INFLUENCE OF SPRING FROST ON APPLE FLOWER BUDS AT VARIOUS DEVELOPMENTAL STAGES

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### Abstract

Spring frost often reduces the yield because of damaged flowers. Spring frost is a dangerous climatic hazard that can be responsible for yield losses to orchard trees. Frost damage is highly dependent on the stage of development of the flower buds. Flower buds samples were collected from 'Granny Smith', 'Golden Delicious Rainiers' and 'Gala Schniga' apple cultivars at different flower buds stages from BBCH 59 (most flowers with petals forming a hollow ball) to BBCH 65 (full bloom on trees), two days after frost that occurred on the morning at 5:00-7:00 of March 31, 2017 when the temperature dropped to -1.5 to -3.3 °C in the apple orchard of company Pollino Agrar near Fruška gora mountain. Pistils in flower buds samples at a hollow ball stage to full bloom were examined individually and classified as dead or alive based on tissue browning. The highest damage on flowers was recorded to cultivar 'Gala Schniga' (75.4%) and the lowest to cultivar 'Golden Delicious Rainiers'. Cultivar 'Gala Schniga' had more than 90% of damaged king flowers. Our results showed that the spring frost damage of pistils was variable according to stages of flower bud development and genotypes.

**Keywords:** cultivar, flowering, damage, bbch scale.

### Introduction

Apple (*Malus × domestica* Borkh.) is the most important of the deciduous tree fruits in terms of production and a focus of research worldwide. Frost, especially spring frost causes more economic losses than any other weather-related phenomenon to apple production. The bud burst phase of orchard trees is the most critical phase in relation to low temperature and frost since the most parts of the bud, especially the ovary, are very sensitive to low temperatures (Farajzadeh et al. 2010). Blossoming is the most sensitive period, e.g. to frost, and determines the fruit set of the apple trees (Chemielewski et al. 2011). Spring frost is a dangerous climatic hazard that can be responsible for yield losses to orchard trees (Hudina and Štampar 2006). In the context of global warming, the general trend towards earlier flowering dates of many temperate tree species is likely to result in an increased risk of damage from exposure to frost (Eccel et al. 2009). A comparison of the phenological data of two periods, Kunz and Blanke (2011) showed that apple cultivars manifested 10 days earlier full bloom.

Species and varieties can exhibit different damage at the same temperature and phenological stage (Lenz et al, 2013). Frost damage is highly dependent on the stage of development of the flower buds (Aygün and San 2005). Temperature fluctuation before and during bloom can also influence flower tolerance to low temperatures since warm conditions may increase flower vulnerability and cool temperatures may decrease it (Rodrigo, 2000). Grab and Craparo (2013) recorded that cultivar 'Granny Smith' had the earliest beginning and the shortest duration of flowering in comparison with cultivar 'Golden Delicious'

At bloom, the first symptom that is observed after freezing is the thawing of the flowers characterized by a brown discoloration at the base of the style; depending on the severity of the freeze, the damage may extend both to the style and to the ovary, resulting in death of fruit abortion (Rodrigo, 2000; Aygün and San 2005).

The flower buds that survive the rest period continue with its development until bloom (Salazar-Gutierrez et al. 2016), but with decreased of yield and fruits quality. The aim of this study was to determine sensitivity of apple bud flowers in different stage of development.

### Material and methods

In this study we investigated sensitivity of flower buds of tree apple cultivars 'Granny Smith', 'Golden Delicious Rainders' and 'Gala Schniga' to the spring frost. Two days after frost which occurred on the morning at 5:00-7:00 of March 31, 2017 when the temperature dropped to -1.5 to -3.3°C in the apple orchard of company Pollino Agrar near Fruška gora mountain, flowers buds samples were collected. Pistils in flower buds samples at a hollow ball stage to full bloom were examined individually and classified as dead or alive based on tissue browning.

The apple cultivars were at different flower buds stages from BBCH 59 (most flowers with petals forming a hollow ball) to BBCH 65 (full bloom on trees). To all cultivars the king flower and flower on position L1. The cultivar 'Gala Schniga' had open flowers in position L2 and L3, while other flowers in the buds were in red balloon stage. The cultivars 'Granny Smith' and 'Golden Delicious Rainders' had opened flowers in L2 position and flowers in other position L3 and L4 were in red balloon stage. The cultivars 'Granny Smith' and 'Golden Delicious Rainders' had a lot of buds flowers in L5 position which were in hollow ball stage. The experiment was built by a random field with five repetitions (20 flowers buds per tree were taken from every part of canopy, five trees were taken for a repeat). Analysis of variance has been done with STATISTICA 7 software package.

Also, we recorded another phenological properties such as time of bud burst, time of beginning of blooming, full and end of blooming. The period between bud burst and blooming was marked by a high daily temperature.

### Results and discussion

The earliest buds burst had cultivar 'Gala Schniga' and the latest 'Golden Rainders' (Table 1). Also, cultivars had same schedule in time of beginning and full blooming. All cultivars had finish of blooming in second decade of April. According to Milatovic et al. (2009) in same agricultural environment in period of 2004-2007 years, cultivars 'Gala Schniga' and 'Golden Rainders' manifested the latest beginning and full blooming.

Table 1. Phenological properties of apples cultivar

| Cultivar          | Buds burst | Beginning of blooming | Full blooming | End of blooming |
|-------------------|------------|-----------------------|---------------|-----------------|
| 'Gala Schniga'    | 12.03.     | 25.03.                | 02.04.        | 12.04.          |
| 'Granny Smith'    | 14.03.     | 27.03.                | 05.04.        | 17.04.          |
| 'Golden Rainders' | 15.03.     | 28.03.                | 06.04.        | 17.04.          |

Because of warming conditions, Kunz and Blanke (2011) showed that apple cultivars expose 10 days earlier full bloom. The cultivar 'Granny Smith' had the longest duration of blooming of 21 days, while 'Gala Schniga' had the shortest of 18 days (Fig. 1).

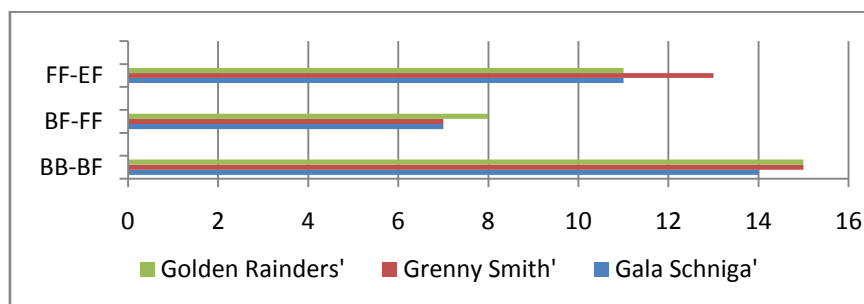


Fig 1. Duration of apple blooming in days (BB – bud burst; BF – beginning of flowering; FF – full flowering; EF – end of flowering)

The cultivar 'Gala Schniga' had the highest percentage of damaged flowers, more than 75.4% on all positions in flower buds, and the cultivar 'Golden Delicious Rainders' had the lowest, only 14% (Fig 2).

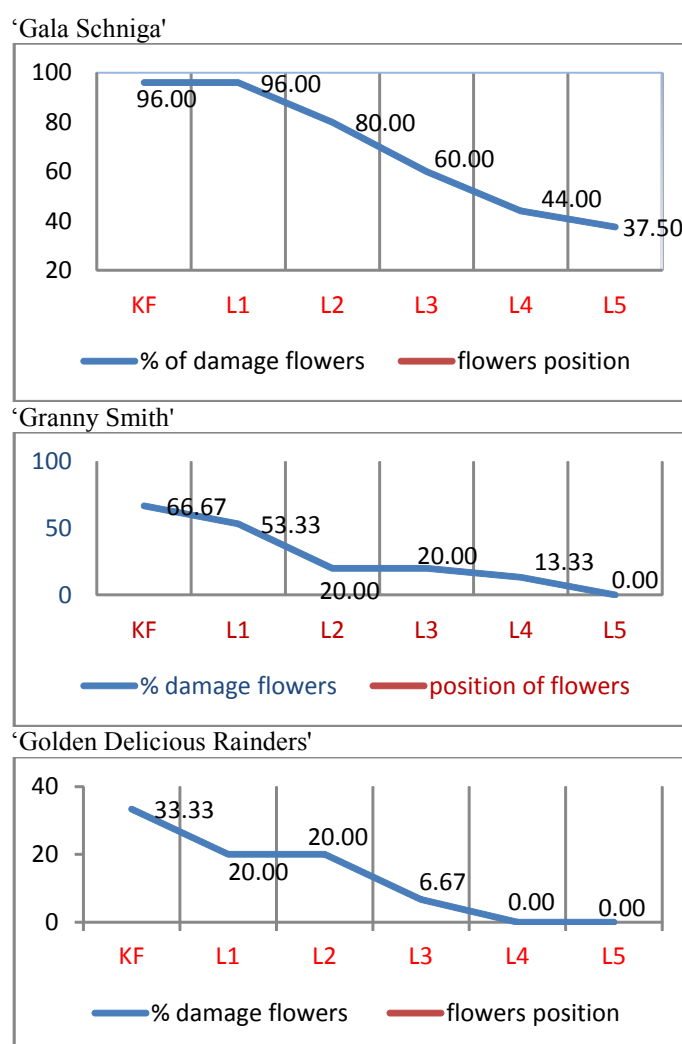


Fig. 2. Damage of flowers of apple cultivars

According to Rodrigo 2000, spring frost hardiness depended to a genotip. Aygün and San 2005, recorded that between seven cultivars 'Golden Delicious' had the highest survival flowers. All cultivars had the highest damage of king flowers (KF). A damage of KF ranged between 33% in 'Golden Delicious Rainders' to 96% in 'Gala Schniga'.

'Gala Schniga' had a same percentage of damage on flowers in L1 position in the clusters. Also, this cultivar had a statistical significant damage of flowers to all positions in the cluster in comparison to the other two cultivars. Statistical significant difference in damage flowers between cultivars 'Granny Smith' and 'Golden Delicious Rainders' were in a position L1 and L4. Jakopic et al. (2015) showed that fruits in this position in clusters might had satisfactory quality.

At the time of appearance of spring frost the cultivar 'Gala Schniga' was at nearly full blooming time, and that might be a reason why this cultivar had the highest damage of flowers. Westwood (1993) recorded that frost damage is highly dependent on the stage of development of the flower buds.

### **Conclusions**

Spring frost caused a very serious and significant damage to flowers buds. The highest damage was recorded to the cultivars 'Gala Schniga', which had a 74% of damaged flowers. Also, the cultivar 'Gala Schniga' had a earliest time of beginning and full blooming. In same agricultural conditions, in aim of avoidance of flowers damage and a better production results we recommend growing late flowering cultivars.

### **References**

1. Aygün, A. and San, B. (2005): The Late Spring Frost Hardiness of Some Apple Varieties at Various Stages of Flower Buds. *Tarim Bilimleri Dergisi* 2005, 11 (3) 283-285.
2. Chemielewski, F.M., Blumel, K., Hennigis, Y., Blanke, M., Weber, R and Zoth, M. (2011): Phenological models for the beginning of apple blossom in Germany. *Meteorologische Zeitschrift*, 20 (5): 487-496.
3. Eccel, E., Rea, R., Caffara, A. and Crisel, A. (2009): Risk of spring frost to apple production under future climate scenarios: the role of phenological acclimation. *International Journal of Biometeorology*, 53 (3): 273-286.
4. Farajzadeh, M., Rahimi, M., Kamali, G.A. and Mavrommatis, T. (2010): Modelling apple tree bud burst time and frost risk in Iran. *Meteorological Applications*, 17 (1): 45-52.
5. Grab, S. and Czaparo, A. (2011): Advance of apple and pear tree full bloom dates in response to climate change in the southwestern Cape, South Africa: 1973–2009. *Agriculture and Forest Meteorology*, 151: 406-413.
6. Hudina, M. and Štampar, F. (2006): Influence of Frost Damage on the Sugars and Organic Acids Contents in Apple and Pear Flowers. *European Journal of Horticultural Science*, 71(4), 161-164.
7. Jakopic, J., Zupan, A, Eler, K., Schmitzer, V., Stampar, F. and Veberic, R. (2015): It's great to be the King: Apple fruit development affected by the position in the cluster. *Scientia Horticulturae*, 194: 18–25.
8. Kunz, A. and Blanke, M.M. (2011). Effects of global climate change on apple 'Golden Delicious' phenology - based on 50 years of meteorological and phenological data in Klein-Altendorf. *Acta Hortic.* 903, 1121-1126.
9. Lenz, A., Hoch, G., Vitasse, Y. and Körner, C. (2013): European deciduous trees exhibit similar safety margins against damage by spring freeze events along elevational gradients. *New Phytologist*, 200 (4): 1166–1175.
10. Милатовић, Д., Ђуровић, Д., Ђорђевић, Б. (2009): Помолошке особине новијих сорти јабуке. *Зборник радова II Саветовања „Иновације у воћарству“*, стр 139-146.
11. Rodrigo, J. (2000): Spring frost in deciduous fruit trees morphological damage and flower hardiness. *Scientia Horticulturae*, 85: 155-173.
12. Salazar-Gutierrez, M.R., Chaves, B. and Hoogenboom, G. (2016): Freezing tolerance of apple flower buds. *Scientia Horticulturae*, 198: 344-351.
13. Westwood, M.N., 1993. *Temperate-zone Pomology: Physiology and Culture*. Timber Press, Portland.

## MINERAL RATIOS IN BEETROOT UNDER DIFFERENT FERTILIZATION

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### Abstract

Besides the absolute values of mineral content in plant tissue, particularly is important their relative relationship. Imbalance in nutrient uptake very often is result of extensive fertilization with one nutrient, which is then in excess, so can prevent uptake of other nutrients what may cause disturbances in metabolism. Therefore, the goal of present research was to determine the influence of fertilization on mineral ratios in beetroot. A field trial (2003-2005) was set up in a hilly part of Croatia according to the Latin square method with four types of fertilization (control, 50 t stable manure ha<sup>-1</sup>, 500 and 1000 kg NPK 5-20-30 ha<sup>-1</sup>). Some investigated ratios ranged as follows: N+P+K 4.08-8.17, K+Ca+Mg 2.83-5.14, K/Ca 6.8-18.1, K/Mg 4.3-19.3, K/(Ca+Mg) 2.6-9.3, (N+P)/K 0.70-1.26, Ca/P 0.7-2.0, Ca/Mg 0.65-1.07, K/Mn 297-789, N/Zn 501-786, P/Zn 41-92, Fe/Mn 1.6-2.8, Fe/Cu 11-23, Fe/(Cu+Zn) 2.1-4.2, Zn/Cu 3.3-7.6, Mn/Zn 1.5-1.8, Mn/Fe 0.38-0.62. In order to achieve high mineral content and its favorable ratio is necessary to combine the organic and mineral fertilizers with foliar fertilization.

**Keywords:** *Beta vulgaris* var. *conditiva* Alef., iron, manganese, nutrient, phosphorus.

### Introduction

Both, macro and micronutrient plant content, should be within a range referred to as sufficient for a productive plant production. Mineral and organic fertilization affects both, vegetative and reproductive organs, but what is more important yield quantity and quality, which is nowadays very important issue. If the plant mineral content is below the optimum range the plant might suffer from excessive or deficient content of nutrients that reduce the yield quantity and quality. To avoid this situation the optimal fertilization design should be applied according to plant needs as well as nutrient soil status. In addition to the optimal content of minerals in soil and plant tissue, relative ratio of minerals is particularly important (Bergmann, 1992). It is commonly known that between ions two types of relations are dominated, antagonism and synergism. The imbalance in nutrient uptake is very common where the abundant fertilization of one single nutrient was applied which is then in surplus and therefore block the uptake of another nutrient which can cause metabolic disorders (Marschner, 1995). The nutrient soil application has to be adapted to the planned production to ensure the annual need for normal growth and development of plants (Karažija, 2013). However, special attention should be paid to the balance of nutrition, as increased doses of mineral fertilizers can cause problems (Miljković and Bišof, 1988, cited by Drenjančević, 2011). Initially, the ratio of minerals was attempted to evaluate on the basis of the plant tissue mineral content and its limit values (Bergmann, 1992). Later, evaluation of N-P-K was performed, and then to a number of other ratios among biogenic elements, which better indicated harmony in plant nutrition. It was followed by the representation of certain elements in a triangular percentage, and in particular for nitrogen, phosphorus, potassium, calcium and magnesium. Then the intensity of nutrition was expressed as the sum of cations (K+Ca+Mg). Recent studies have shown that great attention should be paid to cations, especially potassium, calcium and magnesium (Ćosić i sur., 2010) although the ratio both beetwen microelements, as well as macro and microelements becoming more and more interesting (Ryser, 1982; Pitura and Michalojc, 2015; Murawska et al., 2013; Krzywy and Krzywy, 2001; Tariq and Mott, 2006; Jarnuszewski and Meller, 2013).

Therefore, the aim of this research was to determine and evaluate the effect of organic and mineral fertilization of red beet on mineral ratios.

## Material and methods

### Field work

A field fertilisation trial with beetroot (*Beta vulgaris* var. *conditiva* Alef.), cultivar 'Bikor', was carried out in Brašljevača and Hrvatsko Polje (Croatia) from 2003 to 2005 (Brašljevača in 2003, B-2003; Hrvatsko Polje in 2004, HP-2004 and Hrvatsko Polje in 2005, HP-2005) using the Latin square method with four treatments (unfertilised control, 50 t stable manure ha<sup>-1</sup>, 500 and 1000 kg NPK 5-20-30 ha<sup>-1</sup>). Untreated beetroot seed was sown (22<sup>nd</sup> May 2003, 21<sup>st</sup> May 2004 and 29<sup>th</sup> June 2005) directly into soil with a plant spacing of 0.07 m x 0.40 m and a main plot area of 12 m<sup>2</sup>. Beetroot were harvested (21<sup>st</sup> Aug 2003, 24<sup>th</sup> Aug 2004 and 28<sup>th</sup> Sep 2005) after ~90 days.

### Chemical plant analysis

The edible parts of six plants from each plot at harvest were randomly selected for analyses. Samples of plant material (dried at 105°C) were analysed in triplicate and the results presented as mean values. After digestion of plant material with concentrated HNO<sub>3</sub> (MILESTONE 1200 Mega Microwave Digester), phosphorus was determined spectrophotometrically, potassium by flame photometer, calcium, magnesium, iron, manganese, copper and zinc were analysed by an atomic absorption spectrophotometer (AAS) (AOAC, 1995).

### Chemical soil analysis

Field investigations were carried out on silty loam soil with a soil reaction (pH<sub>H2O</sub>) of 6.1-6.6, with low to moderate humus and nitrogen content, poor in phosphorus and low to rich in potassium (Table 1). Air-dried, ground and homogenized soil was analysed according to following methods: soil pH was determined electrometrically using a combined electrode (pH-meter MA5730) for a soil:water suspension (1:2.5, w/v) (active acidity) (Škorić, 1982); humus by the Tjurin method (JDPZ, 1966); potassium and phosphorus by the Egner-Riehm-Domingo method (Egner et al., 1960) and nitrogen by Kjeldahl method (AOAC, 1995).

Table 1. Chemical properties of the soils collected

| Environment <sup>x</sup> | pH <sub>H2O</sub> | %     |      | AL – mg 100 g <sup>-1</sup>   |                  |
|--------------------------|-------------------|-------|------|-------------------------------|------------------|
|                          |                   | humus | N    | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O |
| B-2003                   | 6.5               | 2.17  | 0.12 | 0.1                           | 6.0              |
| HP-2004                  | 6.1               | 2.65  | 0.13 | 1.5                           | 15.3             |
| HP-2005                  | 6.6               | 3.10  | 0.16 | 6.2                           | 32.8             |

<sup>x</sup>B-2003 – Brašljevača, year 2003; HP-2004 – Hrvatsko Polje, year 2004; HP-2005 – Hrvatsko Polje, year 2005

Comparing climatological conditions in all three investigation years during the growing period, it is evident that the most favourable conditions for normal growth and development of red beet prevailed in year 2004. Precipitation in 2003 was 247 mm and was poorly distributed. Temperatures were between 19°C and 23°C. In year 2004 was less precipitation (176 mm) during the red beet growth, air temperatures ranged from 17°C to 21°C, so this year was favourable for red beet growing because of favourable temperatures, and better distribution of precipitation. Air temperatures in year 2005 (15-20°C) were also within the biological optimum for the growth and development of red beet, but the precipitation was excessive (423 mm) so the weather conditions were unfavourable.

## Results and discussion

As was discussed earlier, at the beginning of thinking about mineral ratios, sum of some nutrients was discussed first. So, regarding both sum of nutrients (N+P+K) and (K+Ca+Mg) fertilization treatments did not affected, but environment did (Tables 2 and 3). Statistically the highest sum of nutrients was

determined in humid environment HP-2005 (8.17 and 5.14, respectively) and was higher than Ryser (1982) reported (3.92-4.54 and 4.23-4.91, respectively) and Neyroud et al. (2007) reported (4.27 and 3.47, respectively).

Ratio between two antagonistic cations (Bergmann, 1992) is very important. Again, the lowest ratio in dry season is determined because of lack of water which is important for Ca uptake (Table 4). All fertilization treatments statistically increased the ratio (11,3-13,1) compared to control treatment in HP-2004 environment (with favourable climate conditions). However, statistically the highest K/Ca ratio was determined in HP-2005 environment (18,1) compared to other two environments which can be explained by leaching of Ca due to excess of rainfall (Petek, 2009). Our results are much higher than literature reported (Pitura and Michalojc (2015) reported 2-4 as optimal K/Ca ratio) which can be explained with relatively high soil potassium content, especially in HP-2004 and HP-2005 environments (Table 1).

Table 5 shows K/Mg ratio in red beet root. Similar situation, as with K/Ca ratio, has been determined with this ratio too. Again, all fertilization treatments had statistical effect on K/Mg ratio in season with favourable climate condition (HP-2004) that ranged from 11,3 to 12,2. In other two environments fertilization treatments had no effect due to lack or excess of water. There are different literature data regarding the optimal K/Mg ratio. Ryser (1982) reported optimal range from 6.8 to 7.9, and Pitura and Michalojc (2015) from 2 to 6 cited from other literature, but in their own results reported mostly 10-20 in lettuce, curly kale and celery. Our findings showed high ratio in HP-2004 and especially in HP-2005 environments probably due to high potassium soil content (Table 1) which suggest very low uptake of magnesium of very low magnesium soil level.

Ratio of cations  $K/(Ca+Mg)$  is also important for optimal plant growth (Table 6). Again, the trend of this ratio is the same as in previous mentioned ratios with potassium. There are different data regarding the  $K/(Ca+Mg)$  ratio. According to different authors cited in Pitura and Michalojc (2015) optimal ratio is 1.62-2.2, although they reported up to 4.02, while Murawska et al. (2013) reported very low ratios, from 0.62 to 0.77, as well as Ryser (1982) from 0.58 to 0.66. Our results showed statistically higher ratio of cations in HP-2004 and HP-2005 environments. On the other side, the situation is completely opposite when the ratio is calculated by dividing by potassium –  $(N+P)/K$  (Table 7). Even though there is sum of different nutrients, the trend is the same. Statistically the highest ratio is determined in dry season in B-2003 environment (1.26) and is lower than reported by Ryser (1982) of 1.30-1.50. Obviously, potassium is prevailed cation in investigated soil and so in uptake by plant too. Unfavourable climate conditions had greater effect on nutrient uptake than the fertilization treatments, so in HP-2004 effect of fertilization was determined only.

Table 8 shows the Ca/P ratio in red beet root. Fertilization treatment statistically significant affected Ca/P ratio in HP-2004 environment only, when climate conditions were optimal. In other two environments (dry and humid) effect of fertilization was not statistically affected Ca/P ratio. The statistically highest ratio was obtained in B-2003 environment (2,0) in dry season when uptake of phosphorus was at low level due to lack of water in rhizosphere which is very important for phosphorus soil mobility because phosphorus is transported in soil mostly by diffusion (Mengel and Kirkby, 1987). Murawska et al. (2013) reported very low Ca/P ratio in maize grain (0.14-0.21). Optimal ratio according to Pitura and Michalojc (2015) is 2.0. Our findings are similar to this value just in dry season, which suggest that in season of favourable climate condition uptake of Ca was low, probably due to low calcium soil content according to soil type. As we reported in our previous paper (Petek et al., 2016), in humid environment (HP-2005) the highest red beet root phosphorus content was determined (3,7 g P kg<sup>-1</sup>) and the highest calcium content in dry environment (B-2003, 4,0 g Ca kg<sup>-1</sup>).

In Table 9 are shown results of Ca/Mg ratios that ranged from 0.60 to 1.16. In humid HP-2005 environment determined ratio was statistically significant the highest (1.07) and was much higher than Murawska et al. (2013) reported (0.27-0.46).

Potassium had the same effect on uptake of micronutrient manganese. The average highest K/Mn ratio (789) was determined in humid HP-2005 environment (Table 10) where is high soil potassium

content. In all three environments the fertilization treatment increased the value of K/Mn ratio compared to unfertilized treatment, in B-2003 and HP-2005 this increase was statistically significant. Bergmann (1992) reported 225 as optimal K/Mn ratio, Ekholm et al. (2007) 870 and Lisiewska et al. (2006) 789. In our research the ratios are determined in very wide range, from 297 up to 789. Low ratio suggest low potassium uptake and high ratio suggest low manganese uptake. As manganese is one of important microelement, both in plant and human metabolism, we suggest as optimal ratio about 500, which realised in HP-2004 environment when favourable climate condition performed. By consulting our previous work (Petek et al., 2016), these result of ratios with potassium are highly expected, especially in HP-2005 environment because, not just that there was high soil potassium content, but also there was high red beet root potassium content ( $47 \text{ mg K kg}^{-1}$ ).

The ratio of N/Zn is shown in Table 11. All values are a lot below recommended value of 1200, reported by Bergmann (1992). Here can be determined similar situation as with K/Mn ratio regarding the effect of fertilization treatments and effect of environments. Again in HP-2005 environment statistically the highest N/Zn ratio (786) was determined. But, as in first two years of investigation N/Zn ratio was quite low, better nitrogen fertilization is needed as Petek et al. (2016) reported quite high zinc content, especially in B-2003 of  $53 \text{ mg Zn kg}^{-1}$ .

Generally, fertilization treatment had positive effect on P/Zn ratio (Table 12) compared to control treatment, but environment had great effect on P/Zn ratio too. The highest ratio was determined in humid season (HP-2005) probably due to relatively good phosphorus uptake in this season. This assumption is backed up with our previous work (Petek et al., 2016) which reported  $3,7 \text{ g P kg}^{-1}$  in red beet root. However, all values are much lower than reported by most literature. Bergmann (1992) reported P/Zn ratio of 110 as optimal, Ekholm et al. (2007) 113 and Kadar (2000) lower than 200. Only Lisiewska et al. (2006) reported 54 which is relatively similar as we determined in dry season, so we think that, due to lack of water, this value is not the optimal one.

Discussing about ratios between microelements (Fe/Cu, Fe/(Cu+Zn), Zn/Cu, Mn/Zn and Mn/Fe) (Tables 13-18) it can be observed the similar trend. Mostly, the fertilization treatments had no statistically significant effect on micronutrients ratios. According to literature data of ratios with Fe [recommended Fe/Cu ratio by Pitura and Michalajc, (2015) is 6-19 and 15.8-29.2 reported by Tariq and Mott (2006)], it can be concluded that red beet had no problems with Fe uptake which very well proved that red beet is rich in iron, as we already reported in our paper ( $96\text{-}270 \text{ mg Fe kg}^{-1}$ ) (Petek et al., 2016). The ratios between micronutrients in plants reported in literature: Zn/Cu 4.9-16.9, Mn/Zn 0.43-5.8, Mn/Fe 0.21-2.5 (Pitura and Michalajc, 2015; Tariq and Mott, 2006). In the present research mentioned ratios are as follows: 3.3-4.2, 1.5-1.8 and 0.38-0.62, respectively. Bergmann (1992) reported that abundant fertilization with nitrogen can cause a lack of potassium in the plant and that also magnesium, manganese, iron and zinc content must be balanced to favorably affect the metabolic processes in the plant. So, it is not always important to achieve the highest mineral ratio, because it means that the second nutrient is in low level in plant tissue. So, we always have to keep in mind the optimum levels of all nutrients.

Table 2. N+P+K ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                            | N+P+K ratio, based on % in DW |         |         |         |
|---------------------------------------|-------------------------------|---------|---------|---------|
|                                       | B-2003 <sup>1</sup>           | HP-2004 | HP-2005 | Average |
| Control                               | 6.28                          | 3.85    | 7.92 b  | 6.01    |
| Stable manure, $50 \text{ t ha}^{-1}$ | 6.60                          | 4.27    | 8.36 ab | 6.41    |
| $500 \text{ kg NPK ha}^{-1}$          | 6.11                          | 4.08    | 7.93 b  | 6.04    |
| $1000 \text{ kg NPK ha}^{-1}$         | 6.19                          | 4.13    | 8.49 a  | 6.27    |
| Average                               | 6.30 B <sup>2</sup>           | 4.08 C  | 8.17 A  |         |

<sup>1</sup>Environments: B-2003 – Brašljevača in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 3. K+Ca+Mg ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | K+Ca+Mg ratio, based on % in DW |         |         |         |
|--------------------------------------|---------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>             | HP-2004 | HP-2005 | Average |
| Control                              | 3.94                            | 2.72    | 4.96    | 3.87    |
| Stable manure, 50 t ha <sup>-1</sup> | 4.12                            | 3.00    | 5.28    | 4.14    |
| 500 kg NPK ha <sup>-1</sup>          | 3.75                            | 2.78    | 5.06    | 3.86    |
| 1000 kg NPK ha <sup>-1</sup>         | 3.75                            | 2.80    | 5.28    | 3.94    |
| Average                              | 3.89 B <sup>2</sup>             | 2.83 C  | 5.14 A  |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 4. K/Ca ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | K/Ca ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                            | HP-2004 | HP-2005 | Average |
| Control                              | 7.2                                            | 9.4 b   | 16.5    | 11.0    |
| Stable manure, 50 t ha <sup>-1</sup> | 6.0                                            | 13.1 a  | 19.2    | 12.8    |
| 500 kg NPK ha <sup>-1</sup>          | 6.8                                            | 13.1 a  | 18.5    | 12.8    |
| 1000 kg NPK ha <sup>-1</sup>         | 7.1                                            | 11.3 ab | 18.3    | 12.2    |
| Average                              | 6.8 C <sup>2</sup>                             | 11.7 B  | 18.1 A  |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 5. K/Mg ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | K/Mg ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                            | HP-2004 | HP-2005 | Average |
| Control                              | 4.1                                            | 9.7 b   | 19.0    | 11.0    |
| Stable manure, 50 t ha <sup>-1</sup> | 4.0                                            | 12.1 a  | 19.2    | 11.8    |
| 500 kg NPK ha <sup>-1</sup>          | 4.4                                            | 11.3 ab | 19.3    | 11.7    |
| 1000 kg NPK ha <sup>-1</sup>         | 4.7                                            | 12.2 a  | 19.5    | 12.1    |
| Average                              | 4.3 C <sup>2</sup>                             | 11.3 B  | 19.3 A  |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 6. K/(Ca+Mg) ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | K/(Ca+Mg) ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|-----------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                                 | HP-2004 | HP-2005 | Average |
| Control                              | 2.6                                                 | 4.8 b   | 8.8     | 5.4     |
| Stable manure, 50 t ha <sup>-1</sup> | 2.4                                                 | 6.3 a   | 9.6     | 6.1     |
| 500 kg NPK ha <sup>-1</sup>          | 2.7                                                 | 6.0 a   | 9.4     | 6.1     |
| 1000 kg NPK ha <sup>-1</sup>         | 2.8                                                 | 5.8 a   | 9.4     | 6.0     |
| Average                              | 2.6 C <sup>2</sup>                                  | 5.7 B   | 9.3 A   |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 7. (N+P)/K ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | (N+P)/K ratio, based on % in DW |         |         |         |
|--------------------------------------|---------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>             | HP-2004 | HP-2005 | Average |
| Control                              | 1.22                            | 0.72 a  | 0.79    | 0.91    |
| Stable manure, 50 t ha <sup>-1</sup> | 1.27                            | 0.65 b  | 0.75    | 0.89    |
| 500 kg NPK ha <sup>-1</sup>          | 1.24                            | 0.70 ab | 0.74    | 0.90    |
| 1000 kg NPK ha <sup>-1</sup>         | 1.29                            | 0.73 a  | 0.79    | 0.94    |
| Average                              | 1.26 A <sup>2</sup>             | 0.70 B  | 0.77 B  |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 8. Ca/P ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | Ca/P ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                            | HP-2004 | HP-2005 | Average |
| Control                              | 2.1                                            | 1.6 a   | 0.8     | 1.5     |
| Stable manure, 50 t ha <sup>-1</sup> | 1.8                                            | 1.0 b   | 0.6     | 1.2     |
| 500 kg NPK ha <sup>-1</sup>          | 2.0                                            | 1.0 b   | 0.7     | 1.3     |
| 1000 kg NPK ha <sup>-1</sup>         | 2.2                                            | 1.2 ab  | 0.7     | 1.4     |
| Average                              | 2.0 A <sup>2</sup>                             | 1.2 B   | 0.7 C   |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 9. Ca/Mg ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | Ca/Mg ratio, based on % in DW |         |         |         |
|--------------------------------------|-------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>           | HP-2004 | HP-2005 | Average |
| Control                              | 0.60                          | 1.05    | 1.16    | 0.94    |
| Stable manure, 50 t ha <sup>-1</sup> | 0.67                          | 0.92    | 1.00    | 0.86    |
| 500 kg NPK ha <sup>-1</sup>          | 0.66                          | 0.87    | 1.05    | 0.86    |
| 1000 kg NPK ha <sup>-1</sup>         | 0.69                          | 1.09    | 1.08    | 0.95    |
| Average                              | 0.65 C <sup>2</sup>           | 0.98 B  | 1.07 A  |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 10. K/Mn ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | K/Mn ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                            | HP-2004 | HP-2005 | Average |
| Control                              | 268 b <sup>2</sup>                             | 491     | 726 b   | 495     |
| Stable manure, 50 t ha <sup>-1</sup> | 352 a                                          | 636     | 840 a   | 609     |
| 500 kg NPK ha <sup>-1</sup>          | 274 ab                                         | 544     | 794 ab  | 537     |
| 1000 kg NPK ha <sup>-1</sup>         | 293 ab                                         | 588     | 796 ab  | 559     |
| Average                              | 297 C                                          | 565 B   | 789 A   |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 11. N/Zn ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | N/Zn ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                            | HP-2004 | HP-2005 | Average |
| Control                              | 545 b <sup>2</sup>                             | 481     | 758 b   | 595     |
| Stable manure, 50 t ha <sup>-1</sup> | 698 a                                          | 501     | 788 ab  | 662     |
| 500 kg NPK ha <sup>-1</sup>          | 583 ab                                         | 474     | 738 b   | 598     |
| 1000 kg NPK ha <sup>-1</sup>         | 695 a                                          | 548     | 862 a   | 702     |
| Average                              | 630 B                                          | 501 C   | 786 A   |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 12. P/Zn ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | P/Zn ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                            | HP-2004 | HP-2005 | Average |
| Control                              | 33 b <sup>2</sup>                              | 57 b    | 90      | 60      |
| Stable manure, 50 t ha <sup>-1</sup> | 55 a                                           | 75 a    | 99      | 76      |
| 500 kg NPK ha <sup>-1</sup>          | 37 ab                                          | 62 ab   | 84      | 61      |
| 1000 kg NPK ha <sup>-1</sup>         | 39 ab                                          | 69 ab   | 96      | 68      |
| Average                              | 41 C                                           | 66 B    | 92 A    |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 13. Fe/Mn ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | Fe/Mn ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|-------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                             | HP-2004 | HP-2005 | Average |
| Control                              | 2.9                                             | 2.4     | 1.6     | 2.3     |
| Stable manure, 50 t ha <sup>-1</sup> | 3.3                                             | 2.5     | 1.7     | 2.5     |
| 500 kg NPK ha <sup>-1</sup>          | 2.7                                             | 2.2     | 1.6     | 2.2     |
| 1000 kg NPK ha <sup>-1</sup>         | 2.4                                             | 2.4     | 1.6     | 2.1     |
| Average                              | 2.8 A <sup>2</sup>                              | 2.4 B   | 1.6 C   |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 14. Fe/Cu ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | Fe/Cu ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|-------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                             | HP-2004 | HP-2005 | Average |
| Control                              | 26                                              | 12      | 18      | 19      |
| Stable manure, 50 t ha <sup>-1</sup> | 23                                              | 11      | 18      | 18      |
| 500 kg NPK ha <sup>-1</sup>          | 23                                              | 11      | 19      | 17      |
| 1000 kg NPK ha <sup>-1</sup>         | 19                                              | 11      | 18      | 14      |
| Average                              | 23 A <sup>2</sup>                               | 11 C    | 18 B    |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 15. Fe/(Cu+Zn) ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | Fe/(Cu+Zn) ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|------------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                                  | HP-2004 | HP-2005 | Average |
| Control                              | 4.4                                                  | 2.8     | 2.1     | 3.1     |
| Stable manure, 50 t ha <sup>-1</sup> | 4.6                                                  | 2.6     | 2.1     | 3.1     |
| 500 kg NPK ha <sup>-1</sup>          | 4.1                                                  | 2.5     | 2.0     | 2.9     |
| 1000 kg NPK ha <sup>-1</sup>         | 3.8                                                  | 2.6     | 2.2     | 2.9     |
| Average                              | 4.2 A <sup>2</sup>                                   | 2.6 B   | 2.1 B   |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 16. Zn/Cu ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | Zn/Cu ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|-------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                             | HP-2004 | HP-2005 | Average |
| Control                              | 5.0 a <sup>2</sup>                              | 3.4     | 7.5 b   | 5.3     |
| Stable manure, 50 t ha <sup>-1</sup> | 4.3 ab                                          | 3.1     | 7.5 b   | 5.0     |
| 500 kg NPK ha <sup>-1</sup>          | 4.7 ab                                          | 3.6     | 8.1 a   | 5.5     |
| 1000 kg NPK ha <sup>-1</sup>         | 3.9 b                                           | 3.0     | 7.4 b   | 4.8     |
| Average                              | 4.5 B                                           | 3.3 C   | 7.6 A   |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 17. Mn/Zn ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | Mn/Zn ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|-------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                             | HP-2004 | HP-2005 | Average |
| Control                              | 1.8                                             | 1.6     | 1.5 ab  | 1.6     |
| Stable manure, 50 t ha <sup>-1</sup> | 1.7                                             | 1.5     | 1.4 bc  | 1.5     |
| 500 kg NPK ha <sup>-1</sup>          | 1.9                                             | 1.4     | 1.4 c   | 1.6     |
| 1000 kg NPK ha <sup>-1</sup>         | 2.0                                             | 1.5     | 1.5 a   | 1.7     |
| Average                              | 1.8 A <sup>2</sup>                              | 1.5 B   | 1.5 B   |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Table 18. Mn/Fe ratio in red beet root dry weight according to fertilizations and years of environments

| Treatments                           | Mn/Fe ratio, based on mg kg <sup>-1</sup> in DW |         |         |         |
|--------------------------------------|-------------------------------------------------|---------|---------|---------|
|                                      | B-2003 <sup>1</sup>                             | HP-2004 | HP-2005 | Average |
| Control                              | 0.35                                            | 0.43    | 0.64    | 0.47    |
| Stable manure, 50 t ha <sup>-1</sup> | 0.32                                            | 0.43    | 0.60    | 0.45    |
| 500 kg NPK ha <sup>-1</sup>          | 0.40                                            | 0.45    | 0.62    | 0.49    |
| 1000 kg NPK ha <sup>-1</sup>         | 0.44                                            | 0.42    | 0.62    | 0.49    |
| Average                              | 0.38 B <sup>2</sup>                             | 0.43 B  | 0.62 A  |         |

<sup>1</sup>Environments: B-2003 – Brašljeva in 2003; HP-2004 – Hrvatsko Polje in 2004; HP-2005 – Hrvatsko Polje in 2005

<sup>2</sup>Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

## Conclusions

Organic and mineral fertilization affected mineral ratios, especially in season with favourable climate conditions. Agroecological conditions affect too on mineral ratios during 3-year field trial. Although, it can be concluded that potassium dominates in treatments fertilized with NPK, especially with 1000 kg NPK ha<sup>-1</sup>, results showed antagonistic effect on other nutrients just in humid season. Based on obtained results it can be concluded that during the dry season deficiency of P, Mg and Zn can be expected, and during the humid season surplus of potassium can be obtained. So producers should be prepared, besides basic soil fertilization, to perform foliar fertilization with mentioned nutrients on time, to avoid deficiencies caused by low nutrient uptake in dry season, or to avoid antagonism with potassium in humid seasons.

## References

1. AOAC. (1995). Official method of analysis of AOAC International. 16th Edition, Vol. I, Arlington, USA.
2. Bergmann W. (1992). Nutritional disorders of plants. Gustav Fischer Verlag Jena, Stuttgart, New York.
3. Ćosić, T., Karažija, T., Čoga, L., Petek, M., Poljak, M., Herak Ćustić, M., Jurkić, V., Pavlović, I. and Slunjski, S. (2010). Istraživanje biljno hranidbenog kapaciteta vinograda u Bolu folijarnom analizom, Study, Department of Plant Nutrition, Zagreb.
4. Egner, H., Riehm, H. and Domingo W. R. (1960). Untersuchung über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Boden. II, Chemische Extraktionsmethoden zur Phosphor und Kaliumbestimmung - K. Landw. Hogsk. Annir. W.R. 1960, 26: 199-215.
5. Ekholm, P., Reinivuo, H., Mattila, P., Pakkala, H., Koponen, J., Happonen, A., Hellström, J. and Ovaskainen M.-J. (2007). Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. *Journal of Food Composition and Analysis*. 20:487–495.
6. Neyroud, J. A., Amiguet, S., Andrey, G. and Evequoz, Ch. (2007). Le diagnostic foliaire en arboriculture: bilan de 20 ans d'étude. *Revue suisse Vitic. Arboric. Hortic.* 39 (5): 307-313.
7. Jarnuszewski, G. and Meller, E. (2013). Mineral element ratios in plants grown on post-bog soil fertilized with zinc and copper. *Fol. Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech.*, 304 (26): 25-32.
8. JDPZ (1966). Priručnik za ispitivanje zemljišta. Knjiga I. Kemijske metode ispitivanja zemljišta, Beograd.
9. Kadar I. (2000). Effect of mineral fertilisation on the element uptake of maize (*Zea mays* L.) on chernozem soil, II. *Novenytermeles*. 49(1-2):128-140.
10. Karažija, T. (2013). Utjecaj organskih gnojiva na ishranjenost, prinos i kvalitetu grožđa cv. Sauvignon bijeli na karbonatnim tlima. PhD thesis, Zagreb.
11. Krzywy, E. and Krzywy, J. (2001). The N:S and Fe:Mn ion ratios as factors indicative of the quality of crop plants obtained under the Waste 7, sulfate hydrate (VI) iron (II). *Fol. Univ. Stetin.*, 221 Agric. 88, 133-140.
12. Lisiewska, Z., Kmiecik, W. and Gebczynski P. (2006). Effects on Mineral Content of Different Methods of Preparing Frozen Root Vegetables. *Food Science and Technology International*, 12:497-503.
13. Marschner, H. (1995). Mineral nutrition of higher plants. Academic Press Limited, San Diego, CA 92101.
14. Mengel, K. and Kirkby, E. A. (1987). Principles of Plant Nutrition. International Potash Institute, Bern.
15. Drenjančević, M. (2011). Fe-kloroza vinove loze na podunavskoj regiji. PhD thesis, Osijek.
16. Murawska, B., Spychaj-Fabisiak, E., Knapowski, T. and Głowacki B. (2013). Ionic equilibrium in maize grain depending on the fertilisation and soil type. *Journal of Central European Agriculture*, 14(4): 1518-1529.

17. Petek, M., Toth, N., Pecina, M., Karažija, T. and Herak Ćustić, M. (2016). Macrominerals in Red Beet Root under Organic and Mineral Fertilization. *Agriculturae Conspectus Scientificus*, 81 (3): 173-179.
18. Petek, M. (2009). Mineralni sastav cikle (*Beta vulgaris* var. *conditiva* Alef.) pri organskoj i mineralnoj gnojdbi. PhD thesis, Zagreb.
19. Pitura, K. and Michalojc, Z. (2015). Influence of nitrogen doses on the chemical composition and proportions of nutrients in selected vegetable species. *Journal of elementology*, 20 (3): 667-676.
20. Ryser, J.P. (1982.). Vers l''utilisation pratique du diagnostic foliaire en viticulture et en arboriculture. *Revue Suisse Vitic. Arboric. Hortic.* 14: 49-54.
21. Škorić, A. (1982): Priručnik za pedološka istraživanja. Fakultet poljoprivrednih znanosti, Zagreb.
22. Tariq, M. and Mott, C.J.B. (2006). Effect of boron supply on the uptake of micronutrients by radish (*Raphanus sativus* L.). *Journal of Agricultural and Biological Science*, 1 (2): 1-8.

## ENERGY CROPS AS ALTERNATIVE AGRICULTURE CROPS FOR BIOMASS PRODUCTION IN MACEDONIA AND BULGARIA

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### Abstract

In this review the focus is on few agricultural energy crops, which means crops that are grown exclusively or primarily for the purpose of producing biomass for energy purposes in an agricultural rather than a forestry context. However, cultivation of most of these crops is restricted to certain regions, e.g. by requirements for a certain climate zones. Having in mind the similar agro ecological conditions in R. Macedonia and Bulgaria, but also needs of the crops for successful growth and development, species as *miscanthus*, *switch grass* and *sweet sorghum* are introduced as a potentially used energetic plant species for this part of the Balkan region.

**Keywords:** *Miscanthus*, *switch grass*, *sweet sorghum*, production, yield.

### Introduction

Energy crops can be classified into those providing solid fuels for direct combustion, thermal processing and electricity generation, and liquid fuels, notably bioethanol and biodiesel. Solid fuel crops include energy coppice, *Miscanthus* and whole-crop cereals. Bioethanol is derived from the fermentation of sugar, starch or, potentially, cellulosic crops. Biodiesel refers commonly to transesterified vegetable oil, derived from oilseed rape or sunflower. So far, energy cropping with ligno-cellulosic crops is not wide spread in most EU countries. From the data published by Panuotsou et al. (2011) there are only some larger cropping areas in Sweden, Poland and the UK. In total the present EU wide perennial cropping area is estimated to be at around 93000 hectares with a total energy potential of 440 KTOE/year. Most of the previous research on energy crops were focused on cost of production for these crops (Khanna et al., 2008; Hallam et al., 2001; McLaughlin & Kszos 2005). Measuring the cost of production is required, but not sufficient to promote adoption of energy crops by farmers to achieve the target levels of cellulosic ethanol production. For many farmers growing of energy crops for bioenergy production is new and analysis should be conducted within the context of technology adoption. The aim of this review is to evaluate species as *miscanthus*, *switch grass* and *sweet sorghum* as a potentially used energetic plant species for this part of the Balkan region.

### Status of bioenergy production/energy crops in R. Macedonia and Bulgaria

*R. Macedonia.* Republic of Macedonia has large amount of biomass from agriculture available to be used as an energy source. The quantity is really large and it could bring to the theoretical generation of 1 500 GWh of electricity (R. Macedonia – IPA Rural Development Program 2014-2020 (2015)). In 2007 Macedonia's petrol company Makpetrol opened the first (and so far the only) biodiesel plant in the country with an annual capacity of 30.000 t. The raw material for the production of biodiesel is oil from oilseed rape exclusively provided by imports. In October 2014 the Macedonian Government joined a Memorandum of Understanding (MOU), to facilitate development of the cellulosic ethanol market in the Pelagonia region between Ethanol Europe and DuPont. According to the terms of the MOU, the Government had to facilitate the project in establishing a viable supply chain using energy crops, increasing local production of cereals and oilseeds, and offering incentives for renewable

biomass electricity for the nation's power grid. So far, with the exception of the one-year field trials with sorghum and switch grass in sub-region of Prilepsko Mariovo (as part of project activities), nothing has been realized.

**R. Bulgaria.** As a member-state of EU, Bulgaria harmonizes its policies and legislation with the European: 1) to increase the share of renewable energy in overall energy by up to 20% EU consumption and 2) for all Member States of 10% minimum share of biofuels in the overall consumption of petrol and diesel fuel for the transport in the EU. Up to now, Bulgaria has followed two main ways for pushing ahead the activities for implementing the EU energy policy: 1) by implementing measures and stimuluses' for increasing the consumption of biofuels and 2) by implementing measures and stimuluses' for increasing the production of raw materials and their processing for producing energy products. Bulgaria has enough land to ensure the production of biofuels with the raw materials required for this purpose, without the food industry being adversely affected. The necessary areas to achieve the mandatory target of 10% biofuels in 2020 amount will be totally 509,001 ha, representing 14.6% of the arable land in 2016. At present, the sown area with industrial oil crops is around 200.000 ha (196,958 ha).

**Miscanthus.** *Miscanthus* is a perennial rhizomatous grass with the C4 photosynthetic pathway. Giant miscanthus - *Miscanthus x giganteus* is a natural hybrid, formed by crossing *Miscanthus sinensis* (diploid  $2n=2x=38$ ) and *Miscanthus sacchariflorus* (tetraploid  $2n=4x=76$ ) (Greef and Deuter, 1993). As a result of his triploidness, *Miscanthus x giganteus* is sterile, so it cannot produce fertile seed (Linde-Laursen, 1993).

**Growth and development.** In practice exists two methods of propagation that are currently used for *Miscanthus* plants – rhizome division and micropropagation. Rhizome division is more used method because it is less expensive and generally produces more vigorous plants. Plants starts to grow from dormant winter rhizomes, when the soil temperatures reach 10-12 °C, and the temperature threshold for leaf expansion of the plants that start to grow is in the range of 5-10 °C (Clifton- Brown and Jones, 1997). From the rhizomes during of April overhead stems appear characterized by a rapid increase, reaching height of about 2.0 m. During the winter they are dried where the water content is around 30%. The harvest is carried out during February by cutting the entire stems. New plants evolve from rhizomes next spring when favorable temperature conditions are created.

**Fertilization.** The cultivation cycle of *Miscanthus x giganteus* is characterized by less demand of mineral fertilizers and pesticides. An amount of 60 kg ha<sup>-1</sup> N was found optimal to support the development of the rhizome system from the second or third year onwards (Greef, 1995). Overall nutrient requirements for N, P and Ca are about 2-5, 0.3-1.1 and 0.8-1.0 kg t<sup>-1</sup> of dry matter respectively (Lewandovski and Kicherer, 1997). The potassium fertilization did not improve the yield of *M. x giganteus* which may be an effect of indifference of the crop for this element or the good potassium supply level in the soil .

**Irrigation.** To obtain high yields of *Miscanthus* in temperate climates, the optimum amount of precipitation is around 800 mm (Schwarz, 1993). However, from the second year onwards, plants develop a more powerful root system and more robust rhizomes, so crops are more tolerant to drought, but also to freezing. In absence of N fertilization, irrigation did not modify biomass yield and the effect of irrigation increased with the increase in N level (Ercolia et al. 1999).

**Weed, pest and diseases.** Weed control in the establishment phase of the crop is essential. Once the crop is mature, weed interference is effectively suppressed (Planting and growing miscanthus). *Miscanthus* species are susceptible to diseases and pests in the areas to which they are native (Asia). To date, there are no reports of plant diseases significantly limiting production, but the crop is known to be susceptible to *Fusarium* (Thinggaard, 1997), to Barley Yellow Dwarf Luteovirus (Christian et al., 1994) and to miscanthus blight (*Leptosphaeria* sp.). There are no reported insect pests in Europe that have significantly affected the production of miscanthus. However, two 'ley pests', the common rustic moth – *Mesapamea secalis* and ghost moth larvae – *Hepialus humuli*, have been reported feeding on miscanthus and may cause problems in the future (Planting and growing miscanthus).

*Harvest and biomass yield.* Harvest is usually carried out in spring (February to April), in order to collect well-dried material. The optimal time for harvesting may be quite short, since the crop will be about to re-start growth in April, and this can add to the costs of harvest. Yields of up to 25 t ha<sup>-1</sup> year<sup>-1</sup> (dry matter) have been obtained from the third year onwards in the spring harvest, even there have been huge differences in biomass yields from 2 t ha<sup>-1</sup> (Hotz et al.), to 44 t ha<sup>-1</sup> (Danalotos et al.).

**Switchgrass – *Panicum virgatum*,** is a C<sub>4</sub> perennial grass native to North America (Moser and Vogel, 1995). The species is polymorphic with two distinct ecotypes: lowland, mostly tetraploid with 2n = 4x = 36 chromosomes, and upland, which are tetraploid and octaploid with 2n = 8x = 72 chromosomes (Brunken and Estes, 1975; Sanderson et al., 1996).

*Growth and development.* Switchgrass reproduces through seeds and spreads vegetatively as well. The base temperature for germination and growth is between 8 and 10 °C, optimum temperature is around 30 °C (Hsu et al. 1985), and maximum temperature can be around 40 °C, but all of these conditions appear to be cultivar dependent. Upland cultivars have been selected from higher latitudes, are thin-stemmed and found in drier conditions, and have greater winter survival potential than the lowland cultivars when grown at the same latitude. Regrowth of bought genotypes begins each spring, and its primary growth period follows through the warm months of June, July, and August. Frost in the autumn stops its annual growth.

*Fertilization.* For maximizing biomass production the optimum N rates for switchgrass varied, from 50 – 110 kg ha<sup>-1</sup> year<sup>-1</sup> (Planting and Managing Switchgrass as a Biomass Energy Crop) up to 160 – 220 kg ha<sup>-1</sup> year<sup>-1</sup> (Is switchgrass a low nutrient input crop or not?). Split applications are more suitable for rates greater than 100 kg ha<sup>-1</sup> or for a two-cut harvest system. Investigations from Muir et al., (2001) and Parrish and Fike (2005), reported little or no yield response of switchgrass to phosphorus fertilization. However in soils with low plant available P, application of 45 kg P ha<sup>-1</sup> increases biomass yield by up to 17% (Kering et al., 2012). The single use of potassium (68 kg ha<sup>-1</sup>) did not affect the yield level. Applied together with N (135 kg ha<sup>-1</sup>) has positive influence of increasing yield, indicating that for maximum biomass production, proper nutrient management (N, P, and K) is required (Maru et al., 2013).

*Irrigation.* Even though irrigation has potential to increase yield, the feasibility of switchgrass production is based primarily on the use of otherwise unproductive and unprofitable agricultural lands, which likely do not have access to cost-effective irrigation. Example of weekly application of 2.54 cm of irrigation delivered by overhead sprinklers during the growing season over a three-year period (2009-2012) did not significantly increase annual biomass yield of switchgrass (Jacobs and King, 2012).

*Weed, pest and diseases.* Weed competition is a major reason for switchgrass stand failure during establishment. In already established stands, weed pressure during the second growing season could be worse than in subsequent years if there was poor site occupancy by switchgrass seedlings during the seeding year. With adequate weed control during the first two years of a stand, subsequent problems can be limited. The two main pests that are of concern in the U.S. are the switchgrass moth and the switchgrass gall midge (Samson et al., 2016). Outbreaks of rust and smut can occur during the establishment year but are generally more likely to occur post-establishment (Sanderson et al., 2012).

*Harvest and biomass yield.* An established stand of switchgrass can be maintained for more than 10 years (Fike et al. 2006), obtain yield from 8–15 t ha<sup>-1</sup> (Monti et al. 2008). Lowland cultivars are recommended to be harvested once per year after the first frost. Upland cultivars may be harvested under a two-cut system, with the first cut in June or July and the second cut after the first frost (Bransby et al. 1999; Cassida et al. 2002).

**Sweet sorghum.** Sweet sorghum – *Sorghum bicolor* (L) Moench is similar to grain sorghum but features more rapid growth, higher biomass production, and wider adaptation. As a C<sub>4</sub> species is more wateruse efficient and can be successfully grown in semiarid tropics, where other crops such as maize fail to thrive (Hlophe, 2014).

*Growth and development.* Sorghum comes in many forms. All are canelike grasses some 50 cm to 6.0 m tall. The plants have a fibrous root system that may penetrate 150 to 250 cm into the soil unless a hard pan is present in the soil. The leaves look very much like those of maize and number 14 to 18, growing on alternate sides of the stem. During a drought, the leaves will curl inward, thus conserving moisture loss through transpiration. The plant is self-pollinated. The crop is grown from seed (Smith and Frederiksen, 2000).

*Fertilization.* Different authors recommend different requirement of nitrogen, from 112, 150, 224 even to 269 kg N ha<sup>-1</sup> depends from many factors (Maughan et al., 2012; Wortmann et al., 2010; Powell and Hons, 1992; Marsalis and Bean, 2001). For phosphorus, according Roy and Khandaker (2010) who evaluate the effect of various levels of phosphorus fertilizer on the yield of sorghum fodder at three cuttings, it may be suggested that sorghum fodder can be cultivated through the application of 80 kg P/ha<sup>-1</sup> and harvested at the age of 66 days at first cutting for maximum production. In combination of N and P an increase yield has been observed with fertilizer application up to 100 kg N + 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> whereas, the quality parameters such as protein content, crude fiber and ash percent had significantly higher with NP application of 100 + 100 kg ha<sup>-1</sup> (Ayub et al., 1999).

*Irrigation.* In its growing period of about 4.5 months, the crop water requirement is 800 mm for two crop cycles. The crop may deplete less water from the soil than maize, and that in general confirms sweet sorghum's 25% less water requirement compared to maize (Hanson, 2014)

*Weed, pest and diseases.* The lack of weed control of sweet sorghum can result in yield losses, emphasizing the need of a good weed management (Leandro et al., 2016). At least 150 insect species have been reported as pests for sorghum worldwide. Many of the sorghum pests can damage crops other than sorghum, such as corn, cotton, and millet. (Chunshan et al., 2011). Like with pests, any disease prone to infecting grain sorghum may also influence sweet sorghum. Diseases that affect sweet sorghum include leaf anthracnose, red stalk rot and maize dwarf mosaic virus.

*Harvest and biomass yield.* Early maturing sweet sorghums, typically mature in approximately 90 days. Certain full-season varieties or hybrids can take more than 150 days to mature (Sweet sorghum production guide). The yield of fresh mass could be from 36 to 45 t ha<sup>-1</sup>, even up to 100 t ha<sup>-1</sup>.

## Conclusions

As biofuels are produced from biomass of crop plants, as indicated earlier, they offer enormous opportunities to improve the income levels of smallholder farmers in bought countries – R. Macedonia and Bulgaria. At community level, farmers can cultivate energy crops that fetch more income while meeting their food needs. Local production of biofuels is projected to have a broad range of positive economic, social and environmental implications. At a national level, producing more biofuels will generate new technologies, new industries, new jobs and new markets assisting economic growth in rural areas besides reducing environmental pollution.

## References

1. Ayub, M., Tanveer, A., Mahmud, K., Ali, A., M. Azam. (1999). Effect of Nitrogen and Phosphorus on the Fodder Yield and Quality of Two Sorghum cultivars (*Sorghum bicolor* L.). Pakistan Journal of Biological Sciences, 2 (1): 247-250.
2. Bransby, D.I., Bouton, J, Conger, B, McLaughlin, S. B., Ocumpaugh, W. R., Parrish, D., Taliaferro, K., Vogel, K., Wallschleger, S. (1999). Developing Switchgrass as a Bioenergy Crop. PotNewCropNewUses. AgronSHS. 282-299.
3. Brunken, J.N., and J.R. Estes. (1975). Cytological and morphological variation in *Panicum virgatum* L. Southwest. Nat. 19:379–385.
4. Cassida, K.A., Evers, G., Grichar, J., Hons, F., Hussey, M.A., Muir, J.P., Ocumpaugh, W.R., Read, J.C., Tischler, C., Venuto, B.C. (2002). Evaluation of Switchgrass Cultivars and Cultural Methods for Biomass Production in the South Central U.S. OakRidgeNL.TAMU.

5. Christian, D.G., Lampty, J.N.L, Forde, S.M.D., Plumb, R.T. (1994). First report of barley yellow dwarf luteovirus on *Miscanthus* in the United Kingdom. *European Journal of Plant Pathology*;100:167-170.
6. Chunshan, G., Wei, C., Xue, F., Jianzhou, Z., Guihua, Lu. (2011). Sorghum Insect Problems and Management. *Journal of Integrative Plant Biology* 2011, 53 (3): 178–192.
7. Clifton-Brown, J.C., Jones, M.B. (1997). The thermal response of leaf extension rate in genotypes of the C4-grass *Miscanthus*: an important factor in determining the potential productivity of different genotypes, *J. Exp. Bot.* 48, 1573–1581.
8. Danalatos, N.G., Dalianis, C., Kyritsis, S. Growth and biomass productivity of *Miscanthus sinensis*\giganteus under optimum cultural management in north-eastern Greece. In: Chartier P, Ferrero GL, Henius UM, Hultberg S, Sachau J, Wiinblad M, editors. (1996). *Biomass for energy and the environment: Proceedings of the Ninth European Bioenergy Conference, Copenhagen, Denmark, 1996*. New York: Pergamon,. p. 548-553.
9. Ercolia, L., Mariottib, M., Masonib, A., Bonaria, E. (1999). Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of energy use in crop production of *Miscanthus*. *Field Crops research*. Volume 63, Issue 1, pp 3-11.
10. Fike, J.H., Parrish, D.J., Wolf, D.D., Balasko, J.A., Green, J.T. Jr., Rasnake, M., Reynolds, J.H. (2006). Switchgrass production for the upper southeastern USA: influence of cultivar and cutting frequency on biomass yields. *Biomass Bioenergy* 30:207–213.
11. Greef, J.M. (1995). Etablierung und Biomassebildung von *Miscanthus x giganteus*. Gottingen, Germany: Cu villier Verlag.
12. Greef, J.M., Dauter, M. (1993). Syntaxonomy of *Miscanthus x giganteus* GREEF et DEU. In: *Angewandte Botanik*, 67, 1993, pp. 87-90.
13. Hagan, A.K, Bowen, K.L, Pegues, M. (2014) Nitrogen rate and variety impact disease and yield of sorghum for biofuel. *Agron J* 106: 1205–1211.
14. Hallam, A., Anderson, I., Buxton, D. (2001). Comparative economic analysis of perennial, annual, and intercrops for biomass production. *Biomass Bioenergy* 21:407–424.
15. Hanson, B.H. (2014). Sweet sorghum (*Sorghum bicolor* (L.) Moench) response to supplemental irrigation in different growth stages. [https://repository.up.ac.za/bitstream/handle/2263/43352/Hlophe\\_Sweet\\_2014.pdf?sequence=1&isAllowed=y](https://repository.up.ac.za/bitstream/handle/2263/43352/Hlophe_Sweet_2014.pdf?sequence=1&isAllowed=y).
16. Hlophe, BH. (2014). Sweet sorghum (*Sorghum bicolor* (L.) Moench) response to supplemental irrigation in different growth stages. in:[https://repository.up.ac.za/bitstream/handle/2263/43352/Hlophe\\_Sweet\\_2014.pdf?sequence=1&isAllowed=y](https://repository.up.ac.za/bitstream/handle/2263/43352/Hlophe_Sweet_2014.pdf?sequence=1&isAllowed=y).
17. Hotz, A., Kuhn, W., Jodl, S. Screening of diferent *Miscanthus* cultivars in respect of yield production and usability as a raw material for energy and industry. In: Chartier P, Ferrero GL, Henius UM, Hultberg S, Sachau J, Wiinblad M, editors. (1996). *Biomass for energy and the environment: Proceedings of the Ninth European Bioenergy Conference, Copenhagen, Denmark, 1996*. New York: Pergamon, p. 523-527.
18. Hsu, F.H., Nelson, C.J., Matches, A.G. (1985). Temperature effects on germination of perennial warm-season forage grasses. *Crop Sci.* 25:215-220.
19. Huisman, W., Kortleve, W.J. (1994). Mechanization of crop establishment, harvest, and post harvest conservation of *Miscanthus sinensis*\Giganteus". *Industrial Crops and Products* 2:289-297.
20. Is switchgrass a low nutrient input crop or not? from: [https://www.ipni.net/ipniweb/pnt.nsf/5a4b8be72a35cd46852568d9001a18da/94e3e3de8f398732852573a20046367d/\\$FILE/PNT-Winter07-5.pdf](https://www.ipni.net/ipniweb/pnt.nsf/5a4b8be72a35cd46852568d9001a18da/94e3e3de8f398732852573a20046367d/$FILE/PNT-Winter07-5.pdf).
21. Jacobs, A.A., King, J.R. (2012). Using fertilization, irrigation, and harvest strategies to maximize ‘Alamo’ and ‘Cave-in-Rock’ switchgrass biomass yield in the Southern Ozarks. In: Booneville Plant

- Materials Center Annual Technical Report. <http://www.plantmaterials.nrcs.usda.gov/pubs/arpmctr11197.pdf>.
22. Kering, M. K., Biermacher, J. T., Butler, T. J., Mosali, J., Guretzky, J. A. (2012). Biomass Yield and Nutrient Responses of Switchgrass to Phosphorus Application. *Agronomy & Horticulture - Faculty Publications*. 541. <http://digitalcommons.unl.edu/agronomyfacpub/541>.
  23. Khanna, M., Dhungana, B. Clifton-Brown, J. (2008). Costs of producing miscanthus and switchgrass for bioenergy in Illinois. *Biomass Bioenergy* 32:482–493.
  24. Leandro, G., Franciele, F.F., André, A., Alexandre, F. da Silva., César, T. F. (2016). Selectivity and efficiency of herbicides in weed control on sweet sorghum. e-ISSN 1983-4063 - [www.agro.ufg.br/pat](http://www.agro.ufg.br/pat) - Pesq. Agropec. Trop., Goiânia, v. 46, n. 2, p. 123-131.
  25. Lewandowski, I., Kicherer, A. (1997). Combustion quality of biomass: Practical relevance and experiments to modify the biomass quality of *Miscanthus × giganteus*, *Eur. J. Agron.* 6, 163–177.
  26. Linde-Laursen, I.B. (1993). Cytogenetic analyses of *Miscanthus giganteus*, and interspecific hybrid. *Hereditas* 119: 297-300.
  27. Marsalis, M.A, Bean, B. (2011). Western forage production guide. United Sorghum Checkoff Program Lubbock, TX 79403.
  28. Maru, K.K., Twain, J.B., Jon, T.B., Jagadeesh M., John A.G. (2013). Effect of Potassium and Nitrogen Fertilizer on Switchgrass Productivity and Nutrient Removal Rates under Two Harvest Systems on a Low Potassium Soil. *Bioenerg. Res.* 6, issue 1: pp 329–335.
  29. Maughan, M., Voight, T., Parrish, A. (2012). Forage and energy sorghum response to nitrogen fertilization in central and southern Illinois. *Agron J* 104: 1032–1040.
  30. McLaughlin, S.B., Kszos, L.A. (2005). Development of switchgrass as a bioenergy feedstock in the United States. *Biomass Bioenergy* 28:515–535.
  31. Mills, H.A., Jones, J.B. Jr., (1996). Plant analyses handbook II. In: Monti A. 2012. Switchgrass – a valuable biomass crop for energy. pp 205.
  32. Monti, A., Bezzi, G., Pritoni, G., Venturi, G. (2008). Long-term productivity of lowland and upland switchgrass cytotypes as affected by cutting frequency. *Bioresource Tech.* 99:7425– 7432.
  33. Moser, L.E., Vogel K.P. (1995). In: Mitchell, R., Fritz, J., Moore, K., Moser, L.E., Vogel, K. 2001. Predicting Forage Quality in Switchgrass and Big Bluestem. University of Nebraska – Lincoln, from: <http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1036&context=agronomyfacpub>.
  34. Muir, J.P, Sanderson, M.A, Ocumpaugh, W.R., Jones, R.M., Reed, R.L. (2001). Biomass production of “Alamo” switchgrass in response to nitrogen, phosphorus and row spacing. *Agron J* 93: 896-901.
  35. O'Neill, N.R., Farr, D.F. (1996). *Miscanthus* blight, a new foliar disease of ornamental grasses and sugarcane incited by *Leptosphaeria* sp. and its anamorphic state *Stagonospora* sp. *Plant Disease* 80:980-987.
  36. Panoutsou, C., Elbersen, B., Böttcher, H. (2011). Energy crops in the European context. Available through: [http://biomassfutures.eu/public\\_docs/final\\_deliverables.pdf](http://biomassfutures.eu/public_docs/final_deliverables.pdf).
  37. Parrish, D.J., Fike, J.H. (2005). The biology and agronomy of switchgrass for biofuels. *Crit Rev Plant Sci* 24: 423-459.
  38. Planting and growing miscanthus. <http://adlib.eversite.co.uk/resources/000/023/838/miscanthus-guide.pdf>.
  39. Powell, J.M., Hons, F.M. (1992). Fertilizer nitrogen and stover removal effects on sorghum yields and nutrient uptake and partitioning. *Agr Ecosyst Environ* 39: 197–211.
  40. R. Macedonia – IPA Rural Development Program 2014-2020. (2015). Available through: [http://ipardpa.gov.mk/Root/mak/\\_docz/Zakonodavstvo.pdf](http://ipardpa.gov.mk/Root/mak/_docz/Zakonodavstvo.pdf).
  41. Roy, P.R.S., Khandaker, Z.H. (2010). Effect of phosphorus fertilizer on yield and nutritional value of sorghum (*Sorghum bicolor*) fodder at three cuttings. *Bang. J. Anim. Sci.* 2010, 39 (1&2): 106 – 115.

42. Samson, R., Delaquis, E., B. Deen, J. DeBruyn and U. Eggimann. 2016. Switchgrass Agronomy 2016. from: [https://www.agrireseau.net/documents/Document\\_93992.pdf](https://www.agrireseau.net/documents/Document_93992.pdf).
43. Sanderson, M.A., R.L. Reed, S.B. McLaughlin, S.D. Wullschleger, B.V. Conger, D.J. Parrish, D.D. Wolf, C. Taliaferro, A.A. Hopkins, W.R. Ocumpaugh, M.A. Hussey, J.C. Read, and C.A. Tischler. 1996. Switchgrass as sustainable energy crop. *Bioresource Technol.* 56:87–93.
44. Sanderson, M.A., Schmer, M.R., Owens, V., Keyser, P. and Elbersen, W. 2012. Crop Management of Switchgrass. *Agronomy & Horticulture - Faculty Publications.* 551. <http://digitalcommons.unl.edu/agronomyfacpub/551>.
45. Schwarz, H., 1993. *Miscanthus sinensis* 'Giganteus' production on several sites in Austria. *Biomass Bioenergy* 5, 413-419.
46. Smith WC., Frederiksen AR. 2000. Sorghum – Origin, history, technology and production. page 11.
47. Sweet sorghum production guide. From: <http://www.lsuagcenter.com/nr/rdonlyres/7fd22fa2-3b95-4c4c-a8f8-01f6974910a7/98173/pub3357sweetsorghumproductionguidefinal1.pdf>.
48. Thinggaard, K. 1997. Study of the role of *Fusarium* in the field establishment problem of *Miscanthus*. *Acta Agriculturae Scandinavica, Section B, Soil and Plant Science* 1997;47:238-241.
49. Wortmann, C.S., Liska, A.J., Ferguson, R.B. 2010. Dryland performance of sweet sorghum and grain crops for biofuel in Nebraska. *Agron J* 102: 319–326.

## POMOLOGICAL AND CHEMICAL PROPERTIES OF SOME WALNUT GENOTYPES IN CENTRAL ANATOLIA

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### Abstract

This study was conducted to determine some pomological and chemical characteristics of Bilecik, Yavuz, Şebini walnut cultivars and Yerli genotype grown in Kırşehir, Turkey. Characteristics of walnut such as nut weight, kernel weight, nut width, nut height and nut diameter and some kernel features were investigated. The results indicated that the average fruit weight ranged from 17.33 g (Yavuz) to 12.83 g (Şebini), the nut width alternated from 37.63 mm (Yavuz) to 31.33 mm (Yerli), the nut height ranged from 52.25 mm (Yavuz) to 41.07 mm (Yerli), and the kernel percentage ranged from 46.07% (Bilecik) to 49.43% (Yavuz). The highest oil content was detected in Yavuz cultivar (63.20%), while the lowest value was obtained from the Bilecik cultivar with 53.90%. Contrary to fat content, the highest protein content was detected in the Bilecik cultivar. Consequently, our data showed that the fruit properties of Yavuz and Şebini cultivars were superior than the others in Central Anatolian ecological conditions.

**Keywords:** Walnut, cultivars, nut, kernel characteristics, Central Anatolia.

### Introduction

Walnut (*Juglans regia* L.), an important fruit crop for Turkey, is the oldest cultivated fruit in the world and grown naturally almost all over Turkey (Sen, 1986; Asma, 2012).

World walnut production has been estimated as 3.462.731 tons of shelled product. The main producing country is the China, which accounts for 1.602.373 tons 46.3% of the world production. The other producing countries are USA (518.002 tons; 15%), Iran (445.829 tons; 12.88%), Turkey (212.807 tons; 6.20%) Mexico (125.758; 3.8%), Ukraine (102.740 tons; 3%), followed by others countries with a lower production (Anonymous, 2017).

Turkey has notably good walnut cultivars. Breeding programs have been launched over the last 40 years to develop new cultivars with uniform fruit quality and high yield quantity (Bayazit et al. 2016). Turkey has a remarkable walnut population of walnut varieties when compared to other parts of the world. The walnut new cultivars have been improved by breeding programs in Atatürk Central Research Institute, Yalova, Turkey in 1971 (Şen, 1980). Several selection studies have been carried out especially dealing with the quality and fruit properties of walnuts grown in Turkey to improve walnut cultivars (Olez, 1971; Sen, 1983; Celebioğlu et al., 1988; Sen and Beyhan, 1993; Ferhatoglu, 1993; Akca and Sen, 1995; Askin and Gun, 1995; Koyuncu and Askin, 1995; Koyuncu and Askin, 1999; Bayazit, 2000; Sutyemez, 2000; Keles et al. 2014; Akça et al. 2015). A number of walnut cultivars have been developed as a result of these breeding programs. The most important walnut cultivars of are Sebin, Bilecik, Yalova 1, Yalova 3, Yalova 4, Sen1, Sen 2, Kaman 1, Kaman 5 and Yavuz in Turkey. In recent years, Maraş18, Sütyemez 1, Akça 1, Akça 2 varieties have been improved and the production is getting widespread day by day. Today, Walnut breeding studies are carried on in different Universities and Research Institutes. However, some foreign walnuts varieties such as Fernor, Chandler, Franquette are also cultivated.

The high quality varieties, determined as a result of these breeding trials in Turkey, do not show desired characteristics except for the regions where they are selected. Sometimes there are some serious problems such as poor fruit quality, inefficiency, frost damage etc. Thus; Çelebioğlu et al. (1988), Tosun and Akçay (2005) reported that some walnut varieties perform better in selected

places, whereas some walnut varieties perform better than selected ones. Similarly, it is not possible to know in advance the performance of the walnut varieties brought from other countries. For this reason, it is necessary to determine suitable walnut varieties for those ecological conditions before planting a walnut orchard, which requires significant labour and money. There are many ways to determine the appropriate variety for an ecological condition. Yield and fruit quality are the most important characteristics.

The Turkish Standards Institution established physical nut and kernel properties of walnut as a quality criteria (Anonymous, 1990; 1991). These properties are nut weight, kernel weight, kernel ratio, shell shape, nut dimensions, and other shell properties.

Some walnut genotypes which are very promising for cultivation have not been examined so far in terms of fruit quality and chemical composition in the Central Anatolian Region. The objective of this study was to determine the fruit quality parameters and chemical composition of some important Turkish walnut genotypes.

### Material and methods

Total of four genotypes, 3 out of 4 were standard cultivars, 'Şebin', 'Bilecik', 'Yavuz', and the rest were promising genotypes, 'Yerli' were included in the study. These genotypes were grown onto seedling rootstocks, and planted at 7 x 7 m in 2008. Fruits of these genotypes were collected from Mucur district of Kırşehir province, which is situated in the Central Anatolia, altitude 1050 meters, in October 2016. The examinations were carried out for one year and trees were eight years old. The location of walnut trees, the sampling days and storage conditions and time until analyses were similar for all genotypes. Physical analyses were quickly determined and kernel samples were kept at -18 °C before chemical analyses. This study was set up with three repetitions in chemical analyses and twenty repetitions in physical analyses.

**Pomological analyses:** Fruits were harvested at their fully mature period in three replicates of 20 fruit in per replicate. Fruit weight (g), fruit width (mm), fruit length (mm), shell thickness (mm), kernel weight (g), kernel percentage (%) were measured. Fruit shape index, fruit shape, shell roughness, shell breaking and size were determined according to Turkish Standard Institution (TSI) 1275/T1 (Anonymous, 2010).

Shape index: nut length / (nut diameter + nut thickness) / 2. This index was evaluated as follows: shape index < 1.25: sample is sphere; shape index 1.25: sample is oval. Kernel ratio (%): (kernel weight / nut weight) x100. Extra: Nut diameter ≥ 27 mm for sphere, nut diameter 26 mm for oval. Class I: nut diameter 24-27 mm for sphere, nut diameter 24-26 mm for oval, class II: diameter 20-24 mm for sphere and oval. Kernel crinkling was determined as fine, medium, bad and empty.

Also, fruit shell and kernel colours were determined using a Color Meter CR-300. The L\* value represents lightness (L\* 0 for black, L\* 100 for white), while a\* scale represents the red/green dimension, with positive values for red and negative ones for green. The color values were measured on three different spots in each of two samples for fruit shell and two different spots each of three samples for kernel. The results were recorded as the mean of these measurements. Chroma  $[(a^*2 + b^*2)^{1/2}]$  and hue angle  $(\tan^{-1} b^*/a^*)$  were also calculated.

**Total fat content:** Total fat analyses were extracted with Hexane in Soxhlet set. Total fat contents of samples were calculated according to the formula given by Akyuz and Kaya (1992):

Total fat (%) = (fat weight (g)/fruit weight (g) in cartridge) x 100.

**Crude protein content** (N×6.25) was determined by using kjeldahl method (Jung et al. 2003).

**Data analysis:** The analysis of variance was constructed according to Steel and Torrie (1980) using SAS (2005). The percentage values were transformed (arcsin) to increase normality. The mean separations were analysed by Tukey test at p<0.05.

### Results and discussion

Good kernel quality is desirable and important properties for walnut cultivars and walnut production. Nut characteristics of 4 walnut genotypes are presented in Table 1. Analyses of variance showed that there were statistically significant differences among the genotypes for all traits.

Table1. Pomological traits of some important Turkish walnut genotypes in the Central Anatolia Region of Turkey in 2016

| Genotypes | Nut weight (g) | Kernel weight (g) | Kernel ratio (%) |
|-----------|----------------|-------------------|------------------|
| Yavuz     | 17.33 a        | 8.56 a            | 49.43 a          |
| Şebin     | 12.83 b        | 6.28 b-c          | 48.93 a          |
| Bilecik   | 16.66 a        | 7.67 a-b          | 46.07 a          |
| Yerli     | 15.54 b        | 5.69 c            | 36.69 b          |
| HSD       | 2.21           | 1.58              | 7.65             |

The average nut weight changed between 17.33 and 12.83 g. The highest value showed 'Yavuz' (17.33 g) followed by 'Bilecik' (16.66 g) and 'Yerli' genotypes (15.54 g). Kernel weight varied from 8.56 g (Yavuz) to 5.69 g (Yerli). It has been noted that the kernel weight of the Yerli walnut genotypes is low.

Kernel ratio varied according to varieties. Kernel/nut ratio ranged from 49.43% (Yavuz) to 36.69% (Yerli), and were lower than 50% in all genotypes. In walnut genotypes, nut weight and kernel weight were found higher than results from other region by other researchers in Turkey (Tosun and Akçay, 2005; Bayazıt and Sumbul, 2012). In contrast, the kernel percentage of the walnut genotypes was low.

Akça and Aydın (2005) determined the nut weight (10.16 and 15.92 g), kernel weight (6.56 and 5.92 g) and kernel ratio (64.80 and 48.56 %) for the Şebin and Bilecik cultivars grown in Tokat province. Our results are better than that shown by Baymış (2008) who studied the Sebin and Bilecik cultivars grown in Kahramanmaraş and found nut weight (11.22 g-13.13g), kernel weight (6.33 g-6.11g), kernel ratio (56.41%-46.53%). This could be due to differences in the ecological, cultural practices, tree age and genetic properties of walnut genotypes grown in different areas. As a matter of fact, the kernel ratio of Şebin walnut cultivars in Ankara ecological conditions is reported as 33.98% by Akkuzu (2001).

Promising walnut genotypes should have a shell thickness of between 0.7 and 1.5 mm (Zhadan and Strukov, 1977). In this research shell thickness of the walnut genotypes determined varied between 1.42 (Bilecik) and 1.83 mm (Şebin) (Table 2). In the previous adaptation researches, shell thickness was 1.06 mm for the Sebin cultivars and 0.93 mm for the Bilecik variety (Baymış, 2008). Similarly, shell thicknesses of the Şebin walnut variety was reported to be 1.85 mm in the Hatay ecologic conditions (Bayazıt and Sumbul, 2012). As a result of this research, the shell thickness values obtained from the walnut varieties were higher than the values obtained from other researches.

Table 2. Nut characteristics of walnut genotypes

| Genotypes | Nut width (mm) | Nut length (mm) | Nut thickness (mm) | Shell thickness (mm) |
|-----------|----------------|-----------------|--------------------|----------------------|
| Yavuz     | 37.63 a        | 52.25 a         | 39.23 a            | 1.54 b               |
| Şebin     | 34.11 b        | 44.44 b         | 35.31 b            | 1.83 a               |
| Bilecik   | 35.40 b        | 45.42 b         | 35.85 b            | 1.42 b               |
| Yerli     | 31.33 c        | 41.07 c         | 33.27 c            | 1.53 b               |
| HSD       | 1.41           | 2.26            | 1.92               | 0.19                 |

Shell thickness is essential for saving kernels from the external effects. In addition, average nut length ranged from 41.07 (Yerli) to 52.25 mm (Yavuz), nut width varied from 31.33 (Yerli) to 37.63

mm (Yavuz), nut cheek varied from 33.27 (Yerli) to 39.23 mm (Yavuz) in the walnut genotypes (Table 2). The pomological characteristics of walnut genotypes were similar to results of other researcher (Baymiş, 2008; Akça and Aydın, 2005; Bayazıt and Sumbul, 2012).

Fruit shape was determined as oval, kernel colour was light yellow and yellow. Kernel removal was easy in the majority of the walnut genotypes.

The ideal nut should have a clean, strong, thin shell, a tight seal and weight between 12 and 18 g. The kernel should be easily removable from the shell, uniformly light in colour, clean, and weight 6–10 g or at least 50% of entire nut weight. Nut and kernel quality is strongly affected by genotypes, environment and their interaction (McGranahan and Leslie 1991; Akça and Ozongun 2004).

The fruit shape index (fruit length/width) of the genotypes between 1.28-1.38, and the fruit shapes were oval in walnut genotypes (Table 3). Shell roughness was smooth for 'Şebin' and 'Bilecik', and medium for 'Yerli', and was rough for 'Yavuz'. 'Şebin', and 'Bilecik' were on ease of shell breaking, but other genotypes were intermediate. The fruits of all walnut varieties in the experiment were included in the extra class.

Table 3: Fruit quality traits of some important Turkish walnut genotypes in in the Central Anatolia Region of Turkey in 2016

| Genotypes | Fruit Shape Index | Nut shape | Shell roughness | Shell breaking | Size  |
|-----------|-------------------|-----------|-----------------|----------------|-------|
| Yavuz     | 1.38              | Oval      | Rough           | Intermediate   | Extra |
| Şebin     | 1.30              | Oval      | Smooth          | Ease           | Extra |
| Bilecik   | 1.28              | Oval      | Smooth          | Ease           | Extra |
| Yerli     | 1.31              | Oval      | Medium          | Intermediate   | Extra |

The percentage of clean yellow kernel should be at least 50% in walnut genotypes according to previous findings. In our study, walnut genotypes had light coloured kernels.

The shell and kernel of all the walnut varieties in the experiment were light coloured. The biggest reason for this is that the temperate climatic conditions prevail in the experiment area, and there is no high summer temperatures.

Table 4. Fruit shell colour traits of some important Turkish walnut genotypes in the Central Anatolian Region of Turkey in 2016 year

| Genotypes | L       | a       | b         | C         | H         |
|-----------|---------|---------|-----------|-----------|-----------|
| Yavuz     | 50.30 c | 11.09 a | 18.09 a-b | 21.23 a-b | 58.39 a-b |
| Şebin     | 59.71 a | 10.25 a | 16.35 b   | 19.31 b   | 57.78 b   |
| Bilecik   | 56.05 b | 10.57 a | 17.36 a-b | 20.34 a-b | 58.56 a-b |
| Yerli     | 52.24 c | 11.35 a | 19.20 a   | 22.31 a   | 59.29 a   |
| HSD       | 2.74    | 1.25    | 2.10      | 2.40      | 1.29      |

Table 5. Fruit kernel colour traits of some important Turkish walnut genotypes in the Central Anatolian Region of Turkey in 2016 year

| Genotypes | L*      | a*        | b*        | C       | H       |
|-----------|---------|-----------|-----------|---------|---------|
| Yavuz     | 40.62 a | 10.99 c   | 21.86 b   | 24.53 b | 62.99 a |
| Şebin     | 39.33 a | 13.93 a   | 24.53 a-b | 28.35 a | 59.88 a |
| Bilecik   | 45.23 a | 12.06 b   | 25.95 a   | 28.68 a | 64.61 a |
| Yerli     | 44.14 a | 11.19 b-c | 25.23 a-b | 27.71 a | 64.33 a |
| HSD       | 7.80    | 0.99      | 3.49      | 2.75    | 6.35    |

Fruit skin L\* value had the lightness value of 59.71 for 'Şebin' (Table 4). Fruit skin a\* values indicating red color, was the highest for 'Yerli' (11.35), while it was the lowest for 'Şebin' (10.25). The maximum hue angle value (h<sub>o</sub>, the higher values are the clearer) was found from 'Yerli' (59.29). Chroma values (C, the lower values are more density) were found highest for 'Yerli' (22.31) genotype.

The kernel L\* value was the lightest for 'Bilecik' (45.23). The kernel a\* values indicating red colour, had the highest for 'Şebin 86' (13.93). C and *ho* values had the highest for 'Bilecik' (28.68 & 64.61, respectively) (Table 5).

In this study, chemical properties of walnuts were also determined. For chemical composition (Fat and protein content), Statistical differences among the genotypes were significant. Table 6 shows the highest protein quantity (20.08%) in Bilecik, the lowest value was found in Yavuz (17.11%). The protein values were in good agreement with the literature (Koyuncu and Askin, 1995; Bayazit and Sumbul, 2012).

Table 6. Fat and protein ratios of walnut genotypes

| Genotypes | Fat (%) | Protein (%) |
|-----------|---------|-------------|
| Yavuz     | 63.20 a | 17.11 b     |
| Şebin     | 60.40 a | 18.45 a-b   |
| Bilecik   | 53.90 b | 20.08 a     |
| HSD       | 5.46    | 2.48        |

Fat contents of the sample (%) were in the range of 53.90–63.20. Yavuz had the highest fat contents. Kahraman (2006) reported oil content in walnut genotypes as 51.70–72.80% and protein content was 15.61–27.50%, respectively.

### Conclusions

In Kırsehir province with climate characteristics, fruit quality features of four walnut genotypes were found high. Especially compared to other research results, the shell and kernel weight of 'Şebin' walnut cultivar was relatively high. In addition, per tree yield was higher in 'Şebin' walnut compared to other varieties. Kernel percentage is very important for quality parameters. The 'Yerli' walnut genotype, grown for many years in the region, is found to be large in fruits. But, the low kernel percentage is the disadvantage of this genotype.

In Turkey, especially, late spring frosts in central and eastern Anatolia regions can damage walnut plants. It was seen that late spring frosts were not effective in walnut varieties in this study.

In conclusion, 'Bilecik' and 'Şebin' genotypes would be choice for fruit quality traits in the Kırsehir province in Central Anatolia Region of Turkey.

### References

1. Akca, Y. and Ozongun, S. (2004). Selection of late leafing, late flowering, laterally fruitful walnut (*Juglans regia* L.) types in Turkey. New Zealand Journal. Crop Horticulture Science. 32, 337-342.
2. Akça and Aydın (2005);
3. Akca, Y. and Sen, S.M. (1995).. Relationship between and yield-nut characteristic in *Juglans regia*. Acta Hort. 442, 81-82
4. Akca, Y., Bilgen, Y. and Ercisli, S. (2015). Selection of promising persian walnut (*Juglans regia* L.) from seedling origin in Turkey. - Acta Scientiarum Polonorum Horticulture 14 (3): 103-114
5. Akcay, M.E. and Tosun, I. (2005). Walnut selection in III. sub-region (Gemlik, Orhangazi, Iznik and Mudanya ) of Bursa province. Bahce 34(1): 57-62.
6. Anonymous (2017). FAO Agricultural Statistical Database, available online at: <http://faostat.fao.org> (Accessed: 22 September 2017)..
7. Asma, B.M. (2012). Pomological and phenological characterization of promising walnut (*Juglans regia* L.) Genotypes from Malatya, Turkey. Acta Sci. Pol., Hortorum Cultus 11(4) 2012, 169-178
8. Askin, M. A. and Gun, A. (1995): Researchs by breeding of Cameli and Bozkurt walnuts (*Juglans regia* L.) research on breeding through selection of walnuts. - Turkey II. National Horticulture Congress, Adana, Tukey, pp. 461-463.
9. Akyuz, N. and Kaya, I. (1992). Food Chemistry Laboratory (in Turkish). Yuzuncu Yıl University, Faculty of Science, Publishing No: 2, Van, Turkey

10. Akkuzu, H.E. and M. Çelik. (2001). Bazı Ceviz Çeşitlerinin Ankara Koşullarında Fenolojik Ve Pomolojik Özelliklerinin Belirlenmesi. I. Ulusal Ceviz Semp., S:69-75, Tokat.
11. Anonymous. (1990). Unshelled walnuts. Turkish Standard Institute T.S.E., TS 1275, Ankara, Turkey.
12. Anonymous. (1991). Walnut kernels. Turkish Standard Institute T.S.E., TS 1276, Ankara, Turkey.
13. Anonymous, (2010). *In Shell Walnut (Juglans regia L.) (in Turkish)*. Turkish Standard Institute. T.S.E., Ankara, Turkey.
14. Anonymous, (2017). FAO statistical database, (Accessed on 10.9.2017). <http://apps.fao.org/page/collections?subset=agriculture>
15. Bayazit, S. and Sumbul, A. (2012). Determination of Fruit Quality and Fatty Acid Composition of Turkish Walnut (*Juglans regia*) Cultivars and Genotypes Grown in Subtropical Climate of Eastern Mediterranean Region. *Int. J. Agric. Biol.*, Vol. 14, No. 3, 419-424.
16. Bayazit, S., Tefek, H. and Caliskan, O. (2016). Walnut (*Juglans regia* L.) Researches in Turkey. Süleyman Demirel University Journal of the Agricultural faculty 11 (1):169-179,
17. Baymış, M. (2008). Assessing of some local and foreign walnut type and warieties (*juglans regia* L.) performances in the Kahramanmaraş ecological conditions. MSc Thesis, university of Kahramanmaraş Sütçüimam, Institute of Natural and Applied Sciences, Department of HorticultureKahramanmaras, 103p.
18. Celebioglu, G., Ferhatoglu, Y. and Burak, N. (1988). Selection and plantation walnuts in Turkey. International Conference on Walnuts, Atatürk Central Horticultural Research Institute, Yalova, pp 83-87.
19. Ferhatoglu, Y. (1993). The characteristics of walnut cultivars obtained through selection. International Walnut Meeting, IRTA – Generalitat de Catalunya Tarragona, Spain, pp 34-36.
20. Jung, S., Rickert, D.A., Deak, N.A., Aldin, E.D., Recknor, J., Johnson, L.A. and Murphy, P. A. (2003). Kjeldahl and dumas methods for determining crude protein contents of soybean products. - *Journal of the American Oil Chemists' Society* 80 (12): 1169-1173.
21. Kahraman, K.A. (2006). The selection of walnuts in Aksaray province. MS Thesis. Selcuk University
22. Keles, H., Akça, Y. and Ercisli, S. (2014). Selection of promising walnut genotypes (*Juglans regia* L.) from inner Anatolia. *Acta Sci. Pol., Hortorum Cultus* 13(3), 167-175
23. Koyuncu, M.A. and Askin, M.A. (1995). A study on determination of main composition of promising walnut genotypes selected from Adilcevaz (Bitlis). *Türkiye II. Ulusal Bahçe Bitkileri Kongresi*, Adana, pp 475-478.
24. Koyuncu, M.A. and Askin, M.A. (1999). A Study on the storage of some walnut trees cultivated in Van lake area. *Turk. J. Agric. Forest.* 23, 785-796.
25. McGranahan, G. and Leslie, C. (1991). Walnuts (*Juglans*). In J. N. Moore and J. R. Ballington Jr.(eds.), *Genetic Resources of Temperate Fruit and Nut Crops*, Vol. 2, 907–951. International Society of Horticultural Sciences, Wageningen, The Netherlands
26. Ölez, H. (1971). Studies on the selection of walnut (*Juglans regia* L.) in the Marmara region. *Bahçe* 4, 7–21 (in Turkish).
27. Sen, S.M. (1986). Walnut growing. Eser Pres (in Turkish),. Samsun, Turkey.
28. Sen, S.M. (1983). Kuzeydogu Anadolu ve Dogu Karadeniz Bolgesi Cevizlerinin Seleksiyon Yoluyla Islahi Uzerinde Arastirmalar. *Doga Bilim Dergisi* 7, 163-170.
29. Sen, S.M. and Beyhan, Ö. (1993). A study on the selection of Darende Walnut. *Acta Horticulturae*. 311, 57-60.
30. Steele, R. and Torrie, J. (1980). *Principles and procedures of statistics*. NewYork: McGraw-Hill.
31. Tosun, I. and Akçay, M.E. (2005). Phenological and pomological properties of some walnut Varieties in yalova ecological conditions *BAHÇE Ceviz* 34 (1): 35 – 39
32. Zhadan, V. M. and Strukov, M. V. (1977). Breeding walnut for fruit size. *Plant Breeding Abstract* 47(11): 918.

**PERFORMANCE OF MONDIAL GALA APPLE CULTIVARS GRAFTED ON M9 APPLE  
ROOTSTOCK IN THE MEDITERRANEAN REGION OF TURKEY**

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**Abstract**

This research was carried out on eight-year-old trees of the Mondial Gala (*Malus domestica* Borkh) cultivars grafted on the M9 rootstock in order to determine its performance in the Mediterranean region with subtropical climatic conditions in Mersin, Turkey. In the study, flowering times, yield and fruit quality parameters were investigated. Mondial Gala planted with planting spaces of 1x3 m. According to the results, first flowering was observed on April 1, full flowering on April 10 and end of flowering on April 17, and fruits were harvested at the end of July. The fruit weight (average 223.2 g), the yield per tree (average 22.6 kg), and yield per trunk cross-sectional area was observed as 0.56 kg/cm<sup>2</sup> at the end of the eighth year. Observed values indicated that Mondial Gala summer apple cultivars were found to be promising for early apple growing in the higher parts of subtropical ecological conditions of Turkey.

**Keywords:** Apple cultivar, phenology, pomology, yield, quality.

**Introduction**

Among pome fruit species, apple is the oldest and most cultivated species. Due to the high level of adaptability and the high number of varieties, apple is the most produced and consumed species among fruit species in temperate climates. *Malus* genus in *Rosaceae* family has more than 30 apple species that it grows in different countries (Özbek, 1978; Way et al. 1990; Deveci 2000).

Wilcox (1962) reported that the origins of apple were Anatolia, Caucasia and Central Asia. Like many fruit species, Turkey is also among the gene centre of apple species (Özbek, 1978; Akgül et al. 2011). The fact that a considerable part of the Asian continent is a gene centre for some species of apple and the presence of various species, subspecies and forms in which it has influenced the spread of apple cultivation in this continent. Europe-wide apple cultivation extends to the southern parts of the northern Scandinavian peninsula. Although, it is prevalent up to the 35<sup>th</sup> latitude in the southern Europe, apple is economically grown at the 58<sup>th</sup> northern latitude in Denmark and 60<sup>th</sup> northern latitude in Sweden. The apple is grown in high places to the south of this latitude. In Africa, Morocco and the Republic of South Africa gained importance in apple cultivation. In addition, the high parts of Central America, New Zealand and Australia have become major apple production centres (Özçağiran et al. 2004).

Apple is one of the most produced and consumed temperate climate fruit species in the world. According to FAO's 2014 data, world apple production was 84.6 million tons. China has a production volume of 41 million tons, accounting for 48.5% of world production. It was followed by the USA (5.2 million tons, 6.1%), Poland (3.2 million tons, 3.8%), Turkey (2.9 million tons, 3.4%) and India (2.5 million tons, 2.9%), respectively (FAO 2017).

Because of its geographical position and ecological conditions, Turkey has a very important place for the cultivation of all fruit species apart from tropical fruit species. In this respect, Turkey is the gene centre of the fruit cultivars and the gene centre of many cultivated species in the world (Ağaoğlu et al. 1997). In Anatolian geography, there is a great variety of fruit richness of pome fruits like apples, pears and quince (Gündüz, 1997; Kaşka, 1997). It is stated that apple variety is around 6.500 in the world and 460 in Turkey (Özbek, 1978). The number of apple varieties in Turkey has increased in recent years.

Apple was produced almost everywhere of Turkey. Amasya, Starking Delicious and Golden Delicious are largely produced apple cultivars in temperate climate regions of Turkey. But, trade value of these cultivars in international market is very low. In newly planted apple orchards, new cultivars are harvested from July to October. By increasing production of mid-season apple cultivars, Mondial Gala and Galaxy Gala would supply more profit and also prevent the accumulation of crops in the late season.

In the present study, phenological and pomological features of 'Mondial Gala' apple cultivar were evaluated at high altitudes of Mersin with subtropical climates of Turkey.

### **Material and methods**

The experiment was conducted in the orchard of private sector at Erdemli/Mersin (Latitude 36 35 27 N; 34 60 39 E; Elevation 750 m) in 2016. Regular agricultural practices were applied in orchards.

The experiment was conducted on eight-year-old 'Mondial Gala' apple cultivar grafted on a dwarf rootstock (M9). Trees were planted in 2008 at a distance 3×1 for M9 rootstocks.

All fruit samples were taken during the maturity stage which were visually determined and harvesting date of the cultivar also was determined. On the experimental trees, the number of fruits was counted, yielded (harvested and weighted per tree in kilograms) and weighted from 30 trees. Trunk diameter was measured at a height of 10 cm and was converted to Trunk Cross Sectional Area (TCSA/cm<sup>2</sup>). Some phenological properties of the cultivar were evaluated by determining in the beginning of flowering, full bloom, end of flowering, duration of flowering, harvest date, number of days from full bloom to harvesting time. Flowering was investigated by the recommendations of the International working group for pollination (Wertheim, 1996). The date of beginning of flowering was taken when 10% of flowers were opened, full when 80% of flowers were opened, end when 90% of petals were fallen. Duration of flowering was determined by the number of days from the beginning to the end of the flowering. The harvesting date is accepted as the time of maturing.

In this study, the pomological methods has been described by Bozbuga and Pırlak (2012). Fruit weight (g) was measured with a sensitive (0.01 g) scale (Precisa XB 2200 C). Fruit length (mm) and diameter (mm) were measured by a digital caliper (Mitutoyo, 0–150 mm). Flesh firmness was tested on two sides of each fruit by an Effegi penetrometer with a 11 mm plunger after removal of the peel and then it was measured as libre.

Total soluble solids (TSS) content were determined with a hand-held refractometer (NOW, 0–32% Brix) and pH (WTW InoLab pH meter) measurements were performed using a pH meter. Acidity (expressed as malic acid %) was determined by titrating with 0.1 N NaOH up to pH 8.10.

Skin colour was measured on opposite sides of the fruit using a Minolta chromameter (model CR-300; Minolta Camera Co., Osaka, Japan), which provided CIE L\* a\* b\* values.

### **Results and discussion**

The beginning of flowering date, the full flowering date and the end of flowering were observed 1 April, 10 April and 17 April, respectively. It is known that the flowering date and period varies depending on cultivars, altitude as well as ecological and cultural conditions.

Kulina et al (2013) stated that beginning of flowering date, the full flowering date and the end of flowering in Mondial Gala/M9, were observed as 24 April, 29 April, 07 May, respectively in Sarajevo region of Bosnia and Herzegovina. İkinci and Bolat (2013) reported that the first flowering of the Mondial Gala apple variety in Sanlıurfa ecology was carried out on 27 March, the full flowering was on 2 April and end of flowering was on 9 April. The average duration of flowering was 16 days. Comparing our results with the results of Kulina et al (2013), time of flowering of Mondial Gala cultivar occurred much earlier in agro–environmental conditions of Erdemli/Mersin. The flowering has occurred relatively later in environmental conditions of Sarajevo because of climatic conditions and later start of growing season, but the process and duration of flowering was in accordance with the characteristics of the examined clone.

The time of maturation of the studied apple cultivar was from 25 of July. Time of maturation depends, primarily, on genetic characteristics and environmental conditions, which may influence earlier or later ripening. As a matter of fact, İkinci and Bolat (2013) reported that Mondial Gala apple cultivar matured 09 August in Şanlıurfa ecological conditions.

At the trial, the trunk cross-sectional area values were established between 23.06 cm<sup>2</sup> and 65.29 cm<sup>2</sup>, mean value was obtained as 40.24 cm<sup>2</sup>. Considering the yield amounts (yield effect) per trunk cross-sectional area unit, the highest value was obtained 0.86 kg/cm<sup>2</sup>, and the lowest value was obtained 0.39 kg/cm<sup>2</sup>, the mean value was 0.56 cm<sup>2</sup>. The number of fruits per tree varied from 92 to 118, with an average of 105. The yield was varied between 6.6 ton/da and 8.4 ton/da, with an average yield of 7.5 ton/da (Table 1).

Table 1. Yield parameters of Mondial Gala apple cultivars budded on M9 rootstock

|         | Trunk cross-sectional area (cm <sup>2</sup> ) | Number of fruits/ tree | Yield (kg tree <sup>-1</sup> ) | kg/cm <sup>2</sup> | Yield (ton da <sup>-1</sup> ) |
|---------|-----------------------------------------------|------------------------|--------------------------------|--------------------|-------------------------------|
| Minimum | 23.06                                         | 92                     | 19.76                          | 0.39               | 6.6                           |
| Maximum | 65.29                                         | 118                    | 25.35                          | 0.86               | 8.4                           |
| Average | 40.24                                         | 105                    | 22.56                          | 0.56               | 7.5                           |

Ten-year-old Mondial Gala varieties grafted on M9 and MM 106 rootstocks under Egirdir conditions, the trunk cross-sectional area between 67.75 cm<sup>2</sup> and 109.12 cm<sup>2</sup>, the yield per tree between 37.44 and 95.5 kg and the yield value per trunk cross-sectional area between 5.25 and 3.25 kg/cm<sup>2</sup> were obtained (Ozongun et al. 2016). In the same study, the tenth-year cumulative yield values of Mondial Gala/M9 and Mondial Gala/MM106 rootstock/variety combinations ranged from 35.17 tons/da to 31.88 tons/da.

The results of fruit physical characteristics of 'Mondial Gala' apple cultivar were given in Table 2. The fruit weight is one of the most important pomological characteristics because it affects a number of other properties, primarily yield. In our study, the minimum and maximum fruit weights were found as 178.7 g and 267.7 g, respectively. The mean fruit weight was 223.2 g. While the minimum fruit width was 72.04 mm, the maximum fruit width was 83.27 mm (Table 2).

Table 2. Fruit properties of Mondial Gala apple cultivar

|         | Fruit weight (g) | Fruit width (mm) | Fruit length (mm) | Flesh firmness (kg/cm <sup>2</sup> ) | TTS (%) | Acidity (%) | pH   | Number of seed |
|---------|------------------|------------------|-------------------|--------------------------------------|---------|-------------|------|----------------|
| Minimum | 178.7            | 72.04            | 59.83             | 1.08                                 | 10.8    | 0.77        | 3.97 | 2              |
| Maximum | 267.7            | 83.27            | 80.91             | 1.5                                  | 12.6    | 1.09        | 4.18 | 10             |
| Average | 223.2            | 77.66            | 70.37             | 1.29                                 | 11.7    | 0.93        | 4.08 | 6              |

Bozbuga and Pırlak (2012) observed average fruit weight, fruit diameter, fruit length, TTS, and seed number in Mondial Gala grafted on M9 was 152 g, 72.2 mm, 60.1 mm, 12.5% and 6.9 respectively in Nigde province. In another study, Baytekin (2006), fruit weights were obtained as 280.18g in Gala/M9 in Tokat province. Atay (2007) determined average fruit weight as 150.98 g in Galaxy Gala cultivar. Kulina et al. (2013) conducted a study in Sarajevo province and observed average fruit weight, fruit diameter and fruit length in Mondial Gala grafted on M9 as 168.36 g, 75.8 mm and 67.9 mm, respectively.

The weight, width, length, flesh firmness, soluble solids content, juice pH and titratable acidity (TA) levels of the Mondial Gala apple variety grafted on M9 under Egirdir conditions were between 147 g, 69.04 mm, 61.73 mm, 9.25 kg/cm<sup>2</sup>, 13.03%, 3.70 and 0.30%, respectively (Ozongun et al. 2016).

Our results share some similarities with some studies, but partly different from others. The difference may be resulted due to cultural practices, ecological conditions as well as variations in fruit formation.

The L value varied between 38.48 and 61.38 and the L value average was 54.70. The fruit skin a\* value, which indicates red colour, changed between -3,95 (negative values are green colour) and 28.09. Its average value in Mersin province ecological conditions was 16 (Table 3).

Table 3. Fruit skin color of Mondial Gala apple cultivar

|         | L     | a     | b     |
|---------|-------|-------|-------|
| Minimum | 38,48 | -3,95 | 19,45 |
| Maximum | 61,38 | 28,09 | 33,25 |
| Average | 54,70 | 12,07 | 26,35 |

### Conclusions

Turkey being the original source of apple has sites suitable for apple production; however, studies in Subtropical conditions of Turkey, particularly in the Mersin province, is not sufficient. In recent years, apple varieties that have matured in the mid-season have begun to be grown in the higher parts of subtropical regions of Turkey. Our preliminary study showed that ‘Mondial Gala’ cultivar, when grafted onto M9 rootstock, have high economic potential in the higher parts of Subtropical regions. Mid-season maturation of these cultivars increases the economic value of fruits in the market. Despite the subtropical climatic conditions of Mersin district, ‘Mondial Gala’ can be evaluated as a high yield producing cultivar.

### References

1. Ağaoğlu, Y.S., H. Çelik, M. Çelik, Y. Fidan, Y. Gülşen, A. Günay, N. Halloran, I. Köksal and R. Yanmaz, (1995). Common Horticultural Plants, Vol. 4, p: 369. Ankara University, Faculty of Agriculture, Education, Research and Development Foundation Publication
2. Akgül, H., Kaçal, E., Öztürk, F.P., Özongun, Ş., Atasay, A., Öztürk, G., (2011). Apple Culture. Adım Ofset, Konya, (in Turkish), 510p.
3. Atay, E. (2007). Determination of fruit growth and development some apple varieties on MM 106 rootstock. M.Sc. thesis (Unpublished). Dept. of Horticulture, Fac. of Agri., Selçuk Univ., Konya, Turkey.
4. Baytekin, S. (2006). Performance of some apple cultivars on different clonal rootstocks in ecology of Turhal district of Tokat province. M.Sc. thesis (unpublished). Dept. of Horticulture, Fac. of Agri., Gaziosmanpaşa Univ., Tokat, Turkey.
5. Bozbuğa F., Pırlak L. (2012): Determination of phenological and pomological characteristics of some apple cultivars in Niğde-Turkey ecological conditions. Journal of Animal & Plant Sciences, 22(1): 183-187.
6. Deveci, L., (2000). Apple growing, Apple's History and Gene Centre, (in Turkish). p; 7-8.
7. FAO, (2017). Agricultural Statistical Database, available online at: <http://faostat.fao.org> (Accessed: 22 September 2017).
8. Gündüz, M., (1997). World trades of pome fruits and evaluation from point of view of Turkey. Pome Fruit Symposium. Edt. M.Büyükyılmaz, M. Burak. Ataturk Horticultural Central Research Institute, Yalova, 295-304.
9. İkinci, A and Bolat, I. (2013). Determination of phenological, pomological and yield characteristics of low chilling apple cultivars budded on M9 and MM 106 rootstocks. IV International Symposium “Agrosym 2013”; 627-636.
10. Kaşka, N. (1997). Importance of apple growing constraints and solution in Turkey Pome Fruit Symposium. Edt. M.Büyükyılmaz, M. Burak. Ataturk Horticultural Central Research Institute, Yalova, 1-12.
11. Kulina, M., Gacesa, B., Stojanovic, M., Alic-Dzanovic, Z. (2013). Pomological Properties of “Gala” apple clones in the region of Sarajevo. IV International Symposium “Agrosym 2013” 257-262.
12. Ozbek, S. (1978). Special Horticulture. Cukurova University Faculty of Agriculture publications number 128 Ankara University Press Ankara (in Turkish). 488 p.

13. Ozcagiran R., Unalan A., Ozek E., İsfendiyoğlu M. (2005). Apple. Temperate fruit species, Pome Fruits. Volume II., Ege University Faculty of Agriculture, Publication No: 556, Bornova, Izmir, pp. 3-72.
14. Ozongun, Ş., Dolunay, E M., Pektaş, M., Öztürk, G., Çalhan, Ö., Atay, E. (2016). Yield and Quality Alterations of Some Apple Cultivars on Different Rootstock Ege Üniv. Journal of Agric. Faculty. 53 (1):35-42
15. Way, R. D., Aldwinckle, H. S., Lamb, R. C., Rejman, A., Sansavini, S., Shen, T., Watkins, R., Westwood, M.N., Toshida, Y. (1990). Apples (*Malus*). In: Genetic resources of temperate fruits and nut crops I. Netherlands, ISHS. 488 p.
16. Wertheim, S.J. (1996). Methods for cross pollination and flowering assessment and their interpretation. Acta Horticulturae 423: 237-241.

## PHYSICAL AND CHEMICAL PROPERTIES OF SOIL IN FRUIT GROWING AREA TOPOLA (SERBIA)

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### Abstract

The aim of the investigation was to assess the level of soil fertility and repair measures in order to enhance the productivity of fruit production in a traditional fruit growing area. (Municipality of Topola, Serbia). The following tests were performed: mechanical composition, physical and chemical properties of soil, total adsorbed base cation, capacity of adsorbed base cation, degree of base saturation, different types of soil acidity, contents of carbon, humus, total nitrogen, easily accessible forms of  $P_2O_5$  and  $K_2O$ . Results obtained have shown that soils from all studied sites are of “heavy” mechanical texture with 75,40-84,90% of physical clay. High capacity of cation adsorption, low to medium hydrolytic acidity and high to almost complete saturation of adsorbed bases, has been measured. In compliance with previous analyses, it has been assessed that soils are neutral and of low acid chemical reaction, with low carbon and medium humus content and total nitrogen. Easily accessible content of  $P_2O_5$  and  $K_2O$  varies depending on the food quantity on individual parcels with low to medium accessibility to parcels with no fruit plantations. Based on the parameters analysed, the tested soils demand repair of mechanical properties, which will facilitate greater nutrient uptake.

**Keywords:** soil fertility, mechanical composition, nutrients,  $P_2O_5$ ,  $K_2O$ .

### Introduction

Municipality of Topola in natural and geographical terms has a good basis for the development of fruit and grape growing. According to the data from Popis poljoprivrede 2012, among the largest areas under plantations is the region of Topola (Keserović, 2012). Orchards are extending to 4,623 ha or 17% of arable land, whereas vineyards on 1.056 ha (4%). Favorable agro-ecological environmental conditions of the hilly landscape of Šumadija contribute to the further development of fruit growing. A downside arising comes from substantial percentage of very clayey soil in certain regions of production, with a an extremely large share of clay

There are over 0.4 million ha of soils with heavy textured mechanical composition and over 0.1 million ha of variously degraded soils. Every year, with different degradation processes, 1,000 new hectares of land is subject to degradation. Excessive or irresponsible use of land leads to productivity fall and ultimately its destruction. As soil represents a complex and dynamic system, changes of its biological, chemical and physical properties occur under the influence of agricultural production (Milivojevic et al. 2012)

Monitoring of soil is carried out by quality and soil fertility control. Intensive agricultural production gradually leads to the depletion and degradation of soil whereby climatic factors have big impact on its fertility. There comes to degradation of its chemical properties such as the loss of nutrients, soil pollution, acidification, salinization and other physical (compaction, deterioration of the structure, formation of crust, etc.) and biological properties (disorder in number and relationship of certain groups of microorganisms, including biological and microbiological activity of soil) (Manojlovic, 2008). The quality of soil with all biological, chemical and physical properties varies in dependence of agro-technical measures applied such as tillage, crop sequence and management of crop residues

(Fuentes et al., 2009). Different agro-technical measures often have significant impact on soil properties resulting in a change of the soil quality (Islam and Weil 2000).

The aim of the research is to determine agro-physical, physical and chemical properties of the soil in fruit growing area of the Municipality of Topola and the repair measures.

### Material and methods

The land was sampled in the fall of 2016, in a rural area of the Municipality of Topola. The samples were collected on parcels that are not under orchards (meadows, plough fields, stubble field, fallow land, fodder) and parcels under different types of fruit species, of two depths: 0-30 cm, 30-60 cm. Sampled parcels were marked with GPS coordinates with number of samples in village areas, determined on the basis of arable land and agricultural production, Popis poljoprivrede (2012).

Agro-mechanical, physical and chemical analyses included examination of: mechanical aggregate composition of soil by sieving and sedimentation of different mechanical fractions JDPZ (1997), determination of the sum of exchangeable adsorbed alkaline cations ( $S \text{ meq } 100\text{g}^{-1}$ ) (method Kappen-a), determination of hydrolytic soil acidity ( $H \text{ meq } 100\text{g}^{-1}$ ), cation exchange capacity ( $T \text{ meq } 100\text{g}^{-1}$ ), the level of saturation with adsorbed bases ( $V\%$ ).

Agro-chemical characteristics of soil are determined by the following methods: pH of the  $\text{H}_2\text{O}$  and 1 MKCl-in (potentiometrically); humus (by the method of Kotzman); total nitrogen (method according to Kjeldahl); readily accessible phosphorus and potassium (AL method,  $\text{P}_2\text{O}_5$  - colourimetrically,  $\text{K}_2\text{O}$  - light photometrically).

### Results and discussion

The results of mechanical and physical composition of the soil chemical analyses are shown in Table 1 and 2 in the area of 4 settlements with the largest percentage of fruit production.

Mechanical composition of all analysed soils is, by texture class, heavy clay. Portion of physical clay fractions is 75.40-84.90%, whereas the share of clay fraction 34.00-52.10%. Content of physical sand fractions is 15.10-24.60% and the share of coarse sand 0.00-0.66%.

Table 1. Mechanical composition of soil, Municipality of Topola

| Table 2. Mechanical composition of soil, Municipality of Topola |       |                                     |          |            |        |       |       |            |
|-----------------------------------------------------------------|-------|-------------------------------------|----------|------------|--------|-------|-------|------------|
| Settlements                                                     | Depth | Content of mechanical fractions (%) |          |            |        |       |       | Soil class |
|                                                                 |       | 2-0,2                               | 0,2-0,02 | 0,02-0,002 | <0,002 | >0,02 | <0,02 |            |
| mm                                                              |       |                                     |          |            |        |       |       |            |
| Šume                                                            | 0-30  | 0.66                                | 21.84    | 29.20      | 48.30  | 22.50 | 77.50 | Heavy clay |
|                                                                 | 30-60 | 0.02                                | 22.28    | 24.60      | 52.10  | 23.30 | 76.70 | Heavy clay |
| Belosavci                                                       | 0-30  | 0.02                                | 24.58    | 41.40      | 34.00  | 24.60 | 75.40 | Heavy clay |
|                                                                 | 30-60 | 0.04                                | 20.76    | 40.90      | 38.30  | 20.80 | 79.20 | Heavy clay |
| Vinča                                                           | 0-30  | 0.00                                | 15.10    | 35.70      | 49.20  | 15.10 | 84.90 | Heavy clay |
|                                                                 | 30-60 | 0.02                                | 18.48    | 32.60      | 48.90  | 18.50 | 81.50 | Heavy clay |
| Blaznava                                                        | 0-30  | 0.00                                | 20.20    | 29.70      | 50.10  | 20.20 | 79.80 | Heavy clay |
|                                                                 | 30-60 | 0.00                                | 19.30    | 28.90      | 51.80  | 19.30 | 80.70 | Heavy clay |

Results in Table 2. indicate that soils have a high cation exchange capacity, low to high hydrolytic acidity and high to almost full saturation with adsorbed bases. Soils at the site Vinča have values of hydrolytic acidity 4.26-5.79 meq  $100\text{g}^{-1}$  and a high level of saturation with adsorbed bases. Similar

research results are also found at the site Šume. Soils in the villages of Belosavci and Blaznava have the most suitable physical and chemical properties.

Table 2. Physical and chemical characteristics of soil, Municipality of Topola

| Settlement | Depth | S                      | H=T-S | T     | V     |
|------------|-------|------------------------|-------|-------|-------|
|            |       | meq 100g <sup>-1</sup> |       |       | %     |
| Šume       | 0-30  | 28.24                  | 4.11  | 32.35 | 87.29 |
|            | 30-60 | 29.38                  | 6.13  | 35.51 | 82.74 |
| Belosavci  | 0-30  | 24.81                  | 3.48  | 28.29 | 87.69 |
|            | 30-60 | 21.06                  | 3.95  | 25.01 | 84.21 |
| Vinča      | 0-30  | 31.83                  | 5.79  | 37.62 | 84.61 |
|            | 30-60 | 29.66                  | 4.26  | 33.92 | 87.44 |
| Blaznava   | 0-30  | 34.05                  | 1.38  | 35.43 | 96.10 |
|            | 30-60 | 35.23                  | 1.42  | 36.65 | 96.13 |

The minimum and maximum values of the soil fertility basic analysis results in the area of 24 settlements in the Municipality of Topola are shown in Table 3. A review of the lowest and highest values of parameters tested within a settlement show the quality of soil and availability of certain nutrients.

Substitutional soil acidity (pH/KCl) in certain areas of the Municipality of Topola is in the class of acid soils (Junkovac, Rajkovac, Mascar, Belosavci, Donja Trešnjevica). At other sites sampled, soils are in the class of low acid (pH / KCl 5.5-6.5) to slightly alkaline (pH / KCl 6.5-7.2). There are no changes within the soil reaction interval with changing of sampling depth.

Active soil acidity on most sites is neutral to slightly alkaline, and in the area of settlements Žabari, Vojkovci, Topola selo, Božurnja, Ovsišće, Vinča, Plaskovac, Donja Šatornja i Blaznava, low to medium alkaline. According to the carbonate content, low carbonate soils are present (0-2% CaCO<sub>3</sub>) at a number of sites. A very high content of carbonate, increasing with the sampling depth to > 25% CaCO<sub>3</sub> is found in the villages of Topola, Božurnja and Ovsišća, whereas other sites are carbonate-free or medium carbonated.

The presence of humus in soil depends on the cadastral culture grown on the sampled soil. Humus horizon (0-30 cm) is medium supplied with humus, and in some localities, results show its high supply. With depth of sampling, the content of humus decreases and the supply is usually low (1-3% humus). The values of humus content in the soils under plantations of different fruit species are, in the humus horizon 71.4% of the samples is humus and 22.9% is low humus. In sub-humus horizon, 80.0% is low humus whereas 20.0% are humus samples.

The presence of total nitrogen corresponds to the content of humus, with mean values prevailing. The results show that half of the land of the total number of tested sites are with very low to low supply of accessible phosphorus (<10 mg 100<sup>-1</sup> g). A good supply of accessible phosphorus is measured at sites with intensive nutrition of fruit plantations and is related to individual sites (of nutritional food) without correlation with other parameters tested.

Soils of the Municipality of Topola are medium to highly supplied with accessible potassium. These research parameters show that the most common area of fruit production in Topola is on soils of smonitsa (vertisol). Soils of smonitsa are potentially fertile, due to deep humus horizon though unfavorable physical and water-physical properties (Miljković, 1996). Sanding, entering of sand is one of physical methods of soil repair. What is more, it is desirable that the sand is the smallest possible so it could be mixed and glued with clay and dust more efficiently (Miljkovic, 2005). (Miljković, 2005).

Degree of evolution, substrate groups and depth of humus horizon are the criterion for the classification of smonitsa into subtypes, varieties and forms (Škorić, 1986). Soils of this type are of heavy texture, due to the large proportion of clay (Gajić and Zivkovic 2002), with only 5% of air. Chemical properties are more favorable. Adsorption capacity is 50 or more ekv. mmol / 100 g of soil, and the base saturation is also high, approx. 90%.

Table 3. Minimum and maximum values of agro-technical analyses by depth of the sampled soil, Municipality of Topola

| Settlement        | Depths | pH        |                  | CaCO <sub>3</sub> | Humus     | N         | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O      |
|-------------------|--------|-----------|------------------|-------------------|-----------|-----------|-------------------------------|-----------------------|
|                   |        | KCl       | H <sub>2</sub> O | %                 | %         | %         | mg 100g <sup>-1</sup>         | mg 100g <sup>-1</sup> |
| Žabari            | 0-30   | 6,63-6,70 | 7,53-7,55        | 2,41              | 3,03-3,74 | 0,15-0,19 | 14,78-26,47                   | 26,82-29,02           |
|                   | 30-60  | 6,65-6,75 | 7,66-7,88        | 2,41              | 2,45-2,95 | 0,12-0,19 | 8,37-12,23                    | 19,97-20,25           |
| Junkovac          | 0-30   | 5,28-5,32 | 6,13-6,27        | 0,00              | 3,80-4,66 | 0,19-0,23 | 4,23-13,75                    | 25,18-26,77           |
|                   | 30-60  | 4,59-5,47 | 6,20-6,61        | 0,00              | 2,33-3,04 | 0,12-0,15 | 4,18-9,58                     | 21,22-25,38           |
| Šume              | 0-30   | 5,94-6,20 | 6,81-7,16        | 0,99-1,53         | 3,54-3,65 | 0,18      | 7,56-11,77                    | >30                   |
|                   | 30-60  | 5,66-6,15 | 6,49-7,29        | 0,85-1,27         | 1,47-2,77 | 0,08-0,14 | 2,43-9,34                     | 26,78->30             |
| Gornja Trnava     | 0-30   | 6,07-6,63 | 7,12-7,51        | 1,13-1,81         | 3,09-5,67 | 0,10-0,28 | 4,62-14,02                    | 20,23->30             |
|                   | 30-60  | 6,13-6,26 | 7,28-7,37        | 0,97-1,53         | 1,56-2,74 | 0,08-0,14 | 2,57-5,36                     | 18,12->30             |
| Svetlić           | 0-30   | 6,04-6,11 | 7,14-7,15        | 0,99-1,81         | 2,56-4,24 | 0,13-0,21 | 2,85-10,37                    | 25,52->30             |
|                   | 30-60  | 5,60-5,81 | 6,97-7,05        | 0,70-1,41         | 2,09-3,65 | 0,10-0,18 | 1,55-3,82                     | 22,07-24,58           |
| Donja Trnava      | 0-30   | 5,87-6,49 | 6,86-7,46        | 1,13-1,41         | 2,12-4,39 | 0,10-0,22 | 3,43-8,52                     | 18,93-32,24           |
|                   | 30-60  | 5,90-5,99 | 6,93-7,53        | 0,70-1,69         | 1,39-2,74 | 0,07-0,14 | 1,61-2,43                     | 15,30-20,96           |
| Natalinci         | 0-30   | 6,12-7,19 | 6,94-8,05        | 1,69-3,24         | 3,33-3,57 | 0,17-0,18 | 4,88-18,17                    | 17,41-24,82           |
|                   | 30-60  | 5,93-6,38 | 6,88-7,26        | 1,11-1,97         | 2,45-3,01 | 0,12-0,15 | 3,43-21,77                    | 18,39-18,78           |
| Kloka             | 0-30   | 5,74-5,85 | 6,59-6,93        | 0,99-1,41         | 3,51-3,57 | 0,17-0,18 | 6,59-9,57                     | 21,46-28,75           |
|                   | 30-60  | 5,48-5,80 | 6,55-6,74        | 0,00-1,11         | 2,06-2,39 | 0,10-0,12 | 3,88-5,06                     | 13,62-22,06           |
| Rajkovac          | 0-30   | 5,19-5,75 | 6,07-6,53        | 0,00-1,25         | 3,27-4,30 | 0,16-0,21 | 11,77-14,18                   | 25,45->30             |
|                   | 30-60  | 5,14-5,80 | 5,99-6,49        | 0,00-1,25         | 2,21-2,86 | 0,11-0,14 | 8,38-9,17                     | 22,37->30             |
| Maskar            | 0-30   | 5,34-5,49 | 6,27-6,53        | 0,00              | 2,59-3,06 | 0,13-0,15 | 2,57-7,83                     | 15,52-19,56           |
|                   | 30-60  | 5,36-5,40 | 6,25-6,35        | 0,00              | 2,27-2,36 | 0,11-0,12 | 2,18-7,86                     | 14,43-20,98           |
| Jelenac           | 0-30   | 5,48-5,97 | 6,78-7,02        | 0,00-0,83         | 2,50-2,95 | 0,13-0,15 | 13,65-17,42                   | 25,63-27,16           |
|                   | 30-60  | 5,23-5,62 | 6,68-6,84        | 0,070             | 1,62-2,18 | 0,08-0,11 | 6,08-16,58                    | 20,17-20,18           |
| Belosavci         | 0-30   | 5,22-6,01 | 6,54-6,95        | 0,00-1,41         | 3,45-4,69 | 0,17-0,23 | 5,49-8,22                     | 27,64-27,76           |
|                   | 30-60  | 5,41-6,17 | 6,54-7,20        | 0,00-1,83         | 2,53-3,42 | 0,13-0,17 | 3,97-4,17                     | 17,05-25,38           |
| Krčevac           | 0-30   | 6,30-6,52 | 7,17-7,30        | 0,83-2,11         | 4,30-4,34 | 0,21-0,23 | >30                           | >30                   |
|                   | 30-60  | 6,44-6,67 | 7,28-7,47        | 0,99-2,54         | 3,51-3,74 | 0,18-0,19 | >30                           | >30                   |
| Donja Trešnjevica | 0-30   | 4,48-5,09 | 5,81-7,09        | 0,00              | 2,83-3,30 | 0,14-0,16 | 2,65-4,07                     | 16,37-17,46           |
|                   | 30-60  | 4,37-4,74 | 5,81-6,10        | 0,00              | 2,09-4,51 | 0,10-0,22 | 1,68-1,83                     | 14,48-16,06           |
| Gornja Šatornja   | 0-30   | 5,89-6,37 | 6,67-7,36        | 1,25-1,41         | 3,95-4,51 | 0,20-0,22 | 1,52-24,78                    | 8,02-14,78            |
|                   | 30-60  | 6,11-6,31 | 6,97-7,27        | 0,99-1,83         | 2,53-3,03 | 0,13-0,15 | 1,00-12,37                    | 7,43-17,49            |
| Vojkovci          | 0-30   | 6,95-7,01 | 7,54-7,81        | 5,64-7,33         | 3,09-3,18 | 0,15-0,20 | 6,35->30                      | 12,54-13,97           |
|                   | 30-60  | 6,86-7,17 | 7,39-7,97        | 5,28-7,75         | 2,01-3,51 | 0,10-0,17 | 1,78-6,02                     | 8,62-18,37            |
| Topola selo       | 0-30   | 6,42-7,23 | 7,39-8,15        | 1,11-14,24        | 2,89-5,66 | 0,14-0,28 | 2,97-22,57                    | 19,18->30             |
|                   | 30-60  | 6,02-7,34 | 7,21-8,28        | 0,83->25          | 1,30-2,68 | 0,06-0,14 | 1,48-15,42                    | 11,35->30             |
| Lipovac           | 0-30   | 4,53-5,98 | 5,64-7,02        | 0,00-1,27         | 2,86-4,45 | 0,14-0,22 | 1,68->30                      | 21,58-28,77           |
|                   | 30-60  | 4,72-5,75 | 6,24-6,43        | 0,00-0,83         | 2,68-3,42 | 0,13-0,17 | 1,55->30                      | 12,63-24,83           |
| Božurnja          | 0-30   | 5,97-7,41 | 7,07-8,19        | 1,69->25          | 3,21-5,63 | 0,16-0,28 | 3,38-29,08                    | 23,76->30             |
|                   | 30-60  | 5,79-7,25 | 7,04-8,35        | 0,85->25          | 2,71-3,80 | 0,14-0,19 | 2,28-24,06                    | 12,78-27,52           |
| Ovsište           | 0-30   | 6,73-6,99 | 7,62-8,03        | 1,81-10,15        | 3,98-4,27 | 0,20-0,21 | 6,02->30                      | >30                   |
|                   | 30-60  | 6,78-7,05 | 7,69-7,86        | 1,81->25          | 1,50-3,65 | 0,09-0,18 | 2,22->30                      | >30                   |
| Vinča             | 0-30   | 6,79-7,04 | 7,52-8,13        | 1,81-7,05         | 2,18-6,01 | 0,09-0,18 | 14,28-28,38                   | 27,83->30             |
|                   | 30-60  | 6,87-7,11 | 7,80-8,21        | 2,26-9,59         | 1,80-3,51 | 0,11-0,30 | 2,83-16,75                    | 19,57->30             |
| Plaskovac         | 0-30   | 6,32-7,02 | 7,06-7,81        | 0,83-1,97         | 3,24-5,45 | 0,16-0,27 | 2,63->30                      | 26,82->30             |
|                   | 30-60  | 5,83-6,98 | 6,94-7,94        | 1,41-2,26         | 1,62-2,62 | 0,08-0,13 | 2,21-6,08                     | 20,39->30             |
| Donja Šatornja    | 0-30   | 6,07-6,89 | 7,61-7,86        | 1,21-3,81         | 2,33-3,36 | 0,12-0,17 | 3,02-25,72                    | 13,87-25,52           |
|                   | 30-60  | 5,54-6,86 | 6,81-7,88        | 0,56-2,82         | 1,71-3,60 | 0,09-0,18 | 8,02-11,24                    | 10,58-19,62           |
| Blaznava          | 0-30   | 6,45-6,91 | 7,05-7,66        | 0,99-3,24         | 3,65-4,83 | 0,18-0,24 | 7,32->30                      | >30                   |
|                   | 30-60  | 6,62-6,94 | 7,31-7,68        | 1,41-2,68         | 1,89-2,95 | 0,09-0,15 | 4,50-22,83                    | 19,62->30             |

The soil reaction is neutral 6,7-7,2, whereas carbonate subtypes are weakly basic with pH ranging from 7-8. Humus content ranges from 3-5%, being rich in nitrogen accordingly. Their phosphorus is low though with good supply of potassium. The quoted results are in accordance with the results of our investigations for the majority of analysed sites.

Certain subtypes of soil are characterized by a low content of phosphorus, which is generally found in acid soils. Availability of phosphorus in acid soils is known to decrease due to its immobilization

with free  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  ions (Debnath et al. 2000). Methods of dealing with low phosphorus content is phosphorization as a measure of ameliorative application of phosphorus fertilizers and liming in order to increase the accessibility of phosphorus from soil reserves. If soils are low in phosphorus, fertilization is carried out by complex fertilizers with high phosphorus, whereas in case of good supply with phosphorus and potassium, the fertilizers with approximately equal relation of the two elements should be used. (Ubavić and Bogdanović 1995).

### **Conclusions**

Soils of the region investigated are heavy clays, with high cation adsorption capacity, low to medium hydrolytic acidity and high to almost full saturation with adsorbed bases.

Acidity of the respective soil is in the class of acid to medium alkaline with prevailing low acid (pH/KCl 5,5-6,5) to low alkaline (pH/KCl 6,5-7,2) soils. With the depth of sampling, there are no changes within the soil reaction interval. Active acidity of soil, on majority of sites has neutral to weak alkaline reaction. According to the analysis results of most of the parameters examined, a significant portion of the area belongs to a carbonated type of soil, with some differences in the content of carbonate influenced by degradation processes and soil evolution in smonitsa. Confirmation of the obtained results and recognition of the microlocality of growing grapes, cherries, peaches, nectarines and others requiring the presence of carbonate are tolerant of the increased carbonate content. The presence of humus in the soil corresponds with the type of soil and surface horizon is medium supplied with humus, which decreases with a depth of sampling. The presence of total nitrogen is in accordance with the content of humus, with mean values of the content prevailing. There is very low to low supply with accessible phosphorus. ( $<10 \text{ mg } 100^{-1} \text{ g}$ ). A good supply of available phosphorus is measured on the sites with intensive nutrition of crop plantations. The soils are well supplied with potassium. All this shows that the soils of the area analysed are potentially fertile, where agromelioration improvement in the structure and mechanical composition, humification and phosphating should be applied.

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### **References**

1. Debnath, A., Bhattacharjee, T. K., Debnath, N. C. (2000): Behaviour of inorganic phosphate fractions in limed acid soils. *J. Indian Soil Sci.* 48: 829-831.
2. Dugalić, G. Gajić, B. (2012). *Pedologija. Agronomski fakultet, Čačak.*
3. Fuentes, M., Govaerts, B., De Leon, F., Hidalgo, C., Sayre, K. D., Etchevers, J., Dendooven, J. (2009). Fourteen years of applying zero and conventional tillage, crop rotation and residue managements systems and its effect on physical and chemical soil quality. *European Journal of Agronomy*, 30: 228-237.
4. Gajić B. and Živković M. (2002). Specifična površina smonica aleksinačke kotline. *Journal of Agricultural Sciences*, 47, (1): 19-27.
5. Islam, K. R. and Weil, R. R. (2000). Land use effects on soil quality in a tropic forest ecosystem of Bangladesh. *Agriculture, Ecosystems & Environment*. 79: 9-16.
6. Manojlović, M. (2008). *Đubrenje u održivoj poljoprivredi. Monografija, Poljoprivredni fakultet, Novi Sad.*
7. Milivojević J., Đekić V., Jelić M. (2012). Fertility of the arable land of the production sites of the town of Kragujevac in private ownership. *Vegetable and vegetable production*, 49 (2): 195-201.
8. Miljković, N. S. (1996). *Osnovi pedologije*, Novi Sad.

9. Škorić, A. (1986). Postanak, razvoj i sistematika tla. Fakultet poljoprivrednih znanosti Sveučilišta u Zagrebu, Zagreb.
10. Ubavić, M. and Bogdanović, D. (1995). Agrohemija, Poljoprivredni fakultet Novi Sad
11. Keserović, Z., Magazin, N, Kurjakov, A., Dorić, M., Gošić, J. (2012). Poljoprivreda u Republici Srbiji. Voćarstvo. Republički zavod za statistiku.

## EVALUATION OF ETHOLOGICAL AND PRODUCTION TRAITS IN VARIOUS GENOTYPES OF MACEDONIAN HONEY BEE SUBSPECIES

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### Abstract

The honey bee colony is a complex society which has a wide range of behaviors. The most important are swarming, colony defense and hygienic behavior. These ethological traits have an impact on the honey yields and are of significant interests of the honey bee breeders. Therefore the ethological characteristics are recognized in selection and breeding programs. According to the breeding programs selection is directed to reduction of swarming and colony defense behavior and increased expression of hygienic behavior. The aim of this research was to evaluate these characteristics in 100 honey bee colonies from four genotypes (A, B, C and D) of the autochthonous honey bee population (*Apis mellifera macedonica*) in one of the registered honey bee queen breeding stations, located in Ohrid region, during 2016. The research included: scoring of the defensive and the swarming behavior of honey bee colonies according to four point system, testing hygienic behavior using Pin-test method and scoring according five point system as well as determining honey yield by weighing of extracted honey in kg per honey bee colony and scoring the honey yield according to the four point system. The results did not show statistically significant differences concerning swarming behavior and honey yield between colonies from the four genotypes. The results have shown statistical significant differences in the average cleaning success (hygienic behavior) after 24 hours, between A and B ( $p=0.0096$ ) genotypes, and in the defensive behavior, between A and D ( $p=0.0166$ ) and between C and D ( $p=0.0333$ ) genotypes.

**Keywords:** *Apis mellifera macedonica*, swarming behavior, defensive behavior, hygienic behavior, honey yield.

### Introduction

Honey bee breeding is a common goal of honey bee breeders and honey bee researches during the last 150 years, but it does not improve as rapidly as the breeding of other live stock. In honey bees (*Apis mellifera* L) the breeding activity is based on the performance evaluation at the level of each honey bee colony (Cauia *et al.* 2010). The evaluation consists, generally, of the appreciation of economic and ethological traits in order to be classified and make selection of the best parents. Most significant ethological traits which have an impact on the productivity of honey bee colonies (honey yield) are swarming, defense and hygienic behavior and thus have been recognized in selection and breeding programs (Ruttner, 1972).

Although swarming is a natural way of reproducing honey bee colonies, beekeepers believe it is a negative characteristic since it decreases the strength of the colony during honey extraction, which reflects the honey yields and other honey bee products. Beekeepers enacted breeding strategies to reduce expression of swarming behavior, in opposition to natural selection (Ruttner, 1972; Möbus, 1983; Poklukar, 1999; Moritz and Southwick, 1992).

Another well-known type of behavior in honey bees is colony defense consisting of recognizing of predators, alerting nest mates and enacting anti-predator behavior (Collins *et al.*, 1980; Moritz *et al.*, 1987; Breed *et al.*, 2004). Improvement of this characteristic is particularly the goal of many selection programs; however, care should be taken and optimal value need to be found between the wish of the beekeepers to work with calmer bees and the danger, calm bees to become prey of

natural enemies (wasps, birds or mammals). Hygienic behavior in honey bees is a heritable trait of individual honey bee workers that confers colony-level resistance against various brood diseases. Hygienic honey bee workers detect and remove dead or diseased brood from sealed cells. However, this behavior is quite rare, with only c. 10 % of unselected colonies showing high levels of hygiene. Beekeepers can potentially increase this by screening colonies for hygiene and breeding from the best. However, the level of hygiene expressed by a colony is variable, which poses a challenge to colony selection (Bigio *et al.* 2013).

The aim of our research was to evaluate these characteristics in the Macedonian honey bee (*Apis mellifera macedonica*) which is an autochthonous subspecies (race) in Republic of Macedonia (Ruttner, 1988b; Kiprijanovska *et al.*, 2012; Uzunov *et al.*, 2009, 2014).

### Material and methods

The investigation was carried out during 2016 in one of the registered honey bee queen breeding stations located in Ohrid region (village Vapila). One hundred honey bee colonies divided in four genotypes (A, B, C and D) of the autochthonous honey bee colonies (*A. m. macedonica*) were evaluated for the following characteristics: swarming behavior, defense behavior, hygienic behavior and honey yield.

The swarming behavior was evaluated during the swarming period (May-June), according to the four-point system (Ruttner, 1972): 4 points = the colony has shown no swarming behavior for the entire season (has not constructed any queen cells); 3 points = queen cells were found in routine control. After the necessary expansion (additional supers) and the breaking up of the queen cells, no more queen cells were constructed; 2 points = queen cells were repeatedly found in routine control and swarming was difficult to control; 1 point = the colony swarmed or swarming could be prevented only by extensive intervention (e.g. nucleus).

The defensive behavior was tested every time the colonies in the apiary were visited and an average score was calculated. This trait was also evaluated according to the four-point system (Ruttner, 1972): 4 points = no protection and no smoke necessary to avoid stings; 3 points = no protection and only a little smoke necessary, no stings; 2 points = much smoke and protection (veil, gloves) necessary to avoid stings and in order to be able to work unimpeded; 1 point = working without a lot of smoke, face protection and gloves is not possible; stings occur even at a great distance from the apiary.

The hygienic behavior was tested using the pin-test (Gramacho and Gonçalves, 2003): 100 cells containing white or pink-eyed pupae pierced through the cell capping with an entomological needle size n° 2 (diameter = 0.45 mm). The removal of the killed pupae by the adult honey bees was estimated after a time interval of 24 hours. Colonies were scored according to the proportion of cleaned brood comb cells 24 hours after killing the pupae on the following five point system (Gregorc and Locar 2010): 5 > 95 %; 4 = 90 – 95 %; 3 = 80 – 89 %; 2 = 70 – 79 % and 1 < 70 %.

The honey yield was determined by weighing the extracted honey in kg per honey bee colony and was scored according to a fourpoint system (Gregorc and Locar 2010): 1 and 2 - below apiary average honey yield and 3 and 4 - above apiary average honey yield.

The data were analyzed using MS Excel program to carry out descriptive statistics on evaluated traits and analysis of variance (ANOVA) and HSD Post-Hoc test to analyze the differences between genotypes.

### Results and discussion

The data for the evaluation of the tested traits are given in Table 1 and the differences in the tested traits between genotypes are given in Table 2.

Swarming behavior – the data in Table 1 show that related to swarming behavior the average scoring values are ranged from 3.87 to 4.0. All honey bee colonies from genotypes A and C have not constructed any queen cells for the entire season. The queen cells were found in three honey bee colonies from genotype B and in one honey bee colony from genotype C, but after the breaking up

of the queen cells no more queen cells were constructed. The results did not show statistically significant differences concerning swarming behavior between colonies from the four genotypes (Table 2) whereas swarming behavior in all 4 genotypes was positively evaluated.

Table 1. Colony scores for observed traits

| Trait                   | Genotype A |        |         | Genotype B |        |         | Genotype C |        |         | Genotype D |        |         |
|-------------------------|------------|--------|---------|------------|--------|---------|------------|--------|---------|------------|--------|---------|
|                         | $\bar{X}$  | Sd     | min-max | $\bar{X}$  | Sd     | min-max | $\bar{X}$  | Sd     | min-max | $\bar{X}$  | Sd     | min-max |
| Swarming behavior (1-4) | 4          | 0      | 4-4     | 3,87       | 0,3378 | 3-4     | 4          | 0      | 4-4     | 3,96       | 0,2    | 3-4     |
| Defense behavior (1-4)  | 3,88       | 0,3316 | 3-4     | 3,48       | 0,7141 | 2-4     | 3,84       | 0,3741 | 3-4     | 3,40       | 0,7071 | 2-4     |
| Hygienic behavior (1-5) | 2,20       | 0,7637 | 1-4     | 3,16       | 1,1060 | 1-5     | 2,44       | 1,1575 | 1-5     | 2,64       | 1,1503 | 1-5     |
| Honey yield (1-4)       | 2,72       | 0,7371 | 1-4     | 2,60       | 0,7637 | 1-4     | 2,56       | 0,5830 | 2-4     | 2,48       | 0,6531 | 1-4     |

Our results correspond to the report of Adam (1968) and Ruttner (1988 a) who reported that a low swarming tendency is one of the main values of the Macedonian honey bees. The low swarming tendency of *A. m. macedonica* was established by Antevski (2015), who reported that the average scoring values in his research is 3.85. Uzunov *et al.* (2014) found variation in swarming tendency of the Macedonian honey bees depending on the origin-low swarming tendency in the Bulgarian population (3.30), higher in the Greek one (2.62) and intermediate in the Macedonian population (3.27) and they explain that this probably reflects the wide range of the *A. m. macedonica* origin which was covered.

Defense behavior - the results have shown statistical significant differences in the defensive behavior, between A and D and between C and D genotypes (Table 2). The results in Table 1 show that genotype D had the lowest average grade (3.40) and three honey bee colonies were evaluate with 2 within this genotype. In genotype B, three honey bee colonies were evaluate with 2 as well, but the average grade was higher (3.48). The honey bee colonies from the genotypes B and D may be characterized as moderately defensive and much smoke and protection is necessary to avoid stings and in order to be able to work unimpeded. Genotype A was the highest scored (3.88), while genotype C had an average grade of 3.84. In these two genotypes, no honey bee colonies were evaluated with a score of less than 3, and they may be characterized as calm.

Our results correspond with the researches made by Uzunov (2013) and Antevski (2015). Uzunov (2013) reported that two genotypes of *A. m. macedonica* were characterized as calm related to defense behavior with an average grade of 3.5. Antevski (2015) also characterized the honey bee colonies from the two genotypes of *A. m. macedonica* as calm, with grades 3.70 and 3.63, respectively.

Hygienic behavior - significant statistical difference between evaluated honey bee colonies was indicated in regard to the hygienic behavior (Table 2). Genotype B was scored as a line with most expressive hygienic behavior whereas genotype A as a line with the least expressive hygienic behavior (Table1). Average percentage of dead pupae removed by the adult honey bee was 83.92 % in genotype B and 75.84 % in genotype A. In genotype B four honey bee colonies were evaluated with 5 (the percentage of dead pupae removed was > 95 %), in genotype C one honey bee colony was evaluate with 5, in genotype D two, and in genotype A no honey bee colonies were evaluate with 5.

According to Spivak and Gilliam (1998 a), colonies that either begin to or completely remove 95 % or more of pupae within 24 hours of death are considered to be “rapid hygienic” and in populations of honey bees that have not been selected specifically for rapid hygienic behaviour, approximately 10% of the colonies will carry this trait. Our results show that on apiary level 8 % of honey bee colonies meet the requirement for rapid hygienic behaviour which gives opportunity for choosing colonies for selection purposes.

Table 2. Analysis of variance of colony scores for observed traits

| Trait                   | One- ANOVA way analysis |                |                         |             |         |         |
|-------------------------|-------------------------|----------------|-------------------------|-------------|---------|---------|
| Swarming behavior       | Source of variance      | Sum of squares | Df                      | MS          | F       | P       |
|                         | Between Groups:         | 0.2605         | 3                       | 0.0868      | 2.2592  | 0.0865  |
|                         | Within Groups:          | 3.6894         | 96                      | 0.0384      |         |         |
|                         | Total:                  | 3.9499         | 99                      |             |         |         |
| Defense behavior        | Source of variance      | Sum of squares | Df                      | MS          | F       | P       |
|                         | Between Groups:         | 4.5100         | 3                       | 1.5033      | 4.7726  | 0.0038* |
|                         | Within Groups:          | 30.2395        | 96                      | 0.3150      |         |         |
|                         | Total:                  | 34.7495        | 99                      |             |         |         |
| Tukey HSD Post-hoc Test |                         |                |                         |             |         |         |
| Genotype                | Mean Difference         |                | 95% Confidence Interval |             | P       |         |
|                         |                         |                | Lower Bound             | Upper Bound |         |         |
| A vs D                  | -0.4800                 |                | -0.8951                 | -0.0649     | 0.0166* |         |
| C vs D                  | -0.4400                 |                | -0.8551                 | -0.0249     | 0.0333* |         |
| Hygienic behavior       | Source of variance      | Sum of squares | Df                      | MS          | F       | P       |
|                         | Between Groups:         | 12.5100        | 3                       | 4.1700      | 3.7315  | 0.0138* |
|                         | Within Groups:          | 107.2800       | 96                      | 1.1175      |         |         |
|                         | Total:                  | 119.7900       | 99                      |             |         |         |
| Tukey HSD Post-hoc Test |                         |                |                         |             |         |         |
| Genotype                | Mean Difference         |                | 95% Confidence Interval |             | P       |         |
|                         |                         |                | Lower Bound             | Upper Bound |         |         |
| A vs B                  | 0.9600                  |                | 0.1782                  | 1.7418      | 0.0096* |         |
| Honey yield             | Source of variances     | Sum of squares | Df                      | MS          | F       | p       |
|                         | Between Groups:         | 0.7500         | 3                       | 0.2500      | 0.5282  | 0.6640  |
|                         | Within Groups:          | 45.4400        | 96                      | 0.4733      |         |         |
|                         | Total:                  | 46.1900        | 99                      |             |         |         |

Honey yield - the average honey yield per colony in the researched genetic lines was very close and ranges between 9.1 kg (genotype A) and 10.5 kg per honey bee colony (genotype C). The minimum

honey yield per bee colony (6.8 kg) was found in genotype A and the highest of 12.4 in genotype C, which is very close to the national average in the last ten years (www.stat.gov.mk, 2016).

There was no significant statistical difference concerning this characteristic (Table 2) which corresponds with the results from the researches made by Uzunov (2013), and Antevski (2015), which also found no differences between the genotypes of *A. m. macedonica* that they investigated in terms of honey yield.

The average scores for this characteristics were also very close and range from 2.48 to 2.72 (Table 1). Low estimates show there are very few honey bee colonies in all four genotypes that differ significantly in yield relative to the average, which limits the possibility of selecting honey bee colonies for selection purposes in relation to this trait.

## Conclusions

Swarming behavior in all 4 genotypes was positively scored, because they showed a very low swarming tendency. Statistically significant differences were not identified between the genotypes.

Defensive behavior in all 4 genotypes was positively scored too, where statistically significant differences were identified between the genotypes. The honey bee colonies from the genotypes B and D may be characterized as moderately defensive and the honey bee colonies from the genotypes A and C may be characterized as calm. Statistically significant differences between the genotypes give opportunity for choosing honey bee colonies for selection purposes related to this characteristic.

The influence of the genotype was determined for the hygienic behavior, where statistically significant differences were identified between genotype A and genotype B. The honey bee colonies which meet the requirement for rapid hygienic behaviour can be selected for producing honey bee queens to achieve high hygienic standards rather quickly.

There is no statistically significant difference between the genotypes in the honey yield which limits the possibility for choosing honey bee colonies for selection purposes in relation to this trait, but with the improvement of other characteristics, an increase in the honey yield can be expected, as many studies have identified the positive correlation between the ethological and production traits of honey bee subspecies

## References

1. Adam, B. (1968) In search of the best strains of bees. Walmar Verlag Zell, Weierbach, Germany.
2. Антевски, В. (2015). Сезонска динамика на развој на македонската медоносна пчела (*Apis mellifera macedonica*) во полошкиот регион. Магистерски труд, Факултет за земјоделски науки и храна, Скопје, Република Македонија.
3. Bigio, G., Schurch, R., Ratnieks, F. L. W. (2013). Hygienic behaviour in honey bees (Hymenoptera: Apidae): effects of brood, food, and time of the year. *Journal of Economic Entomology*, 106(6): 2280-2285.
4. Breed, M. D., Guzman-Novoa, E., Hunt, G. J. (2004). Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. *Annual Review of Entomology*, 49:271-298.
5. Cauia, E., Siceanu, A., Patruica, S., Bur, M., Sapcaliu, A., Magdici, M. (2010). The standardization of the honeybee colonies evaluation methodology with application in honeybee breeding programs. *Animal Science and Biotechnologies*, 43 (2): 174-180.
6. Collins, A. M., Rinderer, T. E., Tucker, K. W., Sylvester, H. A., Lockett, J. L. (1980). A model of honeybee defensive behavior. *Journal of Apicultural Research*, 19:224-231.
7. Goncalves, L. and Gramacho, K. P. (2003). Factores que interfieren en el comportamiento higienico de las abejas. 10° Congreso Internacional de Actualizacion. *Apicola*, Tlaxcala, Mexico, 80-82.

8. Gregorc, A. and Lokar, V. (2010). Selection criteria in an apiary of Carniolan honey bee (*Apis mellifera carnica*) colonies for queen rearing. *Journal of Central European Agriculture*, 11: 401-408.
9. Kiprijanovska, H., Andonov, S., Büchler, R., Drazic, M., Hatjina, F., Kezic, N., Panasiuk, B., Le Conte, Y., Uzunov, A., Wilde, J. (2012). Assessment of the behavioural traits in the international GEI experiment of COLOSS WG4, Workshop WG4 "Honey bee vitality and diversity –Behavioral traits Final results and paper preparing". Ohrid, Macedonia. Proceedings, 6.
10. Möbus, B. (1983). Bee breeding in Germany. In Möbus, B. and van Praagh, J. (Eds). *Pedigree bee breeding in Western Europe*. British Isles Bee Breeders Association, Derby, UK, 6-12.
11. Moritz, RFA, Southwick, EE., Harbo, JB. (1987). Genetic analysis of defensive behavior of honey bee colonies (*Apis mellifera* L.) in a field test. *Apidologie*, 18:27-42.
12. Moritz, RFA. and Southwick, EE. (1992). *Bees as super organisms: an evolutionary reality*. Springer-Verlag, 65-77.
13. Poklukar, J. (1999). Izboljšanje odbire čebel na proizvodne lastnosti z uporabo selekcijskega indeksa. *Zb. Biotehniške fak, Univ. v Ljubljani, Kmetijstvo, Zootehnika*, 74.
14. Ruttner, F. and Ruttner, H. (1972). *Controlled mating and selection of the honey bee*. Apimondia Publishing House, Bucharest, Romania, 87-92.
15. Ruttner, F. (1988a). *Biogeography and taxonomy of honey bees*. Springer-Verlag, Berlin, Germany.
16. Ruttner, F. (1988b). *Breeding techniques and selection for breeding of the honey bee*. British Isles Bee Breeders Association, Derby, UK.
17. Spivak, M. and Gilliam, M. (1998a). Hygienic behaviour of honey bees and its application for control of brood diseases and varroa mites. Part I: Hygienic behaviour and resistance to American Foulbrood. *Bee World* 79: 124-134.
18. Uzunov, A., Kiprijanovska, H., Andonov, S. (2009). Diversity of honey bees (*Apis mellifera* L.) on the territory of Republic of Macedonia. 5th COLOSS Conference 2009. Montpellier, France, Proceedings, 67.
19. Узунов, А. (2013). Биолошки и производни карактеристики на автохтоната медоносна пчела (*Apis mellifera macedonica*) на територијата на Република Македонија. Докторска дисертација, Факултет за земјоделски науки и храна - Скопје, Република Македонија.
20. Uzunov, A., Costa, C., Panasiuk, B., Meixner, M., Kryger, P., Hatjina, F., Bouga, M., Andonov, S., Bienkowska, M., Le Conte, Y., Wilde, J., Gerula, D., Kiprijanovska, H., Filipi, J., Petrov, P., Ruottinen, L., Pechhacker, H., Berg, S., Dyrba, W., Ivanova, E., Büchler, R. (2014). Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. *Journal of Apicultural Research*, 53,2, 248-260.

## PHENOTYPING OF STRAWBERRY BREEDED FROM TURKEY BREEDING PROGRAM

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### Abstract

The standardized phenotyping protocol for strawberries was agreed upon by the breeding team in Oregon, Michigan, New Hampshire, California and Florida and includes four trait categories: phenology and other flower-related traits, plant characteristics, fruit characteristics, and fruit chemistry traits. This study aims to introduce this category to the breeding programs in our country to characterize 15 cultivars of strawberries that are bred in our country. In this context, 25 properties are evaluated. As a result, our cultivars are globose conic in respect of external fruit properties, appearance is above average, achene colour is dark, achene position is even, external colour is red, gloss is average, skin strength is soft, calyx size is above equal, calyx position is flat. In terms of fruit internal properties, firmness is soft, internal colour is light red, depth of internal color is at 51.8% level and average level, aroma is at high level.

**Keywords:** Strawberry, phenotyping characterization, plant qualities, breeding.

### Introduction

Most plant breeding programs revolve around identifying novel and ideal phenotypes. The phenotype is the characteristic of an individual that can be observed and results from the interaction of its genotype with the environment. Success of a breeding program is defined by identifying the optimum traits needed for an individual to be released as cultivar. Most breeding programs still utilize traditional approaches to identify desirable phenotypes. Marker-assisted breeding (MAB) can facilitate and speed up the release of new cultivars. This technology is becoming easier to integrate into a traditional breeding program due to decrease in costs, increased efficiency, and marker availability (Bliss, 2010; Mathey et al. 2013). The U.S. Department of Agriculture-National Institute of Food and Agriculture (USDA–NIFA) Specialty Crop Research Initiative-funded RosBREED project aims to ‘bridge the chasm’ between genomics and traditional rosaceous crop breeding programs (Iezzoni et al., 2010). Large-scale phenotyping protocols have been published for apple (Evans et al. 2011) and peach (Frett et al. 2012) and presentations of the standardized phenotyping can be viewed for all crops (apple, peach, cherry and strawberry) at [www.rosbreed.org](http://www.rosbreed.org). Through the efforts of the U.S. Department of Agriculture-National Institute of Food and Agriculture (USDA–NIFA) Specialty Crop Research Initiative-funded project “RosBREED”, large scale standardized phenotyping protocols were established by breeding teams in diverse environments in the United States (California, Florida, Michigan, Oregon, and New Hampshire) for strawberry to capture phenological and flowering related traits, plant characteristics, fruit characteristics and fruit chemistry (Hancock et al. 2016; Iezzoni et al. 2010; Mathey et al. 2013). The RosBREED’s strawberry group evaluated 890 genotypes that represent the breadth of diversity in the strawberry breeding germplasm and their wild relatives. Within this group was a set of 36 crosses representing eastern and western North American short day and remontant genotypes (Mathey et al. 2017). The objectives are to describe a panel strawberry cultivars breeding programs from Turkey. The traits evaluated were related to fruit quality (shape, appearance, capping, calyx position, percent of filled achenes, achene color, gloss, skin toughness, external and internal color, depth of internal color, flavor, average weight), fruit chemistry (soluble solids, titratable acidity, pH) and plant development (peduncle length, flowering location, presence of anthers, truss size, crop estimate).

### Material and methods

The study was carried out in Mustafa Kemal University Faculty of Agriculture Department of Horticulture between 2015-2016. As a plant material were used to 15 strawberry cultivars (Arnavutköy, Ata77, Bolverim77, Doruk77, Dorukhan77, Ebru, Eren77, Erenoğlu77, Hilal77, Kaşka, Osmanlı, Sevgi, Yalova104, Yalova15, Yalova416) obtained from Atatürk Horticultural Central Research Institute. The experiment was conducted in the glasshouse. The planting were potted with fresh plants at the end of November. Drip irrigation method was used in the study. In this study, 25 phenotypic traits identified in Table 1 were evaluated.

Table 1. Description of the 25 strawberry phenotypic traits (Mathey et al. 2013).

| Trait                                                                           | How trait was evaluated                                                                                                                                         |
|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Phenology</b>                                                                |                                                                                                                                                                 |
| Peduncle length                                                                 | Scored 1-5; 1= divides close to crown, 2= divides at 25% from crown, 3= divides 50% from crown, 4= divides 75% from crown, 5= divides right before flower/fruit |
| Flowering location                                                              | Determined at bloom; Scored 1-2; 1= above or 2= below canopy                                                                                                    |
| Presence of anthers                                                             | Determined at bloom; Scored 1-2; 1= yes, 2= no                                                                                                                  |
| Truss size                                                                      | Number of flowers per truss                                                                                                                                     |
| <b>Plant characteristics</b>                                                    |                                                                                                                                                                 |
| Crop estimate                                                                   | Amount of fruit on plant; Scored 1-9; 1= no fruit; 9= over-cropped                                                                                              |
| Vigor                                                                           | Plant health/growth; Scored 1-9; 1= dead; 9= extremely vigorous                                                                                                 |
| <b>External fruit characteristics</b>                                           |                                                                                                                                                                 |
| Shape                                                                           | Scored 1-9; 1= long conic, 3= globose, 5= globose conic, 7= cordiform, 9= oblate                                                                                |
| Appearance                                                                      | Scored 1-9; 1= very malformed; 9= symmetrical and attractive                                                                                                    |
| Achene color                                                                    | Scored 1-9; 1=dark; 9=very light brown or green                                                                                                                 |
| Achene position                                                                 | In relation to fruit surface; Scored 1-3; 1= sunken, 2=even, 3=protruding                                                                                       |
| Percent of filled achenes                                                       | Percent of all achenes that were filled (10% increments)                                                                                                        |
| External color                                                                  | Scored 1-9; 1= white; 9= "black"                                                                                                                                |
| Gloss                                                                           | Scored 1-9; 1=dull; 9=very glossy                                                                                                                               |
| Skin firmness                                                                   | How easily fruit was abraded when thumb firmly dragged over flesh; Scored 1-9; 1=soft; 9= tough                                                                 |
| Cap size                                                                        | Calyx size in relation to fruit width; Scored 1-3; 1=calyx smaller, 2=calyx equal, and 3=calyx larger                                                           |
| Calyx position                                                                  | Scored 1-5; 1=raised (necked), 3= flat (even with shoulders), 5= sunken                                                                                         |
| <b>Internal fruit characteristics</b>                                           |                                                                                                                                                                 |
| Firmness                                                                        | Firmness of flesh when a fully ripe fruit was compressed between thumb and forefinger Scored 1-9; 1= mush; 9= hard                                              |
| Ease of capping                                                                 | Ease with which cap was removed when pulled by fingers; Scored 1-9; 1= does not remove; 9= very easily removed                                                  |
| Internal color                                                                  | Fruit sliced down the meridian; Scored 1-9; 1= white; 9= "black"                                                                                                |
| Depth of internal color                                                         | Percentage of solid color (10% increments)                                                                                                                      |
| Flavor                                                                          | Perception of sweetness and presence of off flavors; Scored 1-9; 1= not sweet, bad off flavors; 9= very sweet, no off flavors                                   |
| <b>Fruit characteristics measured in the lab including weight and chemistry</b> |                                                                                                                                                                 |
| Fruit weight                                                                    | Average weight of five primary fruit harvested when plant had 30-50% rip fruit, g                                                                               |
| pH                                                                              | pH of fruit puree                                                                                                                                               |
| Soluble solids                                                                  | Brix (g 100 g <sup>-1</sup> ) of fruit puree; determined using refractometer                                                                                    |
| Titrateable acidity                                                             | G L <sup>-1</sup> citric acid of fruit puree; determined using auto-titrator with pH 8.1 end-point                                                              |

## Results and discussion

Some of the genotypes from Turkey strawberry breeding programs are located to cultivar such as 'Arnavutköy', 'Aliso' ve 'Tioga' in the Yalova (Konarlı et al. 1984); again Yalova 'Osmanlı', 'Yalova104', 'Tufts', 'Cruz' ve 'Tioga' cultivars crosses of different combinations of varieties (Erenoğlu et al. 1998). The characterization used in the phenotypic description are presented in Table 1. The mean values of the phenotypic characteristics of the strawberry cultivars from Turkey strawberry breeding programs are presented in Table 2.

Table 2. Mean, minimum and maksimum values for 25 traits of the referans cultivars and breded from Turkey.

| Variable                                                                               | Referans Cultivars (RC) (Hancock et al. 2015) |                  | Local Cultivars (LC) |           |
|----------------------------------------------------------------------------------------|-----------------------------------------------|------------------|----------------------|-----------|
|                                                                                        | Mean                                          | Cultivar Numbers | Mean                 | Range     |
| <b><i>Phenology</i></b>                                                                |                                               |                  |                      |           |
| Peduncle length                                                                        | 3.5                                           | 15               | 3.47                 | 1-5       |
| Flowering location                                                                     | 1.4                                           | 15               | 1.41                 | 1-2       |
| Presence of anthers                                                                    | -                                             | 15               | 1.12                 | 1-2       |
| Truss size                                                                             | 5.1                                           | 15               | 8.06                 | 5-11      |
| <b><i>Plant characteristics</i></b>                                                    |                                               |                  |                      |           |
| Crop estimate                                                                          | 4.1                                           | 15               | 6.38                 | 5-9       |
| Vigor                                                                                  | 4.3                                           | 15               | 6.53                 | 3-8       |
| <b><i>External fruit characteristics</i></b>                                           |                                               |                  |                      |           |
| Shape                                                                                  | 5.8                                           | 15               | 5.50                 | 1-9       |
| Appearance                                                                             | 5.2                                           | 15               | 5.56                 | 3-8       |
| Achene color                                                                           | 4.4                                           | 15               | 2.88                 | 1-7       |
| Achene position                                                                        | 2.2                                           | 15               | 1.88                 | 1-3       |
| Percent of filled achenes                                                              | 88.6                                          | 15               | 92.5                 | 80-100    |
| External color                                                                         | 6.8                                           | 15               | 6.13                 | 2-8       |
| Gloss                                                                                  | 6.1                                           | 15               | 5.75                 | 3-9       |
| Skin firmness                                                                          | 5.4                                           | 15               | 3.94                 | 1-6       |
| Cap size                                                                               | 1.2                                           | 15               | 2.24                 | 1-3       |
| Calyx position                                                                         | 3.3                                           | 15               | 3.0                  | 1-5       |
| <b><i>Internal fruit characteristics</i></b>                                           |                                               |                  |                      |           |
| Firmness                                                                               | 5.6                                           | 15               | 4.19                 | 1-7       |
| Ease of capping                                                                        | 5.3                                           | 15               | 4.94                 | 2-9       |
| Internal color                                                                         | 5.1                                           | 15               | 4.63                 | 1-8       |
| Depth of internal color                                                                | 78.1                                          | 15               | 51.88                | 10-100    |
| Flavor                                                                                 | 4.4                                           | 15               | 7.19                 | 6-9       |
| <b><i>Fruit characteristics measured in the lab including weight and chemistry</i></b> |                                               |                  |                      |           |
| Fruit weight                                                                           | 9.7                                           | 15               | 7.1                  | 3.5-14.0  |
| pH                                                                                     | 3.4                                           | 15               | 3.6                  | 3.25-3.94 |
| Soluble solids                                                                         | 8.9                                           | 15               | 6.1                  | 3.2-8.7   |
| Titrateable acidity                                                                    | 0.9                                           | 15               | 0.82                 | 0.43-1.27 |

In terms of the phenological characters examined local strawberry cultivars, average peduncle length was determined as 3.47. When the flowering location was 1.41, it is possible to say that the flowering location are near to crown, the number of truss size per flower was determined as 8.06 in

our strawberry varieties. In terms of yield estimates of plant characteristics, the value set as 4.1 in RC was 6.38 in LC. Plant vigor, the RC value was 4.3 and the LC value was 6.53. As a result, plant characteristics, LC and RC gave similar results. The value determined by shape of the fruit exterior characteristics was determined as 5.50 in local strawberry cultivars and found to be close to the global conical. In terms of aken position; similar and above; percent of filled achenes; high and filled, external color; similar and red, gloss; similar and bright, calyx size; equal, calyx position; flat, appearance; medium, in terms of aken color, the RC color is clearly defined; LC is determined as dark. Among the fruit exterior characteristics, fruit skin firmness in the RC was determined as hard, but LC was found to be softer (Table 2). It has been determined that there is a significant diversity between RC and LC in terms of fruit interior characteristics. When RC was determined to be harder than YC in terms of firmness, it was determined that LC calyx easily breaks in terms of ease of capping. In terms of internal color and depth of internal color, it was determined that LC is lighter than RC. In terms of taste/aroma, it appears that our LC cultivars are aromatic (Table 2). When fruit weight and chemical properties are examined, fruit weight was determined to be 9.7 g/fruit as average of RC, and this value was determined as 7.1 g/fruit in YC. pH and titratable acidity were similar in both groups; it was found that YC gave a somewhat lower value in terms of soluble solids (Table 2).

### Conclusions

In this study, it was aimed to phenotypically identify 15 strawberry local strawberry (LC) cultivars from our country strawberry breeding programs using reference strawberry (RC) cultivars from different breeding programs. In this context, 26 features were examined. We have determined that we have improved plant characteristics of strawberry breeding programs of Turkey and varieties similar to the varieties used as reference in terms of plant characteristics. As a result, shape of fruit external features; spherical conical, appearance; the albinism is on the middle, fruit color; bold, flowering position; on, external color; red, brightness; medium, skin firmness; soft, calyx size; on the median, calyx position; flat, fruit firmness; soft, internal color; light red, and in terms of taste/aroma, we seem to be improving the varieties at a high level.

### References

1. Bliss, F.A. 2010. Marker-assisted breeding in horticultural crops. *Acta Hort.* 859:339-350.
2. Erenoğlu B, Erbil Y, Ufuk S, 1998. Melezleme yolu ile çilek ıslahı-1: Atatürk bahçe kültürleri merkez araştırma enstitüsü, bilimsel araştırma ve incelemeler, Yayın No:100.
3. Evans, K., Y. Guan, J. Luby, M. Clark, C. Schmitz, S. Brown, B. Orcheski, C. Peace, and W.E. van de Weg, A.F. Iezzoni. 2011. Large-scale standardized phenotyping of apple in RosBREED. *Acta Hort.* 945:233-238.
4. Frett T.J., K. Gasic, J.R. Clark, D. Byrne, T. Gradziel, and C. Crisosto. 2012. Standardized phenotyping for fruit quality in peach [*Prunus persica* (L.) Batsch]. *J Amer. Pom. Soc.* 66:214-219.
5. Hancock JF, Sooriyapathirana SS, Bassil NV, Stegmeir T, Cai L, Finn CE, Van de Weg E, Weebadde CK (2016) Public availability of a genotyped, segregating population may foster marker assisted breeding (MAB) and quantitative trait loci (QTL) discovery: An example using strawberry. *Front Plant Sci* 7:619. doi: 10.3389/fpls.2016.00619
6. Iezzoni, A.F., C. Weebadde, J. Luby, Y. Chengyan, W.E. van de Weg, G. Fazio, D. Main, C.P. Peace, N.V. Bassil and J. McFerson. 2010. RosBREED: enabling marker-assisted breeding in Rosaceae. *Acta Hort.* 859:389-394.
7. Konarlı O, Kepenek K, Aygün H, 1984. Melezleme yoluyla elde edilen yeni çilek çeşitleri. *Bahçe.* 13(2): 5-13.
8. Mathey, M.M., C.E. Finn, S. Mookerjee, K. Gündüz, J.F. Hancock, A.F. Iezzoni, L.L. Mahoney, T.M. Davis, N.V. Bassil, K.E. Hummer, P.J. Stewart, V.M. Whitaker, D.J. Sargent, B. Denoyes, I. Amaya and W.E. van de Weg. 2013. Large-scale standardized phenotyping of strawberry in RosBREED. *J. Amer. Pom. Soc.* 67: 205-216.

9. Mathey, M.M. S. Mookerjee, L.L. Mahoney, K. Gündüz, U. Rosyara, J.F. Hancock, P.J. Stewart, V.M. Whitaker, N.V. Bassil, T.M. Davis, C.E. Finn. 2017. Genotype by Environment Interactions and Combining Ability for Strawberry Families Grown in Diverse Environments. *Euphytica*, 213,112:1-12.

## SELECTION OF APPROPRIATE TRANSPORT FORMULAS FOR ESTIMATION OF TRANSPORT AND DEPOSITION RATES AT BABREKA CANAL

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### Abstract

Siltation is a significant factor, affecting the efficient operation and maintenance of the irrigation canals which influence the hydraulic behavior of the canals and economic benefits of irrigation. In last 25 years a tendency of silting up at the irrigation canals in Bulgaria is observed due to the following reasons: decreased area under irrigation, fluctuation in supply, non-regime section, “berming” of the canal, economic, crime and safety situation. The large number of available bed load and permissible velocity formulas and the significant differences between obtained results enforce selection of appropriate sediment transport formulas in each particular case. In this study discharge and velocity approaches are used for estimation of sediment transport and deposition rates at Babreka Canal, Malka Vitska Irrigation Project in Bulgaria. Seven equations for prediction total bed load transport rates and five equations for minimum permissible velocity are compared. The results show substantial differences in performance. Two formulas for total bed load and two formulas for minimum permissible velocity are selected as appropriate for the studied canal.

**Keywords:** Sediment transport, Irrigation canal, Malka Vitska Irrigation Project, Bulgaria.

### Introduction

Irrigation canals have been designed to ensure a transport capacity equal to or greater than the amount of incoming sediment. In last 25 years a tendency of silting up at the irrigation canals in Bulgaria is observed due to the following reasons: decreased area under irrigation, fluctuation in supply, non-regime section, “berming” of the canal, economic, crime and safety situation. Irrigation canal silting is important problem in achieving effective operation and maintenance and is capable of exerting direct and indirect effects on the hydraulic characteristics and economic benefits of irrigation.

The large number of bed load and permissible velocity formulas available and the significant differences between obtained results enforce the selection of appropriate sediment transport formulas in each particular case.

In this study discharge and velocity approaches were used for estimation of sediment transport and deposition rates at an existing irrigation canal – “Babreka”, Malka Vitska Irrigation Project in Bulgaria. Seven equations for prediction total bed load transport rates and five equations for minimum permissible velocity are compared.

### Material and methods

Determination of bed load rate is important to understand irrigation canal behavior including carrying capacity, sediment deposition, growth of vegetation. Basically irrigation canals are designed on the requirement that all sediment which enters the canal should be transported without sedimentation. Three methods for design stable canals are used: regime method, tractive force method and rational theory (HR Wallingford 1992). The regime design methods are sets of empirical equations derived from observations of canals and natural rivers. Tractive force method is based on a consideration of the balance of forces which act on sediment particle and includes the method of permissible velocity and the method of critical shear stress. The tractive force methods are used for shear stress and sediment transport determination. The rational theory includes the semi-empirical

methods and it is based on the conveying the sediment load through the canal system based on energy dissipation considerations. At least 100 transport rate equations can be found in the literature and the verification of the accuracy of this formulas is mainly based on laboratory and limited field data (Yang et al., 2009).

Among published, seven well-known equations for bed load transport rate determination were selected and compared in this study.

Meyer-Peter and Muller equation (Quesnel, 1974):

$$q_s = \frac{8}{\sqrt{\rho_w}} (\tau_b - 0.047(\gamma_s - \gamma_w)d_{50})^{3/2}, \quad (1)$$

where  $q_s$  is volumetric transport rate of bed load per unit width [kg/s m];  $\rho_w$  - density of water [kg/m<sup>3</sup>];  $\tau_b$  - bed shear stress;  $\gamma_w$  - specific weight of water [kN/m<sup>3</sup>];  $\gamma_s$  - specific weight of sediment [kN/m<sup>3</sup>];  $d_{50}$  - median size of particle size distribution.

Einstein - Brown equation (Hug, 1975):

$$q_s = \sqrt{(s-1)gd^3} \frac{K_{exp} \left( -\frac{0.391}{Fr^*} \right)}{0.465}, Fr^* < 0.182$$

$$q_s = 40\sqrt{(s-1)gd^3} K Fr^{*3}, Fr^* \geq 0.182, s = \frac{\rho_w}{\rho_s}; K = \sqrt{\frac{2}{3} + \frac{36v}{d^3(s-1)}} - \sqrt{\frac{36v^2}{d^3(s-1)}}, \quad (2)$$

where  $s$  is relative density;  $Fr^*$  - dimensionless shear stress or Shields stress;  $v$  - kinematic viscosity [m<sup>2</sup>/s];  $g$  - acceleration of gravity [m/s<sup>2</sup>].

Selim Yalin equation (Hug, 1975):

$$q_s = 0.635\sqrt{(s-1)gd^3} r \sqrt{Fr^*} \left[ 1 - \frac{1}{\sigma r} \ln(1 + \sigma r) \right];$$

$$r = \frac{Fr^*}{Fr_{crit}^*} - 1; \sigma = 2.45 \frac{\sqrt{Fr_{crit}^*}}{s^{0.4}}, \quad (3)$$

where  $Fr_{crit}^*$  is critical Shields stress.

Gomez equation (Gomez, 2006):

$$q_s = \frac{0.0725\gamma QJ}{b d_{50}^{0.51}}, \quad (4)$$

where  $Q$  is water discharge [m<sup>3</sup>/s];  $J$  - energy gradient;  $b$  - canal length [m].

Van Rijn equation (Van Rijn, 1984):

$$q^* = \frac{0.053}{d^{*0.3}} \left( \frac{Fr^*}{Fr_{crit}^*} - 1 \right)^{2.1}, \quad (5)$$

where  $q^*$  is dimensionless bed load transport rate;  $d^*$  - dimensionless particle diameter.

Nagakawa - Tsujimoto equation (Van Rijn, 1984):

$$q_s = 0.02\rho_s Fr^* \sqrt{(s-1)gd_{50}} \left[ 1 - \frac{0.035}{Fr^*} \right]^3, \quad (6)$$

Nielsen equation (Nielsen, 1992):

$$q^* = 12(Fr^* - Fr_{crit}^*) \sqrt{Fr^*}, \quad (7)$$

The minimum permissible velocity or non-silting velocity is the lowest velocity that will not initiate sedimentation and will not allow a growth of vegetation. According Chow (Chow, 1973) the average velocity from 0.6 to 0.9 m/s would prevent sediment deposition and higher velocity than 0.75 m/s would ensure vegetation-free canal. Therefore, the minimum permissible velocity should be ranged of 0.75-0.9 m/s. Non-silting velocity depends on the sediment diameter. Five well-known equations for determination of minimum permissible velocity are chosen for comparison – Zamarin, Grishkan, Roer, Poslavskii and Kennedy equations:

- Zamarin equation:

$$v_{min} = a\sqrt{R} \quad (8)$$

where  $R$  is hydraulic radius;  $a$  – coefficient depend on particle size (Tab. 2).

Tab.2. Coefficient a in Zamarin equation

| d [mm] | 0.1 | 0.2  | 0.4  | 0.6  | 0.8 | 1.0  | 1.2 | 1.4  | 1.6  | 1.8  | 2.0 | 3.0  |
|--------|-----|------|------|------|-----|------|-----|------|------|------|-----|------|
| a      | 0.2 | 0.45 | 0.67 | 0.82 | 0.9 | 0.95 | 1.0 | 1.02 | 1.05 | 1.07 | 1.1 | 1.11 |

- Grishkan equation:

$$v_{min} = kQ^{0.2} \quad (9)$$

where k is coefficient depend on *fall* velocity  $\omega$ .

If fall velocity  $\omega < 1.5$  mm/s,  $k=0.33$ ; if value of  $\omega$  is between 1.5 and 3.5 mm/s  $k=0.44$  and if  $\omega > 3.5$  mm/s  $k=0.55$ .

- Roer equation:

$$v = A \left[ \frac{m+2}{2} (\rho_p - 1) \omega \right]^{0.326} R^{0.473} \quad (10)$$

where A is coefficient equal to 39.3; m - width-to-depth ratio.

- Poslavsii equation (Korpachev, 2009):

$$v_{min} = 0.34 \sqrt{NR^{1/3}} \quad (11)$$

where N is capacity refers to the maximum amount of sediment of a given size that a stream can transport in traction as bedload.

- Kennedy equation (Das, 2012):

$$v_{min} = 0.84h^{0.64} \quad (12)$$

Malka Vitska Irrigation Project is located in Dolni Dabnik Municipality, south Bulgaria and it is owned and managed from Irrigation System SOJSC, Pleven Branch. The Vit River, Krushovits-3 Reservoir, Dolni Dabnik Reservoir and Valchovets Reservoir are the water sources for the irrigation scheme.

Babreka canal is a main concrete lined canal with trapezoidal cross-section. Bottom width of the canal is 2 m, side slope 1:1 and for water discharge  $Q=0.728$  m<sup>3</sup>/s the water depth is  $h=1.08$  m with energy gradient  $J=0.149\%$  (Gadjev, 1989).

In 2009 during the inspection of the Danube Basin Directorate, vegetation and sediment deposition has been found in front of intake sluice gate of the Malka Vitska Irrigation Project. Sediment deposition and vegetation has been found 200 meters upstream from intake from the Vit River. ([http://dariknews.bg/print\\_article.php?article\\_id=423682](http://dariknews.bg/print_article.php?article_id=423682)). Instruction for sediment removing has been given by the Danube River Basin Directorate, but there is no information whether it has been implemented.

At present, a poorly maintained Malka Vitska Irrigation Project is not fully used. The potentially irrigated area on the territory of the Dolni Dabnik Municipality is 9960.2 ha. From them, 1944 ha are not properly irrigated due to amortized hydraulic structures. The real irrigated area is less than 800 ha. Only some vegetables and tobacco are irrigated. There are no water users associations.

## Results and discussion

The bed load transport rate for Babreka Canal is determined and compared in the range from 0.1 mm to 3 mm by seven well-known equations - Meyer-Peter and Muller (1), Einstein – Brown (2), Selim – Yalin (3), Gomez (4), Van Rijn (5), Nagakawa – Tsujimoto (6) and Nielsen equation (7). The bed load transport rate is determined for water discharge  $Q=0.728$  m<sup>3</sup>/s, water depth  $h=1.08$  m, energy gradient  $J=0.149\%$  and Manning's roughness  $n=0.0163$ . The transport capacity of all sediment for Babreka canal is guaranteed when the value of energy dissipation equal to 0.48 Watt/m<sup>3</sup> does not decrease in downstream direction

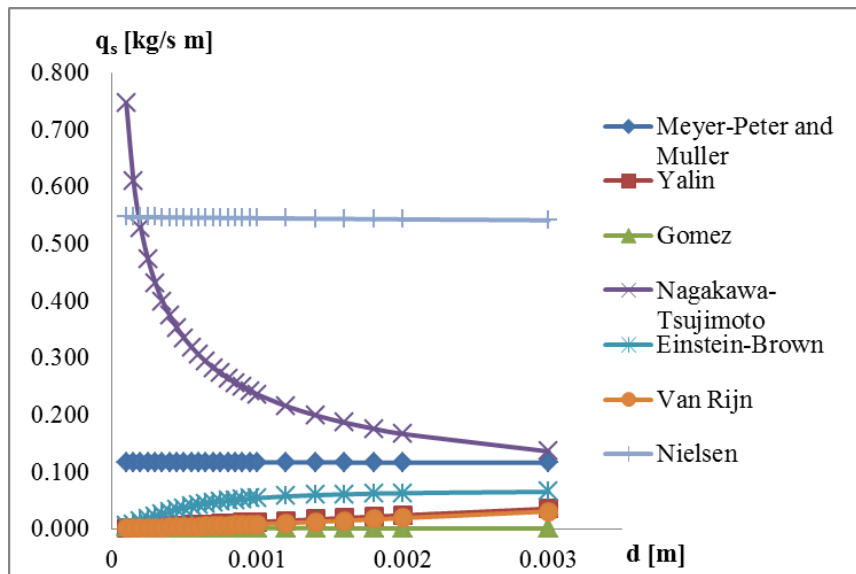


Fig. 1. Bed load transport rate results for Babreka Canal

The results graphically represent in Fig. 1 show that the Nielsen equation (7) and Mayer-Peter Muller equation (1) are more appropriate for diameter particle bigger than 3 mm. It is supposed that the range of smaller diameter particles will incoming into the canal. The results for bed load transport rate, calculated after Nagakawa-Tsujimoto formula (6) have downward trend with particle diameter increasing and this formula was assessed as not appropriate for this case. Gomez equation (4) is low predictor. The similar results were obtained using Van Rijn equation (5) and Yalin equation (3).

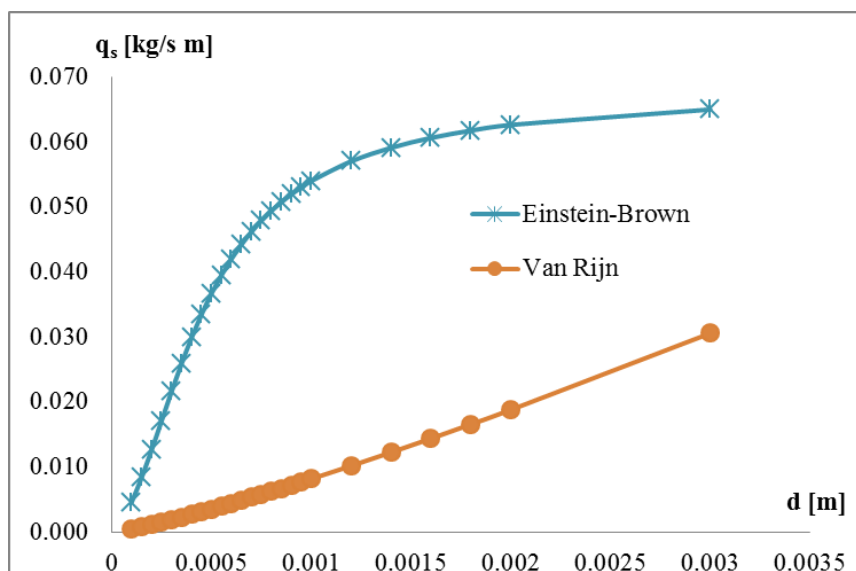


Fig. 2. Bed load transport rate results appropriate for Babreka Canal

In this study the application of Einstein – Brown equation (2) and Van Rijn equation (5) for calculation of bed load transport rate was assessed as appropriate for the studied canal (Fig.2).

The minimum permissible velocity for Babreka Canal was determined in the range from 0.1 mm to 3 mm for water discharge  $Q=0.728 \text{ m}^3/\text{s}$ , water depth  $h=1.08 \text{ m}$ , energy gradient  $J=0.149\%$  and Manning's roughness  $n=0.0163$  using five well-known equations Zamarin (8), Grishkan (9), Roer (10), Poslavskii (11) and Kennedy (12).

The results are shown in Fig. 3. The bed load transport rate results determined using Poslavskii formula (11) have downward trend with particle diameter increasing and equation (11) is not

appropriate for this case. Kennedy formula (12) and Grishkan formula (9) are more appropriate for diameter particle bigger than 3 mm. It is supposed that the range of smaller diameter particles will incoming into the canal.

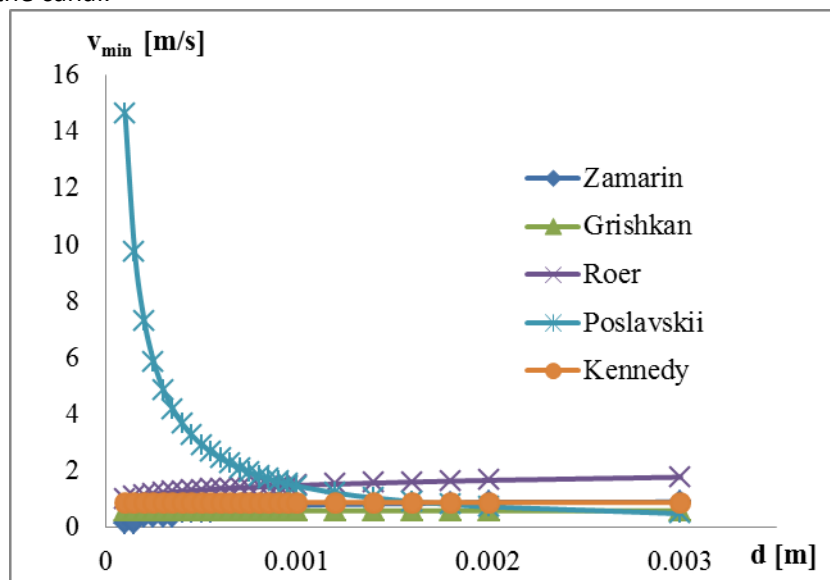


Fig. 3. Minimum permissible velocity results for Babreka Canal

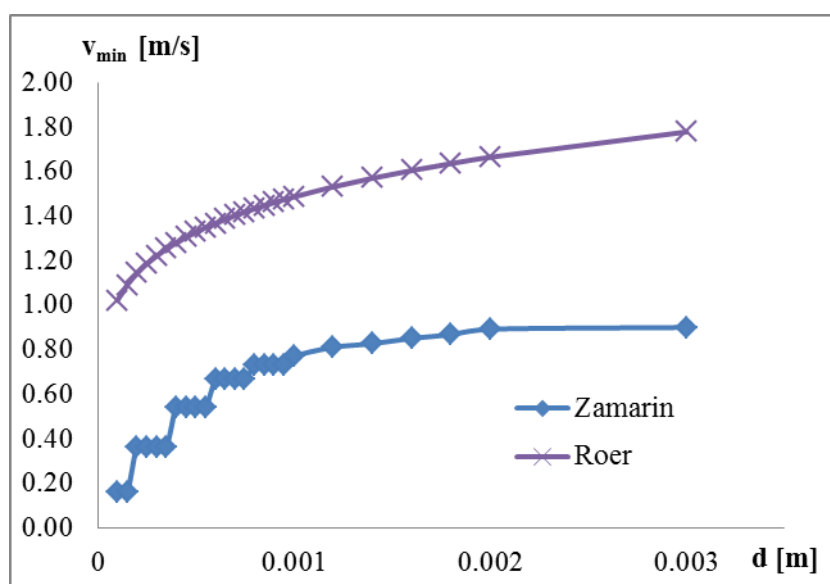


Fig. 4. Minimum permissible velocity results appropriate for Babreka Canal

The application of the Zamarin equation (8) and Roer equation (10) for calculation of minimum permissible velocity was assessed as appropriate for the studied canal (Fig.4).

### Conclusions

The results obtained show substantial differences in the performance of the different formulas. Einstein - Brown formula (2) and Van Rijn formula (5) for bed load transport rate and Zamarin formula (8) and Roer formula (10) for minimum permissible velocity are selected as appropriate for the studied canal.

### References

1. Chow, V. T., (1959). Open channel hydraulics. McGraw-Hill, Inc. New York, N.Y.
2. Das, M.M., (2012). Open Channel Flow, PHI Learning, Delhi

3. Gadjev, G., (1989). Instruction of roughness coefficient selection in open channel, taking into account operating conditions, RIIDHE Project Report (in Bulgarian)
4. Ghazaw, Y.M., (2011). Design and analysis of a canal section for minimum water loss, Alexandria Engineering Journal, Vol. 50, Issue 4, pp. 337-344
5. Gomez, B., (2006). The Potential Rate of Bed-load Transport, Proc Natl Acad Sci U S A. ; 103(46): pp. 17170–17173
6. HR Wallingford, 1992. DORC: User manual. HR Wallingford. Wallingford, UK
7. Hug, M.,(1975). Mécanique des fluides appliqué, Eyrolles, Paris
8. Korpachev V. P. (2009). Theoretical Foundations of water transport timber: monograph. Moscow: Academy of Natural Sciences (in Russian)
9. Nielsen, P., (1992). Coastal Bottom Boundary Layers and Sediment Transport, World Scientific.
10. Quesnel, B.,(1974). Troité d'hydraulique fluviale et tarrentielle appliqué, Eyrolles, Paris
11. Van Rijn, L.C., (1984). Sediment Pick-up Function, Journal of Hydraulic Engineering 110 (10),1984, p.1494
12. Yang, Ch. T., R. Marsooli and M. T. Aalami, (2009). Evaluation of Total Load Sediment Transport Formulas Using ANN, International Journal of Sediment Research, Vol.24, No. 3, pp. 274–286.

**DETERMINATION OF YIELD AND YIELD CHARACTERISTICS OF SOME EARLIER RED LENTIL LINES (*LENS CULINARIS* MEDIC.) IN F7 AND F8 LEVEL**

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**Abstract**

Lentil is one of the most important crops all over the world. Lentil seeds contain high protein, vitamin and essential elements for human healthy. Lentil is produced wide area in Southeastern Anatolia Region of Turkey. This study was aimed to breeding of high yield red lentil varieties to Southeastern Anatolia region of Turkey. Study was conducted in 2011-2012 and 2012-2013 growing seasons under Sanliurfa conditions. Experiment was established to randomize complete block design with 4 replicates. In the study 6 earlier red lentil lines in F7 and F8 level which are obtained from ICARDA and 2 standard varieties were used as a crop material. Statistically differences were found among the tested lines and standard varieties. Flowering period, plant height, first pod height, thousand kernel weight and grain yield were significant at 0.01 levels. According to average of years; the highest grain yield obtained from line 2 (160.14 kg/da), whereas the lowest grain yield was found at line 5 (124.04 kg/da). Some better lines than others were selected in considering to flowering period, plant height, thousand kernel weight and grain yield values.

**Keywords:** Red lentil, earlier red lentil lines, Southeastern Anatolia, grain yield.

**Introduction**

Lentil (*Lens culinaris* Medic.) is an important food source for millions of people all over the world. Lentil belongs to the plant family Leguminosae, it has the ability to fix nitrogen from the air through a symbiotic relationship with bacteria housed in root nodules. At the same time, lentil is incredibly high in protein. In addition, lentil is a good source of vitamins A and B, fiber, potassium, and iron, making it a favorite for people on meat-free diets. In addition, stem and straw of lentil has high quality. It is very important to feed the animal. Moreover, lentil can be grown in rotation with cereal crops.

Red lentil is generally grown in semi-arid regions without irrigation such as Southern Eastern Anatolia region of Turkey. Red lentil is grown in the large areas in Turkey, especially Southern Eastern Anatolia region. Red lentil has 2 354 743 decar sowing area, 345 000 tones production and 147 kg/decar average yield in Turkey. Sanliurfa province which is located in the Southeastern Anatolia Region has the most sowing area and production in Turkey. Sanliurfa province has 1 02 032 decar sowing area, 96 086 ton production and 94 kg/decar average yield (Anonymous, 1997).

Main breeding aims are higher yields, resistance to disease, and suitability for mechanical harvesting, improving seed quality, earliness and resistance to lodge in Southeastern Anatolia Region. Some researchers' results which made in red lentil are given below. Bicer and Sakar (2008) reported that red lentil grain yield ranged from 776.8 kg/ha (FLIP 96-47L) to 3242.3 kg/ha (FLIP 2004-49L) in Diyarbakir conditions. Gupta et al. (1996) conducted a characterization study with 414 lentil lines in India. Researchers reported that the numbers of flowering days ranged from 87 to 143 days, the average weight of 100 kernels were 2.26 g, and the average length of the plant was 28.7 cm. Yilmaz et al. (1996) emphasizes that the highest yield was obtained from a winter-resistant Red 51 variety as 145.1 kg/da in Van ecological conditions. Aydogan et al. (2008) conducted a study to determine grain yield and some yield components of green and red lentils. They reported that the highest and the lowest average yields were 176.2 kg/da and 105.3 kg/da in winter red small sized lentil and spring green large sized lentil, respectively. Oktem and Oktem (2016) conducted a study in

2009-2010 and 2010-2011 growing seasons under Sanliurfa conditions. Researchers reported that genotypes were significant about days to flowering, plant height, first pod height, thousand kernel weight, biological yield and grain yield. Also Oktem (2016) conducted a research was to determine high yield red lentil varieties in 2013-2014 and 2014-2015 growing seasons under Sanliurfa conditions. It was reported that flowering period, plant height, thousand kernel weight and grain yield were found significant at 0.01 level and the highest grain yield was obtained from Sakar variety (186.16 kg/da) while the lowest grain yield was found at Yerli Kırmızı variety (72.82 kg/da). Nazir et al. (2014) conducted an experiment to evaluate performance of newly developed candidate lines of lentil (*Lens culinaris*) in Pakistan during 2011-12. Researchers reported that plant height varied from 29.67 to 38.67 cm.

This study was to aim developing of high yieldly and earliness red lentil varieties to Southeastern Anatolia Region of Turkey.

### Material and methods

This study was conducted in 2011/12 and 2012/13 growing seasons under Sanliurfa conditions. Soil samples were taken from 0 to 20 cm soil layer a day prior to seedling; air dried, passed through a 2.00 mm sieve, and analyzed (Jones 1984). The soil of research field was clay, slightly alkaline, high in lime content, and very low in salt content. Soil properties and climatic data were given in Table 1 and Table 2, respectively.

In the study 6 earlier red lentil lines in F7 and F8 level which are obtained from ICARDA (International Center for Agricultural Research in the Dry Areas) collection and 2 standard varieties, which were Yerli Kırmızı (YK) and Fırat-87 (F-87) were used as a crop material.

Table 1. Some chemical properties of research area soil in 2011/12 and 2012/13

| Year    | Deep (cm) | EC (dS/m) | CaCO <sub>3</sub> (%) | pH       | P <sub>2</sub> O <sub>5</sub> (kg/da) | K <sub>2</sub> O (kg/da) | Org. Mat. (%) |
|---------|-----------|-----------|-----------------------|----------|---------------------------------------|--------------------------|---------------|
| 2011-12 | 0-20      | 62        | 25.8                  | 7.8<br>6 | 1.05                                  | 118.5                    | 0.93          |
| 2012-13 | 0-20      | 58        | 27.3                  | 7.6<br>3 | 2.69                                  | 137.1                    | 2.01          |

Table 2. Some meteorological parameters belong to the growth period of lentil<sup>†</sup>.

| Year | Parameters      | 1     | 2     | 3    | 4     | 5    | 6    | 10   | 11   | 12    |
|------|-----------------|-------|-------|------|-------|------|------|------|------|-------|
| 2011 | Max. Temp. (°C) | 14.6  | 17.8  | 25.2 | 28.5  | 34.9 | 38.9 | 32.7 | 21.4 | 16    |
|      | Min. Temp. (°C) | 0.3   | -0.9  | 2.3  | 4.3   | 11.3 | 17.7 | 8.8  | -0.4 | 0.8   |
|      | Av. Temp. (°C)  | 7.3   | 7.6   | 12.3 | 15.4  | 21.3 | 28.3 | 19.3 | 9.4  | 7.4   |
|      | Humidity (%)    | 62.9  | 64.7  | 46.1 | 59.9  | 46.6 | 30.3 | 53.7 | 53.7 | 57.4  |
|      | Rainfall (mm)   | 58    | 28.2  | 42   | 133.7 | 39.2 | 4.6  | 12.3 | 62.1 | 47.1  |
| 2012 | Max. Temp. (°C) | 14.8  | 16.2  | 21.3 | 32.6  | 33.2 | 42.2 | 37   | 26.9 | 18.3  |
|      | Min. Temp. (°C) | -4.3  | -1.9  | -1.7 | 6.6   | 13   | 17.6 | 11.6 | 7.1  | 1.7   |
|      | Av. Temp. (°C)  | 5.5   | 5.8   | 9.7  | 19.3  | 22.4 | 30.6 | 21   | 14.9 | 8.3   |
|      | Humidity (%)    | 81    | 57    | 47.3 | 42.4  | 40.8 | 21.2 | 48.5 | 65.6 | 73    |
|      | Rainfall (mm)   | 170.9 | 95.8  | 35.8 | 23.3  | 42.3 | 5.8  | 35.2 | 68.4 | 142.8 |
| 2013 | Max. Temp. (°C) | 16.4  | 19.5  | 24.9 | 34.3  | 36.4 | 41.5 | 32   | 27   | 16.9  |
|      | Min. Temp. (°C) | -3    | 2.9   | 0.8  | 7.8   | 11.7 | 17.3 | 10.8 | 5.7  | -2.5  |
|      | Av. Temp. (°C)  | 6.8   | 9.3   | 12.9 | 18.4  | 22.9 | 29   | 19.3 | 14.8 | 6.1   |
|      | Humidity (%)    | 69.5  | 73.6  |      | 44.9  | 43.4 | 24   | 36.0 | 57.5 | 54.6  |
|      | Rainfall (mm)   | 86.8  | 107.2 | 12.1 | 18    | 56.2 | -    | -    | 19.5 | 76.7  |

1.January, 2.February, 3.March, 4.April, 5.May, 6.June, 10.October, 11.November, 12.December

<sup>†</sup>Data collected from the Sanliurfa Meteorological Station (Anonymous, 2014).

Experiment was established to randomize complete block design with 4 replicates. Each parcel was arranged in 6 rows. Plots were 5 m X 0.2 m dimensions. Research area was ploughed firstly (18-20

cm depth) and cultivated (10-12 cm depth) then prepared for planting with a single pass of a disk-harrow. Seeding was made by trial drill and 350 kernel/m<sup>2</sup> seed was used. At sowing, 60 kg ha<sup>-1</sup> of pure N and P, as a 20-20-0 composed fertilizer was applied to each plot. Planting dates were 01.11.2011 and 03.12.2012, respectively. Plants were harvested in May at both years. All tested characteristics were measured on randomly selected 10 plants in the center of each plot. An analysis of variance was performed to evaluate statistically differences between results. Means of the data obtained from research were compared using Duncan test at P≤0.05.

### Results and discussion

Variance analyses were performed separately for each year and combined of years for all traits. Variance analyses of plant height, first pod height, days to flowering, biological yield and grain yield in 2011-2012, 2012-2013 and combined analyses were given in Table 3.

Table 3. Variance analyses of plant height (cm), first pod height (cm), days to flowering (day), 1000 kernel weight (g) and grain yield (kg/da) in 2011-2012, 2012-2013 and combined analyses

| 2011-2012 Growing season |    |              |                  |                   |                    |             |
|--------------------------|----|--------------|------------------|-------------------|--------------------|-------------|
| Mean Squares             |    |              |                  |                   |                    |             |
| Source of Variation      | SD | Plant height | First pod height | Days to flowering | 1000 kernel weight | Grain Yield |
| Replication              | 3  | 0.979        | 6.701            | 8.531             | 2.214              | 83.475      |
| Variety                  | 8  | 56.839**     | 10.966*          | 85.281**          | 215.426**          | 2051.45**   |
| Error                    | 21 | 1.7049       | 4.718            | 3.7217            | 2.030              | 112.87      |
| Total                    | 31 |              |                  |                   |                    |             |
| CV                       |    | 3.59         | 11.55            | 1.51              | 3.64               | 6.38        |
| 2012-2013 Growing season |    |              |                  |                   |                    |             |
| Mean Squares             |    |              |                  |                   |                    |             |
| Source of Variation      | SD | Plant height | First pod height | Days to flowering | 1000 kernel weight | Grain Yield |
| Replication              | 3  | 7.551        | 2.227            | 12.583            | 0.733              | 259.651     |
| Variety                  | 8  | 17.473*      | 17.309*          | 56.50**           | 196.08**           | 1322.20*    |
| Error                    | 21 | 7.6765       | 5.7154           | 2.0357            | 1.331              | 307.74      |
| Total                    | 31 |              |                  |                   |                    |             |
| CV                       |    | 10.03        | 12.03            | 1.37              | 2.67               | 14.70       |
| Combined Analyses        |    |              |                  |                   |                    |             |
| Mean Squares             |    |              |                  |                   |                    |             |
| Source of Variation      | SD | Plant height | First pod height | Days to flowering | 1000 kernel weight | Grain Yield |
| Year (Y)                 | 1  | 1235.82**    | 18.24            | 9096.391**        | 275.5738**         | 35430.2**   |
| Replication              | 3  | 6.08         | 0.56             | 19.1823           | 2.1195             | 97.808      |
| Variety (V)              | 7  | 51.47**      | 10.46*           | 6.9092**          | 395.01**           | 1209.64**   |
| Y X V                    | 7  | 22.80*       | 17.82*           | 0.2844            | 16.497**           | 2164.0**    |
| Error                    | 45 | 4.5413       | 5.0245           | 2.816             | 1.624              | 212.64      |
| Total                    | 63 |              |                  |                   |                    |             |
| CV                       |    | 6.66         | 11.6             | 1.44              | 3.09               | 10.21       |

\*, \*\* indicates statically significant at 0.05 and 0.01 respectively

### Plant height (cm)

According to results of variance analysis; plant height was found statistically significant in both trait years (Table 3). In combined analyses, year, variety and year X variety were statistically significant. Plant height values ranged from 31.7 to 42.4 cm in first year and 25.0 to 30.3 cm in second year (Table 4). The highest plant height values were obtained from Fırat-87 in first year. Yerli Kırmızı and line 1 gave the highest plant height in second year. According to both years of average, the highest plant height values were obtained from standard varieties (Fırat-87 and Yerli Kırmızı). But it was

observed that Firat-87 and Yerli Kırmızı standard varieties had more lodging than others. Hanlan et al. (2006) explained that tall lentil plants have more lodging tendency. Plant height values of lines were smaller than standard varieties in both years. Some results were explained by some researchers. Oktem (2016) explained that the highest plant height value was obtained from Seyran-96 (45.1 cm), while the lowest plant height value was seen at the Kafkas variety (37.3 cm) in Sanliurfa condition. Bicer and Sakar (2007) reported that local varieties had higher plant height than ICARDA lines in Diyarbakir conditions. Nazir et al. (2014) reported that plant height varied from 29.67 to 38.67 cm.

Table 4. Plant height and first pod height values and Duncan groups

| Lines /<br>Varieties | Plant height (cm) |                     |           | First pod height (cm) |          |          |
|----------------------|-------------------|---------------------|-----------|-----------------------|----------|----------|
|                      | 2011-12**         | 2012-13*            | Average** | 2011-12*              | 2012-13* | Average* |
| Line 1               | 34.1 de           | 30.3 a <sup>†</sup> | 32.2 bc   | 19.9 ab               | 22.4 ab  | 21.15 a  |
| Line 2               | 37.9 b            | 28.9 ab             | 33.4 ab   | 19.3 ab               | 21.8 ab  | 20.55 a  |
| Line 3               | 35.6 cd           | 27.3 b              | 31.45cd   | 19.9 ab               | 17.9 c   | 18.90 ab |
| Line 4               | 36.1 bc           | 27.8 ab             | 31.95bc   | 16.0 c                | 22.7 a   | 19.35 ab |
| Line 5               | 31.7 f            | 26.0 b              | 28.85d    | 16.8 bc               | 19.1 bc  | 17.95 b  |
| Line 6               | 32.8 ef           | 25.0 b              | 28.9d     | 18.2 a-c              | 17.8 c   | 18.00 b  |
| YK                   | 40.8 a            | 30.3 a              | 35.5 a    | 19.8 ab               | 18.1 c   | 18.95 ab |
| F-87                 | 42.4 a            | 27.4 ab             | 34.9a     | 20.7 a                | 19.3 abc | 20.00 ab |
| Average              | 36.425            | 27.875              | 32.15     | 18.83                 | 19.89    | 19.36    |
| LSD                  | 1.92              | 3.516               | 2.15      | 3.19                  | 3.516    | 2.26     |

<sup>†</sup>: There is no statistical difference among values annotated with the same letter at P<0.05 according to the Duncan test.

\*, \*\*: indicates statistical significant at 0.05 and 0.01 level, respectively.

#### First pod height (cm)

As can be seen from Table 3, first pod height values were statically significant in both years (P<0.05). In combined analysis; year, variety and year X variety were statically significant (P <0.05). The highest value was obtained from F-87 variety (20.7 cm) in first growing season while the highest value was obtained from Line 4 (22.7 cm) in second growing season (Table 4). According to two years average; first pod height ranged from 17.95 cm to 21.15 cm. Line 1 and Line 2 of first pod height values were higher than standard varieties and other lines. First pod height is important characteristics for machine harvesting. Aydoğan et al. (2002) reported that the first pod height must be at least 12 cm for machine harvesting. Our results were in accord with some researchers results.

Table 5. Plant height and first pod height values and Duncan groups

| Lines /<br>Varieties | Days to flowering (day) |           |           | 1000 seed weight (g) |           |         |
|----------------------|-------------------------|-----------|-----------|----------------------|-----------|---------|
|                      | 2011-12**               | 2012-13** | Average** | 2011-12**            | 2012-13** | Average |
| Line 1               | 123.0 d                 | 100.8 c   | 111.9 d   | 46.5 ab              | 55.3 a    | 50.9 a  |
| Line 2               | 126.0 bc                | 102.3 bc  | 114.15 c  | 47.4 a               | 48.0 b    | 47.7 b  |
| Line 3               | 126.5 bc                | 101.0 c   | 113.75 cd | 26.2 e               | 32.5 e    | 29.35 g |
| Line 4               | 124.5 cd                | 103.3 b   | 113.9 c   | 39.0 c               | 43.1 c    | 41.05 d |
| Line 5               | 128.5 b                 | 103.5 b   | 116 b     | 31.8 d               | 38.4 d    | 35.1 f  |
| Line 6               | 126.0 bc                | 102.8 bc  | 114.4 bc  | 44.8 b               | 47.5 b    | 46.15 c |
| YK                   | 135.0 a <sup>†</sup>    | 109.5 a   | 122.25 a  | 38.0 c               | 39.0 d    | 38.5 e  |
| F-87                 | 135.3 a                 | 110.8 a   | 123.05 a  | 39.4 c               | 42.6 c    | 41 d    |
| Ave.                 | 128.1                   | 104.25    | 116.175   | 39.1                 | 43.3      | 41.2    |
| LSD                  | 2.84                    | 2.174     | 1.69      | 2.10                 | 1.697     | 1.28    |

<sup>†</sup>: There is no statistical difference among values annotated with the same letter at P<0.05 according to the Duncan test.

\*, \*\*: indicates statistical significant at 0.05 and 0.01 level, respectively.

Bicer and Sakar (2007) reported that some pod height of Icarda lines were between 9.67 and 14.67 cm. Erman et al. (2005) stated that first pod height ranged from 10.0 to 16.0 cm. Oktem and Oktem (2016) reported that first pod height values ranged from 27.6 cm and 13.7 cm at small red lentil varieties in Sanliurfa conditions.

#### **Days to flowering (day)**

According to variance analyses days to flowering was significant in both year ( $P < 0.01$ ). In combined analyses, year and variety were significant at  $P < 0.01$  level (Table 3). Days to flowering were between 123.0 day (line 1) and 135.3 day (F.87) in first year, whereas 100.8 day (Line1) and 110.8 day (F-87) in second year (Table 4). According to average of years; days to flowering values changed from 123.05 day (Firat-87) to 111.9 day (line 1). Earlier lines had earlier flowering period than standard varieties. Some researchers' findings were good agreement with our findings about days to flowering period. Turk and Atikylmaz (1999) reported that the lines from ICARDA were earlier than Yerli Kırmızı and Firat-87. Aydoğan et al. (2005) reported that Firat-87 variety had late maturation and late flowering period. Nazir et al. (2014) explained that days to flowering ranged from 80.67 to 114.67 days.

#### **1000 Kernel weight (g)**

According to result of variance analyses; 1000 kernel weight value was found statistically significant in first year ( $P < 0.01$ ) and second year ( $P < 0.05$ ), respectively. In combined analyses, year, varieties and year X varieties were significant ( $P < 0.01$ ). Thousand kernel weight values varied between 26.2 g (Line3) and 47.4 g (Line2) in first year while 32.5 g (Line 3) and 55.3 g (Line 1) in second trait year (Table 5). According to both years of average, the highest 1000 kernel weight value found at line 1 (50.9 g). 1000 kernel weight value of second year was higher than first year value due to rainfall of 2011-2012 growing period higher than 2012-2013 growing period. Erksine and Ashkar (1993) stated that the environment have a significant impact on thousand grain weight. Nleya et al. (2000) emphasize that location and variety effects 1000 kernel weight value.

#### **Grain yield (kg/da)**

According to variance analysis results, grain yield was statistically significant in both years ( $P < 0.01$ ). In combined analyses; year, varieties and year X varieties were found statistically significant at 0.01 level (Table 3). It can be seen from Figure 1 that grain yield values ranged from 128.06 kg/da (Line1) to 190.28 kg/da (Line 2) in first year while varied from 101.6 (Yerli Kırmızı) to 156.7 kg/da (Line 1). According to years of average, the highest grain yield value was obtained from line 2 (160.1 kg/da) whereas the lowest grain yield was found at Line 5 (124 kg/da). Similar findings reported by some researchers. Yılmaz et al. (1996) stated that the highest grain yield was obtained from winter-resistant Red 51 variety (145.1 kg/da). Oktem and Oktem (2016) reported that small red lentil genotypes were significant about days to flowering, plant height, first pod height, thousand kernel weight, biological yield and grain yield. They obtained from the highest grain yield line 1 (228 kg/da), whereas the lowest grain yield was found at Yerli Kırmızı standard variety (138.9 kg/da) in Sanliurfa conditions. Sakar and Biçer (2003), conducted a with 1 line and 4 lentils varieties that the highest grain yield obtain from in Seyran 96. (232.5 kg / da) in Diyarbakır condition.

First year grain yield values were higher than second year values. Rainfall was regularly in the first year especially in the pod binding period. But second year rainfall were insufficient and irregular. Reason of low yields in 2012-2013 growing seasons can be explain as insufficient and irregular rainfall during pod binding period (Table 1). Erksine and Ashkar (1993) reported that variation about 80% on grain yield stemmed from seasonal raining. Silim et al. (1993) emphasized that rainfall effects grain yield especially in pod binding period. If raining is regular in pod binding period, grain yield will be high.

Table 6. Grain yield values and Duncan groups

| Lines /<br>Varieties | Grain Yield (kg/da)   |            |           |
|----------------------|-----------------------|------------|-----------|
|                      | 2011-2012**           | 2012-2013* | Average** |
| Line 1               | 128.06 d              | 156.7 a    | 142.4 b   |
| Line 2               | 190.28 a <sup>†</sup> | 130.0 b    | 160.1 a   |
| Line 3               | 179.44 ab             | 128.0 b    | 153.7 ab  |
| Line 4               | 182.50 ab             | 113.8 bc   | 148.2 ab  |
| Line 5               | 141.39 cd             | 106.7 bc   | 124.0 d   |
| Line 6               | 172.50 b              | 107.7 bc   | 140.1 bc  |
| YK                   | 153.33 c              | 101.6 c    | 127.5 cd  |
| F-87                 | 183.33 ab             | 110.0 bc   | 146.7 ab  |
| Average              | 166.4                 | 119.3      | 142.8     |
| LSD                  |                       | 25.797     | 14.685    |

<sup>†</sup>: There is no statistical difference among values annotated with the same letter at P<0.05 according to the Duncan test.

\*, \*\* : indicates statistical significances at 0.05 and 0.01 level, respectively.

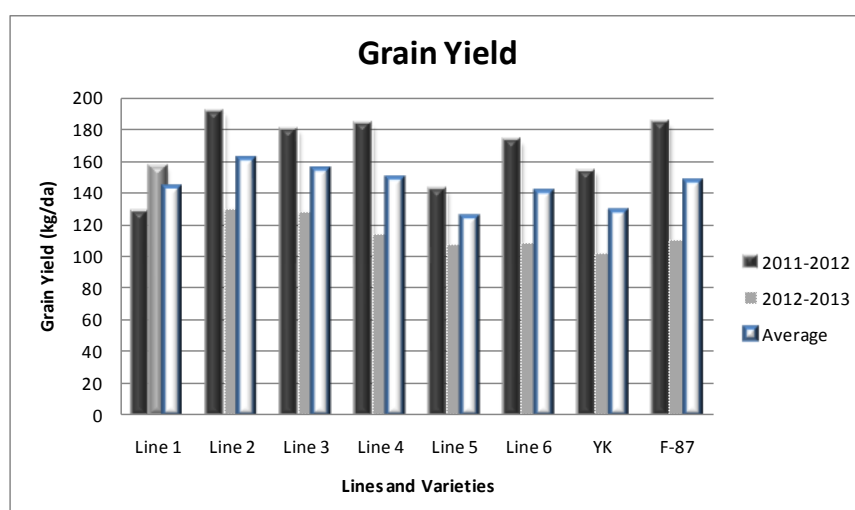


Figure 1. The grain yield values obtained from lines and varieties

## Conclusions

According to two years research results; considering to flowering period, plant height, thousand kernel weight and grain yield; line 1, line 2, line 3, line 4 were found better than others.

## References

1. Anonymous (2014). Climatic Report. Sanliurfa Meteorological Sation, Sanliurfa. 30.01.2014
2. Anonymous (2017). Turkish Statistical Institute. <http://www.tuik.gov.tr> 23.07.2017
3. Aydoğan, A., Karagül, V., Bozdemir, Ç., (2002). Breeding studies of winter lentils (*Lens culinaris* Medik.) in Central Anatolia Region., 11(1-2):1-13 Ref: 32
4. Aydoğan, A., Karagül, V., Bozdemir, Ç. (2005). Adaptation of winter type red lentil varieties in Central Anatolia. Turkey/Sanliurfa GAP IV. Agriculture Congress, 21-23 September, 820-825
5. Aydoğan, A., Karagül, V., Bozdemir, Ç. (2008). Effects of different sowing dates on yield and yield components of green and red lentils (*Lens culinaris* Medik.) J. Field Crops Central Research Institute 17(1-2):25-33
6. Bicer B.T., Sakar D. (2007). ' Determination of yield and yield components in some red lentil lines and cultivars. J.of Fac. of Agric., Omu, 22 (3): 292-296.
7. Bicer, B.T, Sakar D. (2008). Studies on variability of lentil genotypes in Southeastern Anatolia of Turkey. Agrobot. Cluj 36 (1):20-24.

8. Erksine W., Ashkar F.E. (1993). Rainfall and temperature effects on lentil (*Lens culinaris* Medik) seed yield in the Mediterranean environment. J. Agric. Sci. 121:347-354.
9. Erman, M., H. Demirhan, M. Tunçtürk. 2005. Determination of important agricultural and field characteristics of some lentil varieties grown in Siirt ecological conditions in winter. Turkey VI. Field Crops Congress, (I) 237–240, 5–9 September, Antalya.
10. Gupta A., Sinha M.K., Mani V.P., and Dube S.D., 1996. Lens Newsletter. Classification and Genetic Diversity in Lentil Germplasm. Vol.23.
11. Hanlan T. G., Ball, R. A, Vandenberg, A. (2006). Canopy growth and biomass partitioning to yield in short-season lentil. Canadian J of Plant Sci., 86(1):109-119.
12. Jones B.J. (1984) A laboratory guide of exercises conducting soil tests and plant analyses. Madison, Wis. Benton lab., Inc. USA.
13. Nazir Hussain, Muhammad Yaqoob and Abdur Rashid (2014) genetic competition among lentil (*lens culinaris*) candidate lines for yield and yield components under rainfed conditions. Journal of Agricultural Research. Vol. 52 Issue 1 p53-66. 14p.
14. Nleya T., Vandenberg A., Araganosa G., WarkentinT., Muehlbauer F. J., Slinkard E. A. 2000. Produce quality of food legumes: Genotype(G). Environment (E) and (GXE) consideration. Current Plant Science and Biotechnology in Agriculture. R. Knight (ed.).Kluwer Academic Publishers. U.K.
15. Silim S.N., Saxena M.C. and Erksine W. (1993) Adaptation of lentil to the Mediterranean environment. I factors affecting yield under drought conditions. Exp. Agric. 29:9-19.
16. Turk, Z., Atikylmaz N. (2000). A survey on yield and some yield items of lentil (*Lens culinaris* Medik.) Cultivars grown in Diyarbakir ecological conditions. HR. U.. Jour. Agr. Fac., 4 (3-4): 43-52.
17. Oktem, A.G.,2016. Determination of Yield and Yield Characteristics of Some Red Lentil (*Lens culinaris* Medic.) Genotypes Under Sanliurfa Conditions Nevsehir Journal of Sci. and tec. Vol:5 (1) 27-34.
18. Oktem, A.G., Oktem, A. 2016. Determination of Yield and Yield Characteristics of Some Small Sized Red Lentil Lines (*lens culinaris* medik.) in f5 and f6 Level. VII International Scientific Agriculture Symposium, Agrosym 2016“ 662-668.
19. Yılmaz N., Erman M., Kulaz H., 1996. Van Ekolojik Koşullarında Mercimekte (*Lens Culinaris* Medic) Uygun Ekim Zamanının Belirlenmesi. Ondokuz Mayıs Üniversitesi Journal of Agric. 11,(3) : 45-54. Samsun.

## VARIATION OF DEHYDROGENASE ENZYME AKTIVITY AND DIFFERENT PEDOGENETIC DEVELOPMENT ON WEATHERED BASALTIC TOPOSEQUENCES

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### Abstract

Enzymes play the most important role in the decomposition and deterioration of organic matter in the soil. Most of the microbial activity in the soil is determined by measuring the activities of the enzymes. The aim of this study is to examine the changes in dehydrogenase enzyme activity in different pedological development soils formed on the same parent material, but with different slopes, land cover and land use under semihumid climatic conditions. It was carried out on the soil formed on the basaltic parent material in different topographical positions within the Dağköy area of Engiz district, which is located at the south of the Samsun-Bafra Highway. In this study, dehydrogenase enzyme activity was assessed in order to reveal the biological properties of the soil of the study area and it was investigated the dehydrogenase enzyme activity in terms of soil biological property. For this aim, six soil profiles formed on weathered basaltic parent material and located on toposequence of north-south transect were described according to genetic horizon and classified as Lithic Ustorthent, Vertic Haplustept, Typic Haplustept and Typic Haplustert. It was determined that the dehydrogenase changed between 0.073-1.170 µg TPF in soils taken from each profiles. In addition, when the statistical relationship between dehydrogenase enzyme activity and different profiles, land use, and height along the north-south transect was investigated different profiles and different heights were found to be important at % 1 level of effect on dehydrogenase enzyme activity in the soil ( $P=0.006<0.01$ ). Also the effect of different land use patterns were found to be important at % 5 level on the activity of dehydrogenase enzyme in the soil ( $P = 0.048<0.05$ ).

**Keywords:** Soil microorganisms, dehydrogenase enzyme activity, pedogenesisi, toposequence.

### Introduction

Soil microorganisms actively participate in many processes such as mineralization of plant and animal wastes added to soils and biochemical transformation of plant nutrients and also have important effects on soil fertility (Alexander, 1977). For this reason, soil fertility is not only related to the physical conditions and the level of nutrients of the soil, but also the intensity of biological events.

The studies until today reveal that the quality of the soil, the level of fertility and measures to be taken in terms of plant nutrition and the issues related with physical, chemical, biochemical, microbiological, mineralogical, zoological and plant physiology of soil should be considered together (Schinner, 1986). Determination of the biological properties of soils is based on the principle of measuring the activities of microorganisms together with the number and distribution of microorganisms in the soil (Gök ve Onaç, 1995). The food cycle in the soil involves biochemical, chemical and physicochemical reactions and many biochemical events through Soil organisms, plant roots and soil enzymes originating from microorganisms are carried out (Tabatabai, 1982). In this respect, while investigating the microbiological characteristics of soils, the products formed as a result of microbial activity and enzymes secreted during activity are also measured besides of the number and distributions of microorganisms in the soil (Müller, 1965). Dehydrogenase activity, one of the enzymes used in determining the biological properties of soils, is an intracellular enzyme (Trevors, 1984) found in living microbial cells in the soil and it is an indicator used in the assessment

of microbial activity in the soil (Garcia ve ark., 1994). Thus, dehydrogenase activity is a marker of biological redox systems and is used as a measure of the density of microbial metabolism in the soil (Okur, 1997).

The aim of this study is to examine the changes dehydrogenase enzyme activity in soils developed on the weathered basaltic toposequence. Soils formed on the same parent material on the other hand, they founded on different physographical units and land cover-land uses under semihumid climatic conditions within the Dağköy area of Engiz district, which is located at the south of the Samsun-Bafra Highway. Therefore, this paper presents also effects of pedogenesis development, topographic changing, elevation, variation of land cover and land use on dehydrogenase enzyme activity.

## Material and methods

### General Definition of The Research Field

This study was carried out in the south eastern part of the Samsun-Bafra Plain located in the Kızılırmak Delta in the central Black Sea Region of Turkey (Figure. 1). The study area is located approximately 20 km west of the Samsun provincial centre (4594000-4598000 N, 751000-758000 E, UTM/WGS 84 m).

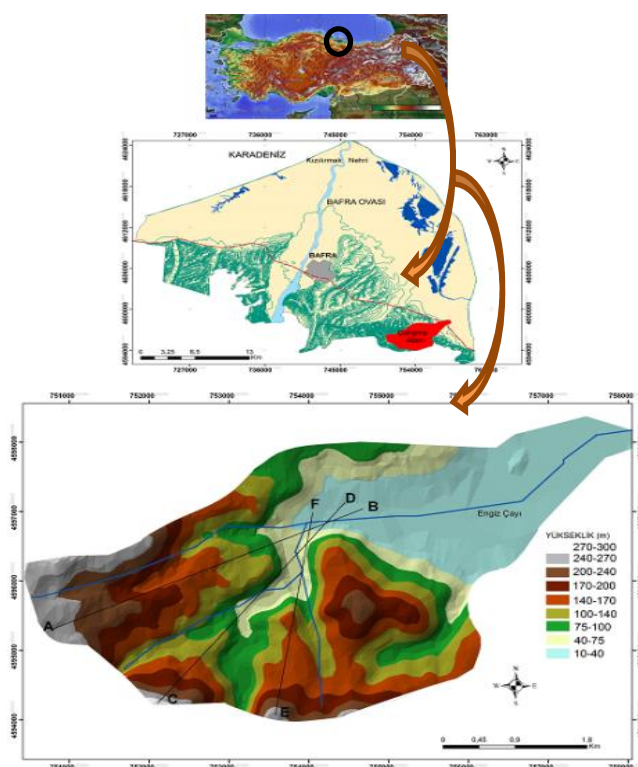


Figure 1. Location map of the study area and cross section with elevation

The Engiz Brook plays a major role in the formation and development of the landscape. Considered, in the soils formed on the basalt parent material there are pastureland and dry farming lands. In addition, only small part of the study area has been covered by forest land. Some forest land was also degraded due to misuse applications. The current climate in the region is semi-humid. The coldest month of the year is February in the study area (5.6 °C) whereas, the hottest months are July and August (22.6 °C). The mean annual temperature, rainfall and evaporation are 13.6°C, 764.3 mm and 726.7 mm, respectively.

The profile pits location were determined by the preliminary field excursion and office works with base data evaluation such as geological, topographical and land use land cover maps after that field work using the GPS tool in order to determine profile coordinates in the study area, profile pits were excavated defined points in field. Soil samples were taken from each horizon of profiles and brought

to the laboratory. After pre-treatment process of soil samples, physical, chemical and biological analyses were performed. Morphological studies were carried out in the field using Soil Survey Staff (1993).

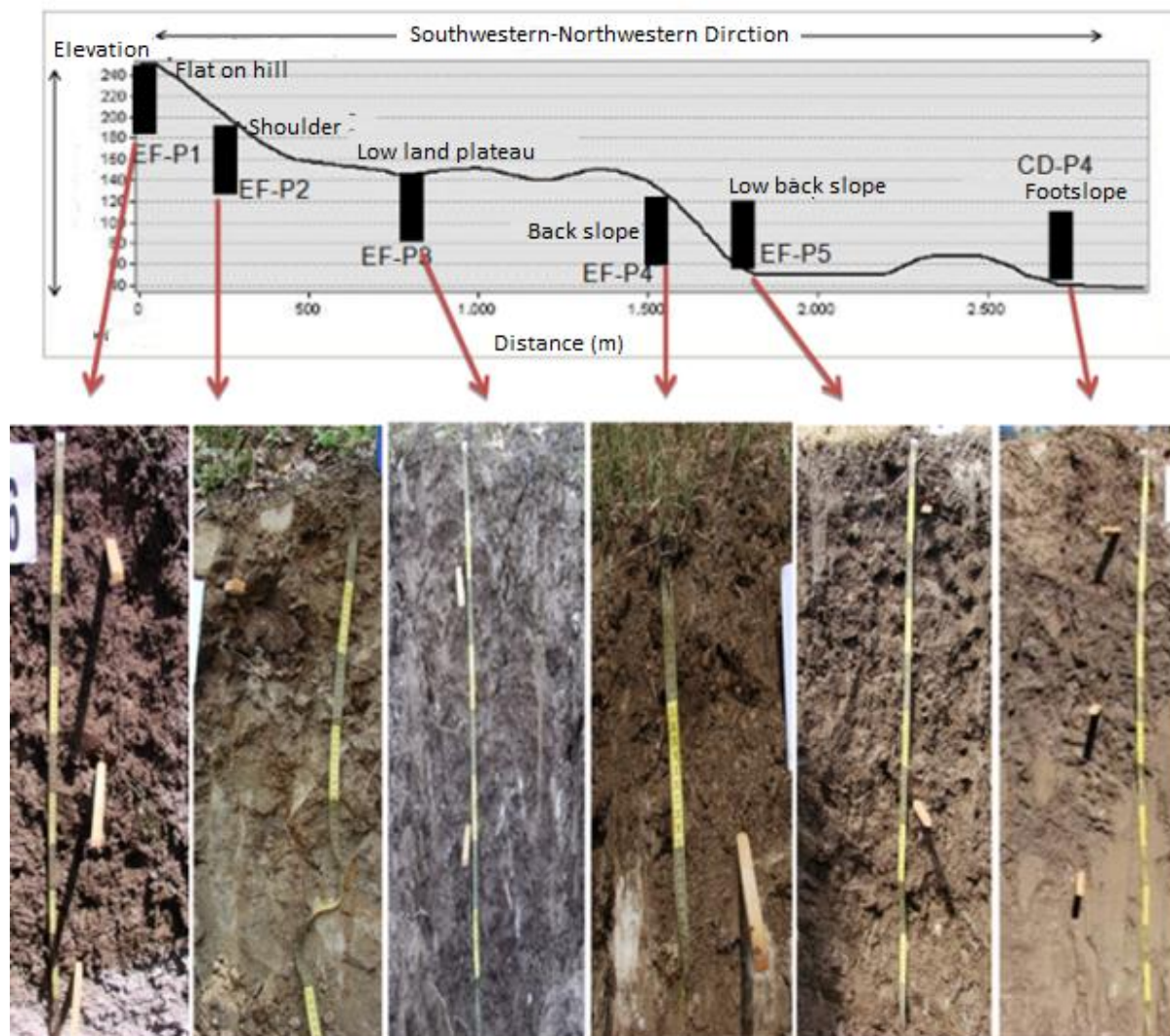


Figure 2. Transect of the four different soil profiles on the same parent material but different topographic positions

#### Physical, Chemical and Biological Analyzes

Texture: in disturbed soil samples (Bouyoucos, 1951); Cation exchange capacity: using sodium acetate (NaOAc) adjusted to pH 8.2 and 1 N ammonium acetate (NH<sub>4</sub>OAc) (Rhoades, 1986), Exchangeable cations (Na and K): using ammonium acetate (NH<sub>4</sub>OAc) adjusted to pH 8.2 (Rhoades, 1986), Ca + Mg was determined by the difference between cation exchange capacity and total exchangeable sodium and potassium. Lime; using Scheibler calcimetry in the determination of free carbonates (Soil Survey Staff, 1993), Soil reaction (pH); Using pH meter in saturation mud (Soil Survey Labrotory, 1992, 2004), Electrical conductivity; (Soil Survey Labrotory, 1992, 2004) using a conductivometer instrument in saturation mud, Organic matter was done according to Walkley-Black method by Jackson's modified (Jackson, 1958).

The dehydrogenase enzyme activity of the soils taken from the profiles was determined by Pepper et al. (1995). For this purpose, glucose and 3% TTC (2,3,5-triphenyltetrazolium chloride) solution were added to the soil sample and incubated for 24 hours at 25 °C. TPF (triphenylformazan) formed at the end of incubation was extracted with methanol and the resulting red color intensity was determined on a spectrophotometer at 485 nm versus the standard TPF series. Each analysis was made in 3 parallels and the findings were expressed in µg TPF g<sup>-1</sup> dry soil.

### Statistical Analysis

SPSS 17.0 package program was used to determine the relationship between dehydrogenase enzyme activity and different profiles, land use and elevation in the soil samples of the study area.

### Results and discussion

#### Physical, Chemical Properties and Classification of Soils in the Study Area

Classification, physiographic position, land use-land cover, elevation, physical and chemical analysis results of different soils (Soil Taxonomy, 1999), located in different topographical positions in the North-South direction are given in Table 1.

Table 1. Classification of the study area soils, physiographic position, land use-land cover, height, physical and chemical analysis results.

| Horizon                                                           | Depth (cm) | pH   | EC dS.m <sup>-1</sup> | Lime % | Organic Matter % | Exchangeable Cations (cmol.kg <sup>-1</sup> ) |                |                                    | Texture % |       |      |       |
|-------------------------------------------------------------------|------------|------|-----------------------|--------|------------------|-----------------------------------------------|----------------|------------------------------------|-----------|-------|------|-------|
|                                                                   |            |      |                       |        |                  | Na <sup>+</sup>                               | K <sup>+</sup> | Ca <sup>++</sup> +Mg <sup>++</sup> | Clay      | Silty | Sand | Class |
| CD-P4/ Typic Haplustert/ Low footslope/ Dry Agriculture / 20 m    |            |      |                       |        |                  |                                               |                |                                    |           |       |      |       |
| Ap                                                                | 0-23       | 7.50 | 0.17                  | 0.20   | 1.65             | 0.22                                          | 1.67           | 40.19                              | 56.2      | 23.1  | 20.7 | C     |
| Bss1                                                              | 23-65      | 7.30 | 0.44                  | 0.98   | 1.26             | 0.25                                          | 1.47           | 39.64                              | 62.6      | 12.8  | 24.5 | C     |
| Bss2                                                              | 65-106     | 8.25 | 0.17                  | 1.10   | 1.09             | 1.33                                          | 1.41           | 37.59                              | 68.4      | 15.8  | 15.8 | C     |
| C                                                                 | 106-146    | 8.14 | 0.11                  | 2.67   | 0.14             | 1.35                                          | 1.40           | 37.03                              | 78.4      | 2.8   | 18.8 | C     |
| EF-P5 / Vertic Haplustept / Low backslope / Dry Agriculture/ 50 m |            |      |                       |        |                  |                                               |                |                                    |           |       |      |       |
| Ap                                                                | 0-15       | 8.28 | 0.26                  | 0.79   | 2.21             | 0.36                                          | 0.36           | 42.97                              | 58.3      | 20.0  | 21.7 | C     |
| Bw                                                                | 15-66      | 8.12 | 0.18                  | 1.26   | 0.67             | 0.47                                          | 0.32           | 42.58                              | 61.9      | 25.6  | 12.5 | C     |
| 2Cr                                                               | 66-106     | 8.20 | 0.25                  | 1.75   | 0.55             | 1.02                                          | 0.26           | 26.49                              | 37.3      | 34.8  | 27.9 | CL    |
| EF-P4 / Lithic Ustorthent / Backslope/ Dry Agriculture/ 135 m     |            |      |                       |        |                  |                                               |                |                                    |           |       |      |       |
| A                                                                 | 0-19       | 7.89 | 0.40                  | 0.39   | 3.37             | 0.16                                          | 0.49           | 40.02                              | 32.5      | 18.6  | 48.9 | SCL   |
| R                                                                 | 19-32      | -    | -                     | -      | -                | -                                             | -              | -                                  | -         | -     | -    | -     |
| EF-P3 / Typic Haplustept / Alçak Plato Düzlüğü / Forest /160 m    |            |      |                       |        |                  |                                               |                |                                    |           |       |      |       |
| A                                                                 | 0-18       | 7.06 | 0.44                  | 1.08   | 1.88             | 0.24                                          | 0.32           | 46.71                              | 54.4      | 22.6  | 23.1 | C     |
| Bw                                                                | 18-57      | 7.31 | 0.42                  | 1.02   | 0.06             | 0.34                                          | 0.09           | 50.11                              | 61.1      | 17.7  | 21.2 | C     |
| Cr                                                                | 57-87      | 7.01 | 0.51                  | 0.39   | 0.57             | 0.58                                          | 0.09           | 39.87                              | 57.5      | 22.6  | 19.9 | C     |
| EF-P2 / Lithic Ustorthent / Shoulder / Degraded Forest / 190 m    |            |      |                       |        |                  |                                               |                |                                    |           |       |      |       |
| A                                                                 | 0-11       | 6.74 | 0.35                  | 0.09   | 1.54             | 0.35                                          | 0.43           | 27.92                              | 22.0      | 21.8  | 56.2 | SL    |
| Cr                                                                | 11-65      | 7.01 | 0.20                  | 0.29   | 0.87             | 0.54                                          | 0.12           | 17.00                              | 14.6      | 9.3   | 76.1 | LS    |
| EF-P1 / Vertic Haplustept / Flat on hill / Pasture / 251 m        |            |      |                       |        |                  |                                               |                |                                    |           |       |      |       |
| A                                                                 | 0-12       | 7.14 | 0.55                  | 0.69   | 3.53             | 0.29                                          | 0.58           | 48.85                              | 61.9      | 23.6  | 14.5 | C     |
| Bw1                                                               | 12-41      | 7.70 | 0.54                  | 0.98   | 1.78             | 0.27                                          | 0.31           | 43.98                              | 49.9      | 27.7  | 12.5 | C     |
| Bw2                                                               | 41-84      | 7.92 | 0.11                  | 0.98   | 1.41             | 0.64                                          | 0.29           | 51.62                              | 47.9      | 32.3  | 19.8 | C     |
| 2Ck                                                               | 84-105     | 7.94 | 0.38                  | 6.37   | 1.29             | 0.63                                          | 0.09           | 43.74                              | 40.0      | 40.8  | 19.1 | C     |

EC: Electrical conductivity, OM: Organic matter, C:Clay, Si:Silty, S:Sand

Vertic Haplustept and Typic Haplustept have deep soils due to their formation on gentle slope lands. Their textures are heavy and clay changes between %54.4 and %61.9, %47.9 and %61.9 on the surface soil and the subsurface horizons, respectively. On the other hand, Lithic Ustorthent located on the slope lands has clay content changes between %22.0 and %32.5. The content of organic matter varies between %1.88 and %3.53 in the surface layers of the Vertic Haplustept and Typic Haplustept soils, but this ratios decrease with increasing depth. In Lithic Ustorthent soil, organic matter values change between %1.54 and %3.37. All soil has low contents of lime in the genetic horizons and slightly alkaline reaction. There is no salinity problem in the soils.

Profiles coded as EF-P4 and EF-P2 were characterized as young soils because they do not have a sub-surface diagnostic horizons and were classified in the Entisol order. These soils on sloping lands have particularly shallow soil depth resulted from covered with low vegetation and misapplication farming practices. In this case, these soils do not have enough pedogenetic processes due to

exposure to the resulting erosion. Therefore, there are usually no diagnostic horizons except for a lithic contact in a depth of 50 cm below the surface and ochric epipedone on the surface of these soils. These soils were classified under the Ustorthent and Lithic Ustorthent subordinate due to the moisture regime in the orthent subordinate due to their location on the slope land. On the other hand, due to the fact that profiles coded as EF-P5, EF-P3, EF-P1 and CD-P4 located on the low slope topographical positions such as footslope, low land plateau and flat on hill have more advanced soil formation than the Entisol with the diagnostic horizon (cambic-Bw) and slicensides (Bss). These profiles were classified in Inceptisol order except for CD-P4 that were classified as Vertisol due to slicenside formation. the result is that the soil moisture regime is ustic Ustept subordinate and Haplustept were placed in large group. EF-P5 and EF-P1 were classified as Vertic Haplustept due to their vertical properties on the surface like cruct features and also EF-P3 is classified as Typic Haplustept due to their large group properties. In the soil of CD-P4 profile, the amount of swelling clays is too much (along the profile %50 or more), it has cracks extending from the surface in the dry seasons and are placed in the Vertisol order due to the appearance of sliding surfaces in the profile. Due to the ustic moisture regime, it has been placed in the ustert subordinate and in the large group of haplustert and Typic Haplustert subgroup due to the fact that all the properties of the large group are carried.

### Biological Properties of Soils in The Study Area

The determined dehydrogenase activity in the soil is an intracellular enzyme has been frequently used in the assessment of the microbiological activity of that soil and it shows the total amount of oxidative activity of soil microflora (Skujins 1973; Trevors 1984; Kızılkaya ve Aşkın, 2006; Aşkın ve Kızılkaya, 2009). Analyzes performed to determine the dehydrogenase enzyme activity of the soils taken from the profiles were carried out in 3 replicates and the results of this analysis are given in Table 2.

Table 2. Concentrations of dehydrogenase enzyme activity characteristics of soil samples taken from the profiles

| Horizon                                                           | Depth (cm) | Dehydrogenase Activity ( $\mu\text{g TPF g}^{-1}$ ) |
|-------------------------------------------------------------------|------------|-----------------------------------------------------|
| CD-P4/ Typic Haplustert/ Low footslope/ Dry Agriculture / 20 m    |            |                                                     |
| Ap                                                                | 0-23       | 1.010                                               |
| Bss1                                                              | 23-65      | 1.170                                               |
| EF-P5 / Vertic Haplustept / Low backslope / Dry Agriculture/ 50 m |            |                                                     |
| Ap                                                                | 0-15       | 0.534                                               |
| Bw                                                                | 15-66      | 0.701                                               |
| EF-P4 / Lithic Ustorthent / Backslope/ Dry Agriculture/ 135 m     |            |                                                     |
| A                                                                 | 0-19       | 0.360                                               |
| EF-P3 / Typic Haplustept / Alçak Plato Düzlüğü / Forest /160 m    |            |                                                     |
| A                                                                 | 0-18       | 0.819                                               |
| Bw                                                                | 18-57      | 0.073                                               |
| EF-P2 / Lithic Ustorthent / Shoulder / Degraded Forest / 190 m    |            |                                                     |
| A                                                                 | 0-11       | 0.147                                               |
| EF-P1 / Vertic Haplustept / Flat on hill / Pasture / 251 m        |            |                                                     |
| A                                                                 | 0-12       | 0.840                                               |
| Bw                                                                | 12-41      | 0.332                                               |

As a result of dehydrogenase analysis of soil samples taken from each horizon of the profiles, it was determined that the dehydrogenase activity that decreased with the soil depths increase were generally lowered in the soil. Dehydrogenase activity is an indicator particularly used in the evaluation of aerobic microorganisms. It is therefore an expected result that high dehydrogenase activity was determined in the surface soil layers due to dominant aerobic conditions. Similarly, Lorenz and Kandeler (2004), Dengiz et al. (2007), Kızılkaya et al. (2007, 2010), Marinari and Antisari

(2010), Antisari et al. (2010), Babu et al. (2010) have also found that topsoil has higher microbial activity.

#### Relationships between Profile, Land Use, Elevation and Dehydrogenase Enzyme Activity

The results of the biological analysis considered within this study were evaluated statistically using three parameters which are soil profiles, land use-land cover and the elevation. Results were presented in Table 3.

According to the ANOVA test result, different profiles and different elevation along the North-South direction were found to be important at %1 level of the effect on dehydrogenase enzyme activity from biological properties of soil ( $P = 0.006 < 0.01$ ). According to the DUNCAN test, it was determined that the highest dehydrogenase enzyme activity along the toposequence is in profile coded as CD-P4 and the lowest dehydrogenase enzyme activity is in profile coded as EF-P2. In terms of height, it was determined that the highest dehydrogenase enzyme activity was 25 m high and the lowest dehydrogenase enzyme activity was 190 m high. It was found that the different land use patterns throughout the EF line were significant at 5% level of the effect on dehydrogenase enzyme activity in the soil ( $P = 0.048 < 0.05$ ). It has been determined that the highest dehydrogenase enzyme activity is in dry agriculture areas and the lowest dehydrogenase enzyme activity is in degraded forest soil.

Table 3. Changes in dehydrogenase enzyme activity depending on the profile, elevation and land use of soils taken from the profiles located on the north-south direction

| Profiles    | Mean<br>+<br>Standard Error | Elevation<br>(m) | Mean +<br>Standard Error | Land Use        | Mean +<br>Standard<br>Error |
|-------------|-----------------------------|------------------|--------------------------|-----------------|-----------------------------|
| CD-P4       | 1.01±0.168a                 | 25.00            | 310.85±8.694a            | Dry Agriculture | 0.72±0.094a                 |
| EF-P5       | 0.61±0.044ab                | 50.00            | 176.27±14.360ab          | Degraded forest | 0.58±0.114ab                |
| EF-P4       | 0.36±0.009bc                | 135.00           | 88.42±6.714b             | Pasture         | 0.34±0.117b                 |
| EF-P2       | 0.14±0.011c                 | 160.00           | 193.29±85.496ab          | Significant     | 0.048                       |
| EF-P1       | 0.58±0.114abc               | 190.00           | 170.87±6.835ab           |                 |                             |
| EF-P3       | 0.45±0.165bc                | 251.00           | 194.75±53.277ab          |                 |                             |
| Significant | 0.006                       | Significant      | 0.045                    |                 |                             |

#### Conclusion

This study was carried out in different pedologically developed soils formed on decomposed basaltic parent material in different topographic positions within the Dağköy area of Engiz district located to the south of Samsun-Bafra highway. In addition, it was examined that the changes of dehydrogenase enzyme activity and soil development under the same parent material, different slope and land use. For this purpose, six profile pits were described on the toposequence in the north-south direction. All profiles were defined according to the horizon principle and classified as Lithic Ustorthent, Vertic Haplustep, Typic Haplustept and Typic Haplustert. When the land is examined in terms of the topographic features, it can be said that where the slope is less or low, the developing period of the soil is more advanced level than the places with more slopes due to soil transportation by means of erosion. As a result of the statistical analysis, it was found that different profiles and different elevation along the North-South direction within the basin were significant at %1 level of effect on dehydrogenase enzyme activity from biological properties of soil ( $P = 0.006 < 0.01$ ). Different land use forms were found to be significant at %5 level of the effect on dehydrogenase enzyme activity in the soil ( $P = 0.048 < 0.05$ ). In this case, in order to increase productive ability and to conserve for sustainability of soil, it is necessary not only to take some measurements which can be successful implemented, but also to know and investigate all properties soil in terms of physical, chemical, pedological as well as biological variations. Improving the biological properties as well as the physical and chemical properties in reaching the highest yield power of the soil, which constitutes the main source of plant and animal nutrients, has great importance. For this reason, it is necessary to

concentrate on biologic activities of the soils, that is, applications to increase the natural microbial population and avoid of applications to reduce biological activity. Therefore, the determination of the microbiological and biochemical characteristics of soils with extensive and detailed microbiological studies has a very large importance for the sustainability of agricultural soils.

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### References

1. Alexander, M., 1977. Introduction to soil microbiology. 2<sup>nd</sup> Edition John Wiley. Sons. Inc. New York, USA, 115-147.
2. Antisari, V.L., Marinari, S., Dell'Abate M.T., Baffi, C., Vianello, G., 2010. Plant Cover and Epipedon SOM Stability as Factors Affecting Brown Soil Profile Development and Microbial Activity, *Geoderma*, 161, 212-224.
3. Aşkın T, Kızılkaya R, 2009. Soil basal respiration and dehydrogenase activity of aggregates: a study in a toposequence of pasture soils. *Zemdirbyste-Agriculture* 96(1), 98-112.
4. Babu, V.S.M., Parma, V., Kumar, Anil S., 2010. Enzymes Activities in Soils under Central Dry AgrClimatic Zone of Karnataka, India as Influenced by Soil Depth, Organic and Conventional Management Systems, *European Journal of Soil Biology*, 3(1), 50-53.
5. Bouyoucos, G.J., 1951. A recalibration of the hydrometer method for making mechanical analysis of soils. *Agronomy Journal* 43, 434-438.
6. Dengiz, O., Kızılkaya, R., Göl, C., Hepşen, Ş., 2007. Effects of Different Topographic Positions on Soil Properties and Soil Enzymes Activities, *Asian Journal of Chemistry*, 19(3), 2295-2306.
7. Garcia, C., Hernandez T., Costa F., Ceccanti, B., 1994. Biochemical parameters in soils regenerated by the addition of organic wastes. *Waste Management and Research* 12, 457-466.
8. Gök, M., Onaç, I. 1995. "Hilvan ve Baziki Ovalarında Yer Alan Yaygın Toprak Serilerinin Bazı Mikro Biyolojik Özellikleri". *Toprak İlmi Derneği İlhan Akalan Toprak ve Çevre Sempozyumu*. Ankara.
9. Jackson, M. L. 1958. *Soil Chemical Analysis*. Englewood Cliffs, New Jersey: Prentice Hall Inc.
10. Kızılkaya R, Aşkın T, 2006. The spatial variability of soil dehydrogenase activity: a survey in urban soils. X.Congress of Croatian Society of Soil Science on Soil Functions in the Environment. June 14-17, 2006. Sibenik, Croatia. Abstract Book (ISBN 953-6135-54-X). p.68.
11. Kızılkaya, R., Dengiz, O., Hepşen, Ş., Başkan, O., 2007.  $\beta$ -Glucosidase Enzyme Activity and Its Relationships with Physico-Chemical Properties in Çatalkaya Basin, Ankara, Ninth Baku International Congress, Energy, Ecology, Economy, 7-9 June, Baku, Azerbaijan.
12. Kızılkaya, R., Dengiz, O., Alpaslan, T., Durmuş, M., Işıldak, V., Aksu, S., 2010. Changes of Soil Microbial Biomass C and Basal Soil Respiration in Different Land Use and Land Cover. International Soil Science Congress on Management of Natural Resources to Sustain Soil Health and Quality, Sayfa: 1039-1046, 26-28 May, Samsun, Turkey.
13. Lorenz, K., Kandeler, E., 2004. Biochemical Characterization of Urban Soil Profiles from Stuttgart, Germany, *Soil Biology and Biochemistry*, 37, 1373-1385.
14. Marinari, S., Vittori Antisari L., 2010. Effect of Lithological Substrate on Microbial Biomass and Enzyme Activity in Brown Soil Profiles in the Northern Apennines (Italy), *Pedobiologia*, 53, 313-320.
15. Müller, G., 1965. *Bodenbiologie*. VEB Gustov Fischer Verlag Jena.
16. Okur, N., 1997. *Toprak Enzimleri Ders Notları*. Ege Üniversitesi Ziraat Fakültesi Toprak Bölümü, Bornova, İzmir.
17. Pepper, I. L., Gerba, C.P., Brendecke, J.W., 1995. *Environmental Microbiology: A laboratory anual*. Academic Press, New York, pp. 175.
18. Rhoades, J. D. 1986. Cation Exchange Capacity, Chemical and Microbiological Properties. *Methods of Soil Analysis, Part II*. Madison: ASA and SSSA Agronomy Monograph, No: 9.

19. Rowell, M.J., Ladd, J.N., Paul, E.A. 1973. Enzymatically Active Complexes of Proteases and Humic and Analogues. *Soil Biol. Biochem.* (5): 699-703.
20. Schinner, F. 1986. "Veröffentlichungen der Landwirtschaftlich-Chemischen Bundesanstalt Linz / Donau". Seminar: Die Anwendung Enzymatischer und Mikrobiologischer Methoden in der Bodenanalyse.
21. Skujins J, 1973. Dehydrogenase: An indicator of biological activities in arid soil. *Bulletin Ecological Communication (Stockholm)* 17: 97-110.
22. Soil Survey Staff. 1992. Procedures for Collecting Soil Samples and Methods of Analysis for Soil Survey.
23. Soil Surv. Invest. Report, Washington D.C., USA: I. U.S. Gov. Print. Office.
24. Soil Survey Staff. 1993. Soil Survey Manual, USDA Handbook, Washington D.C., No: 18.
25. Soil Survey Staff, 2004. Soil Survey Laboratory Methods Manual Soil Survey Investigations Report, USDA, No:42.
26. Tabatabai, M.A., 1982. Soil Enzymes. In *Methods of Analysis, Part 2, 2nd Ed.* A. L. Page Et Al (Eds). *Agronomy J.* (9): 903-947.
27. Trevors, J.T., 1984. Rapid Gas Chromatographic Method to Measure H<sub>2</sub>O<sub>2</sub> Oxidoreductase (Catalase) Activity in Soil, *Soil Biology and Biochemistry*, 16, 525-526.

**INDICATORS OF RURAL POVERTY IN THE REPUBLIC OF MACEDONIA  
- MULTIDIMENSIONAL POVERTY ANALYSIS**

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**Abstract**

Rural poverty presents an escalating problem for post-communist countries struggling with perpetual transition towards market economy. As a country deriving from such economic background, Republic of Macedonia is classified as one of the poorest countries in Europe. The rural population in the country is faced with different obstacles such as: limited access to institutions, infrastructure, finances, and other aspects which not only impede these population's living conditions but also obstruct the development and growth of the rural areas. In order to provide detailed description of the rural poverty situation as well as to identify the vulnerable and deprived group in the rural areas in the Republic of Macedonia, this paper represents an initial record of the poverty conditions in the rural areas. The poverty features are presented through the multi-dimensional poverty analytical tool and the indicators and dimensions of poverty on micro and macro (country) level in order to describe the socio-economic, environmental, political and institutional context. The comprehensive multi-dimensional analysis of the poverty dimensions in the Republic of Macedonia gives overview of the rural poverty situation and highlights several groups, suffering from multiple deprivations such as rural woman and agriculture households, which are part of an extremely vulnerable group, with the highest risk of going under the poverty base lines.

**Keywords:** agriculture, deprivation, post-communist, socio-economic aspects, vulnerable groups.

**Introduction**

"Most people in the world are poor" (Schultz, 1980), therefore it is essential that economists understand the socio-economic aspects that affect poverty. Furthermore, "most of the world's poor people earn their living from agriculture" (Schultz, 1980), so discovering the economics behind the agricultural production is believed to provide a comprehensive picture of poverty in the rural areas. However, economists often tend to use narrow and strict economic models in determining who is poor, with a proxy such as income as a dominantly accepted welfare measure (Barrett, 2004). Nevertheless, the concept of poverty is complex and as such requires inter-disciplinary multidimensional measurement approach (Bourguignon and Chakravarty, 2002; Duclos et al., 2006). The concept is even more complex when we consider rural poverty. The problems faced by the rural population are diverse and include a wide range of supplementary issues that affect rural poverty such as climate, culture, markets and such (Mahmood, 2001).

The poorest countries of Europe are usually those that were severely affected by the collapse of the socialistic economic systems. Coming from such system, Macedonia is one of the poorest countries in Europe (World atlas, 2017). Since its independence in 1991, the country has undergone dramatic socio-economic reforms. The country has gradually improved its economy over the years with successful implemented policies. However, in spite of the reforms and development, the country has a high unemployment rate (27.3% in 2015) high poverty rates and a large 72% of the people have reported living in challenging living standards. Approximately 45% of R. Macedonia's population live in the rural parts of R. Macedonia and the rural areas take about 87% of the country's total area. The

inhabitants of the rural areas in Macedonia are the most deprived groups that lack access to institutions, infrastructure, access to adequate inputs and markets, lack of financial resources for investment and improvement of their incomes and living conditions. This is especially evident for the inhabitants of the hilly-mountainous areas where 43% of the people are facing difficulties of providing resources for food (IFAD, 2017).

This paper derives from the “Multidimensional poverty analysis Republic of Macedonia” (Tuna and Petrovska-Mitrevska, 2017) report and the aim is to sublimate the data on rural poverty, by representing the general rural poverty situation, as well as identifying the vulnerable and deprived groups of people leaving in the rural areas. This records may serve as a base for creation of effective and targeted policies in order to deal with rural poverty which is an important for the economy and the society as a whole. Identifying the micro and aspects of rural poverty would provide a base in constructing effective strategies and policy recommendations for poverty reduction, which is a central premise for the rural development in the country, but also a key component of the Europe 2020 strategy (Eurostat, 2017). The paper includes brief description of the general macro-economic indicators and conditions in the rural areas in the Republic of Macedonia, short description of the analytical tool and approach, as well as the poverty indicators on macro and rural level.

### Material and methods

Since this is the first attempt to depict this important issue in Macedonia, the analysis is based on official data sources such as the State statistical office (reports, censuses and other indigenous resources), World Bank, EU Commission Reports, Reports from: Freedom House, Transparency International, UNDP, Surveys of the Center for research and policy making, NGO “Solidarnost”, NGO “Macedonian Platform Against Poverty”, “NGO Federation of farmers in Republic of Macedonia”; as well as other literature and strategic documents in relation to the topic in the country and the Western Balkan region.

In order to be construct detailed understanding of the poverty conditions in the rural areas, we first need to emphasize that “poverty” is a complex concept which generate different overlapping repercussions on the poor, such as: isolation, disability, vulnerability and powerlessness (Figure 1). This is the so called “Magic circle of poverty” or the poverty syndrome/trap. Poverty is the powerful determinant that that triggers this vicious circle and contributes to malnutrition, fragile health, lack of power and voice, decreased productivity and exclusion from the labor market, reduced earnings, lack of adequate education and distance from the educational, health and other important institutions, as well as other types of exclusions and isolations which puts this individuals in a degraded situation (Chambers, 2012).

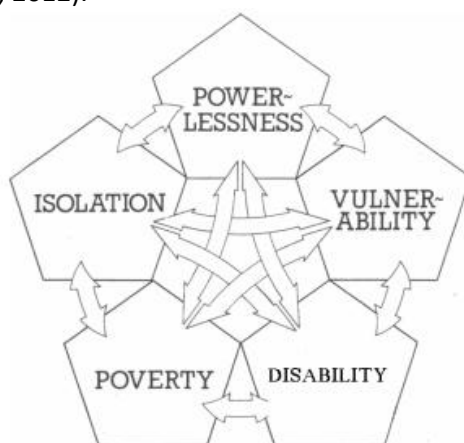


Figure 3. The poverty trap (Chambers, 2012)

To comprehend the complexity of the poverty concept, the methodological tool applied in the structure of this paper includes a multidimensional poverty model developed by the Swedish International Development Agency (SIDA, 2017). It is a model that reflects on the poverty from a

micro and a macro (institutional) perspective and provides a comprehensive description and identification of the poor individuals, deprived groups, as well as the wider causes that affect their position, such as the economic and social context, the political and institutional context, the conflict/peaceful context, and the environmental context (Figure 2). The main idea supporting this multi-purpose tool is that poverty is a complex concept and state, depicting not only the lack of material resources, but also involving series of other interlinked dimensions of poverty. More specifically, the primary objective of this tool is to identify “WHO” lacks: resources, opportunities, power and voice or human security and “WHY” (SIDA, 2017):

1. **RESOURCES** - not having access to or power over resources that can be used to sustain a decent living standard and improve one's life. Resources can be both material and non-material – e.g. a decent income, capital, being educated or trained, professional skills, being healthy, time and spare time, social and family networks, marital status, etc.
2. **OPPORTUNITIES and CHOICE** – the possibility to develop and/or use your resources so as to move out of poverty. Access to e.g. social services (including accessibility to services), to infrastructure, to capital, to land, social status, or to natural resources affects the opportunities and choices.
3. **POWER AND VOICE** - the ability of people to express their concerns, needs and rights in an informed way, and to take part in decision-making that relate to these concerns. Power is a relational concept that allows us to better understand socio-cultural hierarchies and relations of which gender is one, others include for example age, caste, class, religion, ethnicity, race/skin colour, ability/disability and sexual identity. Reinforcing forms of discrimination based on such economic and socio-cultural relations may increase an individual's poverty in this sense.
4. **HUMAN SECURITY** - violence and insecurity are constraints to different groups' and individuals' possibilities to exercise their human rights and to find paths out of poverty.

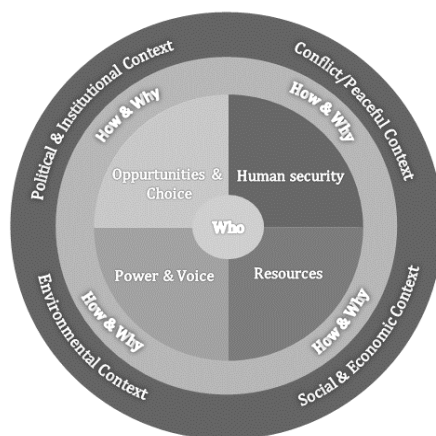


Figure 2. Multi-Dimensional Poverty analytical tool – four dimensions of poverty (We Effect programme instructions 2018-2021)

## Results and discussion

**Macro perspectives of poverty in R. Macedonia** - people living in poverty are particularly exposed to risks related to climate change and environment degradation as well as conflict and tensions adding to their vulnerability. This section includes analysis of the development context or the: political and institutional context, economic and social context, environmental context, conflict and peaceful context (SIDA, 2017).

**Environmental context** – The impact environmental factors, in particular climate change on poverty are large, mostly because poor people are exposed to hazards more often, lose more of their possessing when affected, and receive less support from family and friends, financial systems, and governments (World Bank, 2012). In fact, disasters can push people into poverty, and so disaster risk management can be considered as a poverty reduction policy. Since poverty reduction policies

reduce people's vulnerability, they can be considered part of the disaster risk management toolbox. "Agricultural production is inseparably tied to the climate conditions" (World Bank, 2012: 10), making agriculture one of the most climate-sensitive of all economic sectors. In countries such as Republic of Macedonia, the risks of climate change for the agricultural sector are a particularly immediate and important problem because the majority of the rural population depends directly or indirectly on agricultural incomes for their sustainability and have a relatively lower ability to adapt. In R. Macedonia, certain crops are more vulnerable than others, including: wheat, as the most important cereal crop, grape, tomatoes, alfalfa and apples (Cukaliev, 2014).

**Conflict/Peaceful context** – The country was faced with series of difficult political crisis since 2001. According to the EU Report on the Republic of Macedonia democracy and rule of law have been constantly challenged, in particular due to state capture affecting the functioning of democratic institutions and key areas of society. "The country suffers from a divisive political culture and a lack of capacity for compromise" (CSWD, 2016).

**Political and Institutional context** – Concerning the institutional context, Macedonia has a legal framework against discrimination and protection of human rights, however reality shows a serious lack of implementation in the part of equality and non – discrimination. Nevertheless, corruption remains prevalent in many areas and continues to be a serious problem in the Republic of Macedonia and freedom of expression and the situation of the media remain a serious challenge in the current political climate (CSWD, 2016). The National Strategy for Poverty and Social Exclusion Reduction in the Republic of Macedonia was adopted in 2010, and is one of the key strategic policy documents that should contribute to poverty reduction and social inclusion in the country. The strategy also mentions the agriculture in development programs for economic empowerment of young women farmers, for education of women and for improving the position of certain minorities – underage marriages, families with many children and people, who live at a greater physical distance from larger settlements in the municipality. An additional problem is transmission of poverty and exclusion. Series of strategic documents defining the objectives of the development of social protection were recently adopted or are in the process of adoption in the country, including the National Employment Strategy 2016-2020, the National Strategy for Deinstitutionalization 2008-2018, the Program for Social Inclusion, the National Housing Strategy and the National Strategy for Reduction of Poverty and Social Exclusion. What is positive is that the state financial program in the country is targeting greater participation of women. Yet, the most traditional social norms are visible in the rural areas, and the rural women, who have a difficult life living in poor conditions, limited offer in terms of public services and are likely to leave the village, unless they are offered new economic opportunities and possibilities for employment.

**Social and economic context** – The level of absolute poverty regarding the percentage of the population living with daily income below 1.9 USD is quite low, with 1.38% of people belonging to this category of citizens. The average monthly net wage paid per employee recorded in January 2017 in R. Macedonia was 22,750 MKD (close to 370 EUR). Informal work especially affects young workers and the long-term unemployed. According to the most frequent status of economic activity, the rate of poor employed citizens is 8.9%, while the rate of poor pensioners is 7.3%. At the same time the pensions have been rising for the last five years, reaching 30 percent of the overall central government spending's in 2016 (World bank, 2017). Data from the World Bank show steady increase of the Gini index in the Republic of Macedonia from 28.13% in 1998 to 44.05% in 2008. According to the State Statistical Office analysis, the Gini coefficient (a measure of inequality in income distribution) amounted to 33.7% in 2015, marking a slight decrease of the income distribution compared to 2010, when this index was 40.8 (State Statistical Office, Laeken indicators of poverty-2010).

Regarding the primary and secondary education in the country is free and compulsory, however the poor population is the category of population that abandoned further secondary or higher education. In regards to the literacy, even though the official figures indicate that 95% of the Macedonians are literate, less than 25% of the poor have gained education higher than primary

school level. Many have no schooling at all or only incomplete elementary education; there is an over-representation of women in this group: 73% of the illiterates or those without formal schooling are women.

*Rural sociology* - Approximately 45% of R. Macedonia's population (985,000 inhabitants) lives in the rural parts of R. Macedonia, and this is about 87% of the country's total area. As the state has become increasingly absent in these parts during the last decade, many villages have fallen into decay. The consequences of this neglect are particularly severe for the vulnerable groups living in the hilly and mountain areas. As much as 43% of households in these areas state that they do not have enough resources to provide for food. Due to different socio-economic influences, the migration has a rising trend in the country. The percentage of the population that migrated in the 90's was 11.1%, rising to 16.8% in 2000, and 21.3% in 2010. Some rural areas are experiencing uncontrolled out-migration which has completely emptied villages in a very short time. Already in 1998 it was reported that as many as 121 villages had no longer inhabitants and this trend continues over the years. Close to 21% of the total number of Macedonia's villages have less than 50 inhabitants, and in 104 villages, there are only 10 people or less. Moreover, the number of civil associations and organizations in rural areas are decreasing. A diminishing social capital deprives rural populations of their possibilities to cope with a deteriorating situation, and forces many to move to the cities. Agriculture is the main occupation of the people living in the rural areas, and the share of agriculture, forestry and fishery in the total Gross Value Added is around 10% in the last 10 years. There were 192,675 registered farms in 2007, and this number has decreased to 170,900 agricultural holdings in 2013 (Agricultural Statistics Database, Macedonia 2016). The rural economy in Macedonia is generally represented by small enterprises (including micro enterprises) that focus their economic activity on local and regional markets. These enterprises, which mainly belong to the primary agricultural production, are predominantly located in the rural regions. Low level of vertical integration poses a serious problem for the agricultural production process in Macedonia, which in most cases means complete absence of contractual relations, resulted in uncertainty which prevents long-term planning, and often leads to a disparity of supply and demand and market disorder

**The four dimensions of rural poverty are:** resources, opportunity and choice, power and voice, human security. Being poor in terms of resources means lack of access or power to resources which can be used to maintain decent living standards and improve one's life. Being poor through lack of power and voice relates to the ability of people to communicate their concerns, needs and rights in an informed way, and to take part in decision-making that relate to these concerns. Being poor in terms of human security implies to the fact that violence and insecurity are constraints to different groups' and individuals' possibilities to exercise their human rights in their struggle with poverty (SIDA, 2017).

**Resources – material and non-material** – According to the source of household income, poverty is highest among households living on social assistance (90.6%), followed by those with incomes from agriculture (57.4%) and those with incomes from temporary work (57.3%) (SSO, 2015). The largest problem for the poor are paying the household bills including electricity costs and food and they can barely manage to make ends meet. According to a survey by the Center for Research and Policy Making (2011), the average living standards of the rural families was 5,424 MKD/month (88 EUR), compared to 25,771 MKD/month (420 EUR) earned by the population living and working in the cities. The agricultural sector is traditionally characterized by the lowest salaries in the country, which in 2017 amounted to 16,740 MKD (around 270 EUR) (SSO, 2017). The lowest pensions are also typical for the agricultural sector. The minimal pension received by farmers was 3,744 MKD. The changes in the remittances inflow show large fluctuations over the years, however they present a significant part of the GDP (2.8% -2002; 4.2% - 2007; 4.1% - 2012) (World Development Indicators, 2017). Around 12% of the rural population in Macedonia is without any education, over 42% have primary education and only about 38% finished secondary education. In total, 77% of employees in Macedonian agriculture have the economic status known as full or part-time "unpaid family workers"(LFS, 2012).

**Opportunities and choice** – Inadequate development policies pertaining to sewage systems, clean drinking water, roads and transportation networks together with agricultural production problems of increasing input costs and market uncertainties have resulted in widespread stagnation and caused many young people to abandon farming and move to urban areas. In isolated areas particularly, there is an extremely low availability of health centres, schools, and cultural facilities. Educational standards and institutions are also low in the rural areas. Additionally, most of the illiterate population is located in the rural areas which are also most affected and have the highest degree of poverty.

Agriculture, its income and related processing and service activities are still sustainable source of income for the rural population (17.9% of the total active population in 2015). However, 77% of these employees in agriculture have the economic status known as *full or part-time "unpaid family workers"* (LFS, 2016). In general, labor-intensive, low-earning jobs in sectors such as agriculture are particularly prone to undeclared work. The share of informal employment in agriculture is significant – between 86.1% and 82.4% of all agricultural employment, and therefore were not covered by any social or legal protection (LFS, 2012). At the same time, the efforts to promote entrepreneurship and handy crafts in rural areas are often constrained by the low educational status of the workforce and lack of professional experience. New businesses are constrained by the low income and low purchasing power of rural residents as well as the saturation of activities that require low initial capital (small shops, restaurants, services). Currently the lack of initial capital is a significant barrier to the development of sustainable businesses in rural areas in particular (CRPM, 2012).

The investment or crediting opportunities for the rural population or more specifically the population involved in the agricultural production, are very limited and are mostly provided by commercial banks (30% through trade credits – seeds, fertilizers etc.); the rest 30% belong to governmental support programmes. In addition to the slightly improved rural financial services, the supply of financial assets for financing investments in agriculture does not satisfy the current demand. The financial institutions still consider agriculture as a high-risk sector. On one hand, financial institutions do not have appropriate experience for a proper analysis of the farm financial result, as well as for the risk assessment to repay the investment. On the other hand, they face high administrative costs to process the credit demands which refer to small amounts (CRPM, 2012).

**Power and voice** – Relations between actors in the market are usually confrontational rather than contracting with disproportionate distribution of the realized value, which is an unfavourable situation for the primary agricultural producers since they are small and isolated and lack bargaining power. This is mostly due to the damaged social capital and the low levels of membership in cooperatives. The reforms of the system after the Macedonian independence in the early 90's, introduced reforms and restructuring in the agricultural cooperatives which were heavily supported by foreign donors and institutions. At the time, the agricultural sector was losing pace and losing its market positions both domestically and regionally and farmers' integration was of great importance. Still, the donor experiences, enhanced by the government efforts through different types of support to strengthen the cooperative movement, failed to deliver the expected results and farmers are still reluctant to the cooperation idea, regardless of their size and unfavourable position in the agricultural value chain, with low power and voice to influence and create policies. The number of registered cooperatives in 2016 is 30 (Kakkamis, 2016), that is much lower than in the late 80s (about 200). As a post-socialist country, Macedonia is not excluded from the pattern of low social capital and negative viewpoint towards cooperatives and cooperation in general. There are around 35 registered agricultural cooperatives in Macedonia in 2017, most of which are in some form operational. All of the cooperatives in Macedonia are of micro-small size, with only one larger cooperative of vegetable producers exceeding 400,000 EUR/year, few exceeding 100,000 EUR/year and the rest are either with very low incomes or are inactive. The average number of members per cooperative is also very small (12 members per cooperative), with an average production capacity of 77 ha arable land per agricultural cooperative, and 6.2 ha per cooperative member. Vegetable production is the sub-sector in which the largest number of cooperatives exist, i.e. 11 cooperatives

with 158 members in total. Wheat, other cereals and fodder constitute the sub-sector where seven agricultural cooperatives (85 members) function (Kakkamis, 2016).

The rural woman is an important and largely deprived group of the population in terms of power and voice. In most instances rural woman is not aware of their rights and do not see anything discriminating in this traditional discrimination. Only 5% of surveyed households reported that a woman has the right of ownership of the house and this percentage was higher than all other types of resources (CRPPRW, 2012). Another important issue which denies woman of power and voice is the discrimination the young rural women on the labor market. Young women in rural areas aged 20-24 years (59%) and 25 – 29 years (43%) are faced with the highest unemployment rate, and 64% of rural women are characterized as “officially inactive”. According to the survey made by Center for research and policy making, the most common reason for rural women inactivity is due to child care and household liabilities (stated by 43% of inactive women in rural areas). However, 47% of women are unemployed and still work on family farms, creating handicraft products, but their work is often unpaid. The level of awareness of gender discrimination is relatively low in rural areas, and this impedes the realization of other activities related to gender equality, such as equal representation in politics, economy and equitable distribution in society and at home. Women in rural areas are rarely referred to regarding public issues.

**Human security in the rural areas** – A political crisis lasting two years now in Macedonia is causing economy crises, which in turn generates even more poverty. Most of the poor people are either unemployed, or poor people living on social assistance, or are small family enterprises that frequently face problems with the sale and low prices of their agriculture products. The vulnerability is further aggravated by the weak social security system and economic insecurity, especially distinct for the rural population (EU Commission, 2016).

### Conclusions

The aim of this paper is to depict the general rural poverty situation and identify the vulnerable and deprived groups of people leaving in the rural areas. The comprehensive multi-dimensional analysis of the poverty dimensions in the Republic of Macedonia highlighted several vulnerable groups in the rural areas, suffering from multiple deprivations. One way to categorize them is to classify them in the following groups:

- Traditionally poor people, included in this category are: rural and agriculture households, which are the groups with the highest risk to go underline of poverty. The main characteristics of this group are:
  - Low level of education of the household members,
  - Relatively small economic potential because their main source of income comes from agriculture activities.
- Chronicle poor households which are the most vulnerable part of the population and in the rural areas include the agriculture households without permanent income.
- According to the age and gender, the most vulnerable categories among the ones mentioned above include: young people in the urban and rural areas and women in rural areas

The agricultural households are identified as one of the most vulnerable group of households in the rural areas, mostly because agricultural production is the main source of income for the rural population. This type of production is inextricably tied to the climate conditions, thus is one of the most climate-sensitive of all economic sectors. In countries such as R. Macedonia, the risks of climate change for the agricultural sector are a particularly immediate and important problem because the majority of the rural population depends either directly or indirectly on agriculture for their livelihoods. The rural poor will be disproportionately affected because of their greater dependence on agriculture and their disability to adapt and reorient in order to gain different source of income (World Bank, 2012). Farmers’ position and ability to adapt is especially vulnerable because of their small scale, and accordingly lack of voice and power to create or influence the creation of policies, as well to establish favorable position and bargaining power in the agricultural value chains.

In this respect there is an urgent necessity to strengthen and motivate the cooperative movement in the country in order to ease the process of formation of effective agricultural cooperatives as a base for sustainable agriculture and development of the rural areas. Strategies for developing rural areas will not only contribute for reducing rural poverty, but could also contribute in the revival of the rural areas and reduce the significant problem of migration from the rural to the urban areas, and in a more general level, the emigration problem in the country. In order to achieve this, it is primarily important to that access to infrastructure is and public services is facilitated to the rural population. This is especially important for the rural woman which lack access to basic and specialized health service, lack access to education and day-care child services that are basic for strengthening their economic stability and independence. It is an imperative that rural women are actively enabled to express their needs and take active participation in decision making both at the local and central level. Mechanisms for participation at the local level should be particularly strengthened, because not only rural women but also rural men have shown little awareness, and limited confidence that their voices matter and will be heard, much less that they will be taken into account in local planning and when making decisions.

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### References

1. Barrett, C. B. (2005). Rural Poverty Dynamics: Development policy implications. *Agricultural Economics* (2004), 43-58.
2. Bourguignon, F., Chakravarty, S. (2003). The measurement of multidimensional poverty. *Journal of Economic Inequality* 1, 25-49.
3. CRPM. (2012). Centre for Research and Policy making, Perspectives of Rural Women. (Центар за истражување и креирање политики, „Перспективите на жените во руралните средини“).
4. <http://crpm.org.mk/wp-content/uploads/AboutUS/UnWomenPNZVRSMK.pdf>.
5. Cukaliev, O. (2014). Adaptation to climatic changes in the agricultural sector in R. Macedonia. USAID project for adaptation to climatic changes. Skopje. R. Macedonia.
6. Duclos, J. Y., Sahn, D., & Younger, S. (2006). Making multidimensional poverty comparisons. *The Economic Journal*, 116, 943–968.
7. EU Commission. (2016). EU COMMISSION STAFF WORKING DOCUMENT “The Former Yugoslav Republic of Macedonia 2016”. Report. IFAD, (2017). Rural poverty portal. <http://www.ruralpovertyportal.org/country/home/tags/macedonia>
8. Chambers, R. (2012). Rural development: Putting the last first (Pearson Education Limited). Ars Lamina. Skopje. R. Macedonia (VII-264).
9. CSWD. (2016). COMMISSION STAFF WORKING DOCUMENT. The former Yugoslav Republic of Macedonia 2016 Report. The European Economic and Social Committee and the Committee of the Regions. 2016 Communication on EU Enlargement Policy.
10. LFS. (2012). Labour Force Survey 2012. Statistical Review: Population and Social Statistics. Statistical Review: Population and Social Statistics. State Statistical Office 2013: 745 (2.4.13.06).
11. LFS. 2016. Labour Force Survey (2016). Statistical Review: Population and Social Statistics. Statistical Review: Population and Social Statistics. State Statistical Office. Skopje, May, 2017: 867 (2.4.17.02).
12. Mahmood, H. K. (2001). Rural Poverty in Developing Countries Implications for Public Policy, International Monetary Fund. Economic issues 26.
13. SIDA. (2017). Dimensions of Poverty Sida’s Conceptual Framework. Sida 62028en.

14. SSO. (2015). State statistical office of R. Macedonia – Statistical review: Survey on Income and Living Conditions: 863 (2.4.16.15).
15. SSO. (2017). Average monthly net wage paid per employee, July 2017. Issue 21.09.2017.
16. Schultz, T.W. (1980). Theodore W. Schultz - Prize Lecture, Lecture to the memory of Alfred Nobel, December 8, 1979. The Economics of Being Poor. [https://www.nobelprize.org/nobel\\_prizes/economic-sciences/laureates/1979/schultz-lecture.html](https://www.nobelprize.org/nobel_prizes/economic-sciences/laureates/1979/schultz-lecture.html)
17. Tuna, E. and Petrovska-Mitrevska, B. (2017). Multidimensional poverty analysis, Republic of Macedonia. Project “Institutional support of FFRM” (We effect – Swedish Cooperation Centre). Propoint. Skopje. R. Macedonia.
18. World Bank. (2012). Reducing the vulnerability of FYR Macedonia’s agricultural systems to climate change, Impact assessment and adaptation options. Sustainable development department, Europe and Central Asia Region. April 30, 2012.
19. World Bank. (2017). Personal remittance, received 1996-2016. The World Bank data, Macedonia FYR. (April 28, 2017)
20. [https://data.worldbank.org/indicator/BX.TRF.PWKR.DT.GD.ZS?locations=MK&name\\_desc=true](https://data.worldbank.org/indicator/BX.TRF.PWKR.DT.GD.ZS?locations=MK&name_desc=true)
21. Worldatlas. (2017). Poorest countries in Europe 2015 (April 25, 2017). <http://www.worldatlas.com/articles/the-poorest-countries-in-europe.html>

## SUPPLY CHAINS AS NETWORK- THE CASE OF ORGANIC PRODUCTION IN MACEDONIA

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### Abstract

The absence of relations between the primary producers and markets has been identified as one of the primary problems in the supply chain for producing organic products in Macedonia. Nevertheless, the organisation and network structure of the supply chains are often responsible for the economic performance, competitiveness and information diffusion between the trading firms and their partners-farmers producing organic products. In this regard, analysing supply chains through social network analysis lenses helps to identify the actors and the important factors that contribute in the information diffusion across the supply chains. The aim of this paper is to identify and map the information diffusion channels of the largest distributors of organic products in Macedonia, by mapping the structure of their social networks. The Social Network Analysis includes networks on horizontal level - farmer's decision to get involved in organic farming, or transfer the conventional production into organic, and vertical level – information provided by the distribution and trading partners in the organic value chain. The results show that information and resources are often embedded in the farmers' personal network, however farmers producing organic products are a closed group with very few relations among themselves indicating to a low level of trust in sharing or receiving information. Most of the identified relations among the actors in the analysed network are based either on commercial basis (distributors) or friendship (other farmers). The identified absence of relations among farmers on horizontal level can seriously impede the transfer of information and decrease the motivation for entering, maintaining and introducing innovations in the organic production, thus further examination and improvement of the information channels is necessary.

**Keywords:** distribution channels, information, relations, Social Network Analysis.

### Introduction

The socio-economic aspect is of key importance in organic production. Organic production provides large number of small producers a chance to be involved in production, since it requires minor investments in terms of preparations, materials and equipment. On the other hand, organic products are sold at higher prices that allow adequate incomes for producers and compensation for increased production costs (MOPF, 2015). This involvement of small producers has direct contribution to their subsistence in the rural areas where they live, and indirect contribution to the regional development and the economy of the country (MOPF, 2015).

Organic production in the Republic of Macedonia is evoking rising interest among the agricultural producers in the country. This is observed in the growing of the area and farmers producing organic products. Namely, in 2005, the total area under organic production was 266 ha (50 organic producers), and in 2016 this area mounted to 3,240 ha (533 organic producers). However, this trend reached its pick in 2011 and has a decreasing trend. Still, the changing preferences and increased awareness of the consumers in the country, as well as the awareness of the producers to meet the increased demand for fresh and locally produced organic products; which are often marketed at higher prices and provide better incomes for the farmers, make this segment of agriculture attractive for the enthusiastic farmers (MAFWE, 2016). Nevertheless, even though the number of organic farmers in the Macedonia is increasing, and institutions and bodies to provide coordination among the organic producers exist (such as the Macedonian Organic Producers Federation –

MOPF); information regarding organic production is still scarce and uncommonly shared among the farmers, and between the farmers and the other actors involved in the supply chain for organic products. The economic performance and competitiveness of firms buying and selling organic products and their partners-farmers producing organic products is often dependent on the supply chains organisation and network structure. Most of the research in the links in the supply chains is focused on the analysis of the primary producer-processor relationship, and the network aspect of the supply-chains has received less consideration (McKitterick et al., 2016). The relational structure and power dynamics between the primary producer-processor, as well as the other actors in the supply chain becomes a key aspect for identifying the factors influencing information diffusion (Medicamento and Degennaro, 2006). The absence of relations between the primary producers and markets has been identified as one of the primary problems in the supply chain for producing organic products in Macedonia. Therefore, the aim of this paper is to identify the information diffusion channels for producing organic products among the different actors in the supply chain, by mapping the structure of their social network. More specifically, the aim is to map the information reach or among all involved actors in the organic production (including the distribution, trading and other partners in the organic value chain) on horizontal and vertical level. Social Network Analysis (SNA) provides description and graphic representation of the commercial relations and information channels among the actors in the supply-chain for organic products.

### Material and methods

The data was collected in February-March 2017 through a combined face-to-face and telephone survey with farmers listed as suppliers for the two major retailers of organic products in Macedonia which are the sales channels “Beorg” – organic Bazaar and cooperative of organic products buyers and “Nasha Dobra Zemja”. The farmers’ in this sample are producing different types of products and are dispersed all over the country, which to some extent represents a limitation in the proposed research methodology. The survey was conducted through a short questionnaire, specially designed for this purpose and the selected method of analysis. Social Network Analysis is a specific methodological approach which requires distinctive type of questions in order to construct and map relations among the pre-defined network of actors. To achieve this task, it is necessary to construct a list which will include as many of the farmers/suppliers to these sales channels. Such list was available, however farmers were often difficult to reach, have ceased the cooperation with these sales channels, or have ceased to produce organic products in general. Table 1 (Channels of information) summarizes the general questions and the “Name generating table”, which is a tool enabling collection of information on each farmer’s personal networks; relation to other actors in the supply chain (alters) as well as the relations that alter (nominated farmers) have among them (Wasserman and Faust, 1994).

Table 4. Channels of information framework

| <b>PART I. GENERAL QUESTIONS</b>      |                                                                                                                  |                                                                                                                                                                    |
|---------------------------------------|------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Q1</b>                             | Motivation for starting with organic production.                                                                 | Higher prices, ensured market for organic products, subsidies, future potential of the organic production, other.                                                  |
| <b>Q2</b>                             | Where do farmer sell their organic products?                                                                     | Authorized trading partner, green market, specialized stores, individuals, restaurants, personal consumption, other.                                               |
| <b>Q3</b>                             | The most important source of information?                                                                        | Open question.                                                                                                                                                     |
| <b>Q4</b>                             | Does the trading partner provide important information regarding:                                                | Production of organic products, prices, distribution channels, no information, other.                                                                              |
| <b>PART II. NAME GENERATING TABLE</b> |                                                                                                                  |                                                                                                                                                                    |
| <b>Q5</b>                             | Nominating persons with whom the farmer discusses important issues regarding the production of organic products. | Relation to each of the nominated alters, if they are also producing organic agricultural products, whether the nominated persons are members of cooperatives etc. |

A total number of 28 farmers were interviewed. Each of the surveyed farmers were asked to nominate certain number (most often three to five) of other people (farmers, trading partners, specialized stores for organic products, certifying bodies, associations), with whom they discuss or share information on important issues regarding the production of organic products. The number of nominations is usually given as motive for more nominations, since limiting this number could lead to measurement errors (Lin, 2005). Following the nominations from the interviewed farmers, the information network was constituted of 69 actors (stakeholders). The “name generator” also includes a part titled as the “name interpreter” which refers each nominee’s attributes in regards to their type of relation and production (Table 1, part II) (Borgatti et al., 2013). The structure of the relations in the different types of networks may help the understanding and predicting the behaviour of the existing actors (stakeholders) (Medicamento and Degennaro, 2006).

The analysis was executed using adjacency matrices (NxN in terms of number of nodes). The actors in the network were coded (1 in the case of an existing relation and 0 when the relation was absent). The total network of all the participants in the information channel network) and the structure of the information network are presented through sociograms. The SNA data was analysed using UCINET, a specialised software tool for analysing social structures (Borgatti et al., 2002), and their visualisation was presented using NetDraw (Borgatti, 2002).

### Results and discussion

The first part of the questionnaire addressed the general motivation for starting with producing organic agricultural products, the channels of distribution, as well as the sources of information regarding organic production.

**Motives.** The major incentive to start with organic production among the interviewed farmers in this part of the study was the identified need and awareness of these farmers for the benefits of the organic food. The expected, higher prices and future prospective that this type of agricultural production provides for the primary producers are some of the important motives pointed out by the farmers. Demotivating fact is the absence of awareness on the consumer side and in most instances treating/pricing agricultural organic products by the wholesalers and final consumers as conventional. Absence of institutional support and quality controlling mechanisms from the certification institutions was also pointed as one of the discouraging factors for the organic producers and their incentives to invest in expanding and improving the production.

**Distribution channels.** The departing point in objective 4 was to analyse the ego networks of “Nasha Dobra Zemja” and “Beorg”, hence their supplying partners (farmers) were included in this research. Nevertheless, these were not the only transacting partners identified by the primary producers as their trading partners. Farm producers are in most instances not tied to one buyer only, thus in order to secure their sales and in a way disperse the risk, they are using different procurement practices and sale channels: green markets, individuals, wholesalers, specialized stores etc. Around 50% of the farmers in our sample sell their products to “Nasha Dobra Zemja”, yet many different specialized stores and wholesalers, and other types of distribution channels co-exist in this supply chain (See figure 1).

**Major sources of information.** Internet was pointed as the major source of information for the farmers. Farmers are often unaware or lack information on the other producers that produce organic products, being aware only for the producers of similar products through different types of Farmers’ associations. Unfortunately, most of these associations were indicated to be un-functional or fail to fulfill their main purpose which is to provide ground for cooperation and provide information for its members.

The trading partner does not provide any information for the organic producers, other than the required quantities and the offered price for their production

**Networking.** When the farmers’ producing organic products are spatially closer and are producing the same or similar products, then the probability that they are connected is higher. Since no information was provided by their trading partners Nasha Dobra Zemja and Beorg – the two major

retailers of organic products in Macedonia - and because of the dispersion of the farmers included in this study, there are not many relations on horizontal farmer level..

The information and communication network constructed applying Social Network Analysis tools includes all the identified actors in the sample supply-chain for organic products. The constructed network is based on the perception of the interviewed farmers, and it is a network with sparse relations, especially among the primary producers themselves. The network represents not only the personal relations when it comes to information, but also the existent commercial (relational) ties. The network analysis includes two important characteristics of social action: first, every actor (stakeholder) is part of a certain system, which influences its actions and decisions; and second, every actor's position in the network represents its influence, power, or in this case, ability to transfer information (Medicamento and Degennaro, 2006).

The network of farmers in the selected sample of organic farmers is constituted of 24 components which indicate a relatively compact network, fact supported by the fragmentation measure which is very close to 0 (0.027, see Table 2), meaning that all the actors in the network belong to the same component. This fragmentation also contributes to the low level of density, which shows the probability that a relation (tie) exists between any pair of nodes in the network, and is one of the primary indicators of social cohesion of the network. The density measure is relative to the network size and in bigger networks is expected to express lower values (Borgatti *et al.*, 2013). In accordance to the low density measures, we can conclude that the information transfer through the network expresses extremely small values. The average degree of the nodes or the farmers in the network is also very low, mostly due to the significant number of outliers (nodes without relations), and dyads (separate pairs of nodes) (see Figure 1). The reciprocity value which is 0.111, shows that that 1/10 of the ties in the network are reciprocal (ties between the nodes in both directions), but this is mostly due to the fact that not all of the actors in the selected network were available for the survey. The majority of these reciprocated ties are between the isolated pairs of nodes (see Figure 1).

Table 5. Cohesion network measures - commercial relations and network of information transfer

|                              | Values | Range and explanations                                                                                                                                                            |
|------------------------------|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Average degree</b>        | 0.882  | o Average number of ties of each node                                                                                                                                             |
| <b>In degree (H-index)</b>   | 5      | o Average of ties received by each node                                                                                                                                           |
| <b>Density</b>               | 0.009  | o Values closer to 1 - better connectedness of the actors in the network                                                                                                          |
| <b>Components</b>            | 24     | o Number of component comprising the network                                                                                                                                      |
| <b>Component ratio</b>       | 0.228  | o 1- every node is isolate, 0 – there is one component                                                                                                                            |
| <b>Connectedness</b>         | 0.027  | o 1 – each node belongs to the same component, 0 – every node is in a different component                                                                                         |
| <b>Network fragmentation</b> | 0.973  | o 1- all nodes are at distance1 from each other (complete graph), 0 – all nodes are isolates                                                                                      |
| <b>Average distance</b>      | 1.318  | o The time length for information diffusion across the network                                                                                                                    |
| <b>SD distance</b>           | 0.466  | o Sees distances beyond actors' direct relations                                                                                                                                  |
| <b>Diameter</b>              | 2      | o The longest path of the information flow (between the furthest nodes in the network)                                                                                            |
| <b>Distance - Breadth</b>    | 0.977  | o Average distance among nodes when certain nodes in the networks are removed (when all nodes are distance 1 from each other - complete graph, and 0 when all nodes are isolates) |
| <b>Reciprocity</b>           | 0.111  | o Average reciprocated ties (ties in both directions)                                                                                                                             |
| <b>Dyad reciprocity</b>      | 0.059  | o Reciprocity between pairs                                                                                                                                                       |

The “distance” measure analyses the shortest path between the more distant nodes, and if the connecting relations are absent, then those nodes would be unreachable (Wasserman and Faust, 1994). The average distance in the studied networks has a value of 0.977, indicating that the

network contains relatively close relations in terms of informational flow (Kadushin, 2012), and each actor in the network might be reached in one step, which is logical since all the actors in the network are able to reach other farmers or stakeholders in the network through the common sales trading partner, certification institution, association etc. Because of the expectation of larger network disconnections, we also included the measure of “breadth”, or the distance weighted fragmentation which shows the average distance among nodes in the case of removing certain nodes in the network (Borgatti and Everett, 2006). The majority of the nodes in the network are at distance close to 1 point to a complete graph. The diameter of the graphs shows the maximum distance on which the information in the network can travel between any pair of nodes in the network, or how distant are the remotest two actors in the network, which in this case is very low, and all actors in the networks are reachable in two steps.

Very few representatives of institutional bodies were identified in this network (Faculty of Agricultural Sciences and Food - Skopje, Faculty of Forestry - Skopje, MAFWE, foreign experts), few associations (AOPSE-Association for organic production in south-east region, ASN-Association in Sveti Nikole). Nonetheless, these relations were based on friendship and personal contacts, rather than on institutional (formal) level of cooperation, thus are presented as horizontal level information sources. Important role in the transmission of information was assigned by the farmers to the two certification institutions (BIOSERT and PROSERT). Clearer picture of the presented measures and the structure of the commercial relations and information channels among the actors in the supply-chain for organic products is presented in Figure 1.

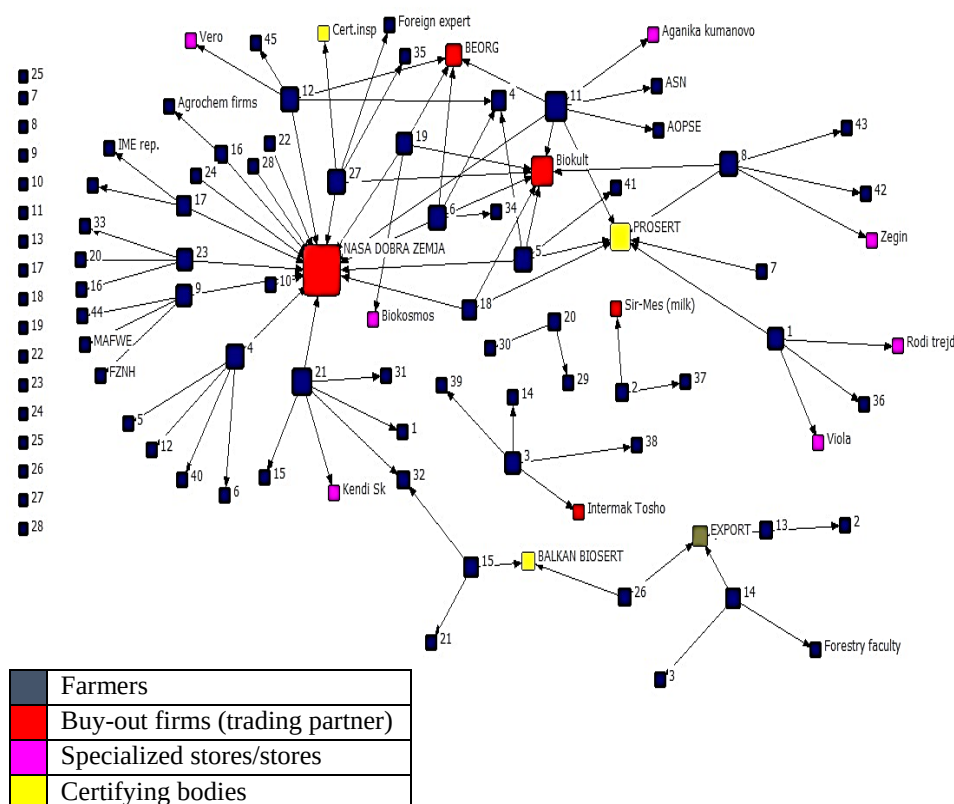


Figure 4: Network aspect of the commercial relations and information channels among the actors in the supply-chain for organic products

An alarming detail that is evident on the relational map (Figure 1) is the large number of outliers which indicates to the fact that many of the farmers involved in the organic production are alienated and do not communicate with other farmers or other identified parties in the supply chain for distribution of organic products. In this figure (socigram), it is noticeable that farmers which are the blue rounded squares have very few relations on horizontal level i.e. among themselves. Farmers have more relations with their trading partners; however these are purely commercial relations with no reversal information for the farmers, other than the quantities and prices.

Nodes that occupy more central in the network and were indicated as important actors in providing information are the certifying institutions (their representatives). There are also, many outliers who did not report any relation or sharing of information with the other actors in the supply chain, suggesting that many of the producers are often alienated, or were unable to identify any person from their immediate or wider surrounding to obtain or share information on different aspects regarding the organic production.

#### **Conclusions**

The aim of this paper was to identify the key actors in the information diffusion network of the supply chain regarding organic production on horizontal farm and vertical level. This included the motives and information diffusion among the farmers, their distribution channels, trading and other partners in the organic value chain. In regards to the motives for organic production, farmers generally have high awareness about the benefits and future prospects of the organic production, however are largely unmotivated by the absence of institutional support and quality controlling mechanisms from the certification institutions. On the other side, representatives of the certification institutions were identified as an important source of information. The access to information and resources are often embedded in the personal networks of farmers, though farmers producing organic products are a closed group of farmers with very few relations among themselves and low level of trust in sharing or receiving information. The identified absence of relations among farmers on horizontal level can seriously impede the transfer of information on various aspects (market, prices, labour etc), innovations and decrease the motivation for starting and maintaining the organic production. As certification bodies and contact persons were identified as one of the most involved in the day-to-day communication with the farmers, a possible prospect would be to engage them as "information brokers". In order to effectively include and use their position in the information relations, one possibility is to find a way to upgrade their capacities and knowledge with novel information on the newest trends and technologies in the organic production.

The absence of extension support and advice is evident, hence particular attention should be paid in developing this segment of communication. Although institutions and organizations that should support of access to information as well as information transfer are present, they are not perceived as mediators in the case of the organic producers in the country. Most of the identified relations among the actors in the analysed network are mostly based on commercial or friendship basis, and the obvious necessity of improvement of the informal information channels should be further examined and strengthened. There is a need to find instruments which will enhance communication channels among the farmers and their major trading partners. The absence of information from the major sales channels Beorg – Organic Bazaar and cooperative of organic products buyers and Nasha Dobra Zemja should also be subject of further analysis. These partners are connected to the farmers through commercial relations and can be used as information and communication brokers for bypassing the absence of relations among the farmers and transfer of various types of information through the supply chain network for organic products.

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## References

1. Borgatti, S. P., Everett, M. G and Freeman, L. C. (2002). UCINET for Windows Software for Social Network Analysis, Harvard MA: Anal Technologies. "
2. Borgatti, S. P. and Everett, M. G. (2006). A graph-theoretic perspective on centrality. *Social Network*, 28: 466-484.
3. Borgatti, S. P. and Everett, M. G and Jeffrey C. (2013). *Analyzing Social Networks*. SAGE Publication Ltd. London/UK.
4. Lin, N. (2005). A Network Theory of Social Capital. *Handbook on Social Capital*, edited by Dario Castiglione, Jan van Deth and Gugli elmo Wolleb. Oxford University Press.
5. Kadushin, C. (2012). *Understanding Social Networks, Theories, Concepts and Findings*. Oxford University press.
6. Medicamento, U. and Degennaro, B. (2006). *Social Networks and Supply chain Management in rural Areas: A case Study Focusing on Organic Olive Oil*. MPRA Paper No. 14558 (Munich Personal ReOPEc Archive), posted 12 April 2009.
7. Wasserman, S. and Faust, K. (1994). *Social Network Analysis, Methods and Applications*. New York, USA: Cambridge University Press.
8. MOPF. (2015). Macedonian Organic Producers Federation. <http://www.fpopm.com/>.
9. MZSV. (2016). *Organsko proizvodstvo sostojba*. [http://www.mzsv.gov.mk/files/Organsko%20proizvodstvo\\_sostojba%202016.pdf](http://www.mzsv.gov.mk/files/Organsko%20proizvodstvo_sostojba%202016.pdf)

## EFFECTS OF DIFFERENT GRAFTING METHODS AND TIMES ON GRAFTING SUCCESS AND PLANT DEVELOPMENT IN SARI ALIÇ HAWTHORN GENOTYPE (*CRATAEGUS AZAROLUS* L.)

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### Abstract

The study was carried out to determine the different grafting methods and times on the grafting success and plant development in ‘Sarı Aliç’ hawthorn genotype (*Crataegus azarolus* L.) on *Crataegus monogyna* L. rootstock in Hatay province, eastern Mediterranean region of Turkey. T and chip budding and whip grafting methods were conducted on February 15, March 01, March 15, April 01, April 15, May 01 and May 15 dates in 2016. The grafting take and bud sprout percentages, graft shoot development and shoot diameter were also investigated. The results of the study showed that graft success and plant development were affected by grafting times and methods. The mean highest bud sprout percentage (71.91%) was found in whip grafting whereas mean graft shoot development (55.18 cm) and diameter (5.57 mm) were detected in T budding. The bud sprout ratio was the highest on April 01 and April 15 (84.44% and 77.77%, respectively). The results showed that whip grafting method applied within March and April months was very successful for growing of ‘Sarı Aliç’ hawthorn genotype in Hatay, Turkey ecological conditions.

**Keywords:** Hawthorn, budding, grafting, plant development.

### Introduction

*Crataegus* species form genus known as hawthorns that belongs to subfamily Maloideae of the Rosacea (Evans and Campbell 2002). The genus is closely related to the genera *Pyracantha* M. Roem, *Mespilus* L. and *Hesperomeles* Lindl. (Hummer and Janick 2009). Several hawthorn species are grown for their eatable fruit in Asia, America, and the Mediterranean countries. In Europe, the fruit, leaves, and flowers of the hawthorn are traditionally employed in the treatment of heart problems due to their antispasmodic, cardiogenic, hypotensive, and antiatherosclerotic effects, as well as being ingredients in wine, conserve and sweets (Ljubuncic et al. 2005, Caliskan 2015).

Turkey have different climatic conditions for the hawthorn species in the diverse regions. The regions have at least one characteristic species and other secondary or common species with domestic genotypes. *C. monogyna* Jacq is widespread in Turkey. Hawthorn species are mainly distributed in temperate zone areas of Turkey. Some hawthorn species are also growing in low altitudes such as 300 and 500 m areas of subtropical ecology such as Hatay in Turkey (Caliskan et al. 2016). Caliskan et al (2012) reported some genotypes of the *C. azarolus* are grown for large and delicious fruits in Belen, Hatay province, eastern Mediterranean region of Turkey. The promising genotypes are found in the region because of its fruit size (fruit weight >10 g), as well as high total soluble solids, total phenolic, antioxidant activity and antioxidant capacity contents. The genotypes of this species are commonly grown in Belen County and the fresh fruits have been sold with the higher prices in local markets.

A most important limiting factor for the hawthorn growing is propagation. Vegetative propagation of hawthorn species is not a usefully method because of their rooting percentage is very lower. At the present time, rootstock production is carried out with seeds, and then grafting and budding methods are used for new plants. However, there is no detail studies for the grafting methods and times on the grafting success and plant development in hawthorn. This study was designed to determine optimal grafting methods and times for hawthorn grown in Hatay, Turkey ecological conditions.

### Material and methods

The study was conducted at Fidanlı located Samandağ, Hatay province (Turkey). The scions of 'Sarı Alıç' hawthorn genotype was taken from main plants which were selected Belen, Hatay in February and stored at 4°C in a refrigerator. In the study, *Crataegus monogyna* L. seedling produced with seed propagation was used as rootstock.

T and chip budding and whip grafting methods were conducted on February 15, March 01, March 15, April 01, April 15, May 01 and May 15 dates in 2016. The budding and grafting methods on rootstock were applied from soil surface at a height 15-20 cm. Cultural applications such as removal of suckers below graft point, weeding, irrigation and fertilization were fulfilled at regular intervals. A mini data logger (Testo 174T Temp/RH, Germany) was used for the daily mean temperature and relative humidity measurements (eight hours intervals).

The grafting take and bud sprout percentage (%), graft shoot length (cm) and diameter (mm) were investigated. The bud sprout percentage was observed within 30 days following grafting. The shoot length and diameter were measured at the end of vegetation period (December 15). The shoot length was measured by meter from the graft point and the shoot diameter was evaluated by digital caliper at a height 5 cm above the graft point.

Data were analyzed using SAS software and procedures (SAS 2005). Variance analysis was formed with Tukey's Honestly Significant Difference (HSD) method at  $p < 0.05$ . The two-factorial arrangement on a completely randomized design was constructed with three replications. Each replication included 15 plants. The data expressed as percentage were transformed using the to the arc-sin $\sqrt{x}$  transformation.

### Results and discussion

Climatic data included daily mean temperature (°C) and relative humidity (%) were recorded during February 10 to December 31, 2016 year (Figure 1). In the experimental area, daily mean temperature ranged between 2.8 and 38.5 (°C) and daily mean relative humidity varied between 22.8 and 99.9%. Generally, mean temperature and relative humidity varied throughout the year except for June, July and August. Hartmann et al. (2011) indicated that temperature and relative humidity values are very critical pending the 30 days following grafting methods. The period is important for formation of callus cells and wound closure. In addition, Yilmaz (1992) showed that the temperature at the grafting times should be 26 and 28°C for optimum development of callus cells. The maximum mean temperature (38.5°C) was recorded in June whereas the mean temperatures were ranged between 10 and 20°C in February and March, ranged between 12 and 25°C in April and ranged between 15 and 29°C in May at the grafting application times. The relative humidity values changed from 30 to 70% at the same times.

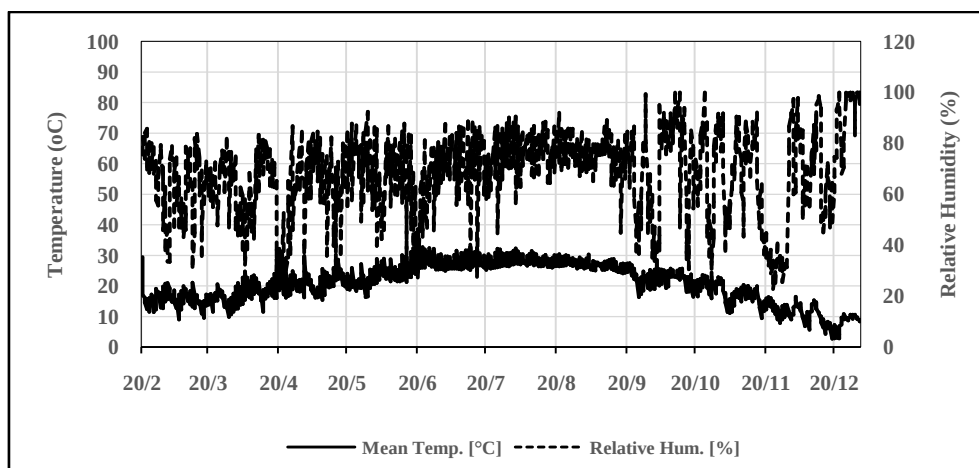


Figure 1. Variation of mean temperature (°C) and relative humidity (%) during the days after grafting in 2016

The influence of T and chip budding and whip grafting on the grafting take and bud sprout percentages, graft shoot length and diameter values were shown in Table 1. The data showed that grafting success and plant development in 'Sarı Aliç' hawthorn genotype were statistically significant ( $p < 0.05$ ) depend on grafting method and time. Grafting success was not obtained from T budding method on February 15. The result may be due to the application date was not enough for activity of plant sap in phloem cells. However, the highest bud sprout ratio (86.67%), graft shoot length (94.33 cm) and shoot diameter (8.40 mm) were found in T budding application on April 15. Grafting take and bud sprout ratios were showed differences in chip budding. The highest grafting ratio was detected for chip budding on March 01 and March 15 whereas the highest bud sprout ratio for chip budding was observed on April 01. Bud sprout ratio for chip budding was the lowest on May 01. The highest graft shoot length and shoot diameter values were measured for chip budding on April 01 while it was lowest on February 15 (50.67 cm and 3.15 mm, respectively) and May 15 (41.33 cm and 3.75 mm, respectively). Grafting take ratio for whip grafting was ranged between 26.66 (May15) and 100.00% (March01 and April 15) and bud sprout ratio was ranged between 23.33 (May 01) and 93.66% (April 15). The longest graft shoot length was detected for whip grafting on February 15 (72.08 cm) whereas it was the lowest on May 15 (26.44 cm). Graft shoot diameter values for whip grafting based on various grafting times did not found statistically important.

Table 1. The influence of T, chip and whip grafting based on grafting times on grafting success and plant development in 'Sarı Aliç' hawthorn genotype

| Grafting Method           | Grafting Time | Grafting take ratio (%) | Bud sprout ratio (%) | Graft Shoot length (cm) | Graft Shoot diameter (mm) |
|---------------------------|---------------|-------------------------|----------------------|-------------------------|---------------------------|
| T budding                 | 15 February   | 0.00 b                  | 0.00 c               | 0.00 d                  | 0.00 d                    |
|                           | 01 March      | 80.00 a                 | 60.00 ab             | 50.33 c                 | 6.66 ab                   |
|                           | 15 March      | 86.67 a                 | 26.67 bc             | 46.78 c                 | 4.83 b                    |
|                           | 01 April      | 100.00 a                | 86.68 a              | 61.50 bc                | 2.74 c                    |
|                           | 15 April      | 93.33 a                 | 86.67 a              | 94.33 a                 | 8.40 a                    |
|                           | 01 May        | 100.00 a                | 53.33 ab             | 70.43 b                 | 8.36 a                    |
|                           | 15 May        | 86.70 a                 | 66.67 ab             | 62.89 bc                | 8.00 a                    |
| Chip budding              | 15 February   | 91.67 ab                | 46.67 abc            | 50.67 c                 | 3.15 e                    |
|                           | 01 March      | 100.00 a                | 60.00 ab             | 32.44 e                 | 4.04 cd                   |
|                           | 15 March      | 100.00 a                | 40.00 bc             | 56.04 b                 | 4.21 bc                   |
|                           | 01 April      | 93.33 ab                | 80.00 a              | 63.00 a                 | 5.64 a                    |
|                           | 15 April      | 86.67 ab                | 53.33 abc            | 54.00 bc                | 4.50 b                    |
|                           | 01 May        | 80.00 ab                | 20.00 c              | 53.44 bc                | 3.97 cd                   |
|                           | 15 May        | 73.33 b                 | 53.33 abc            | 41.33 d                 | 3.75 d                    |
| Whip Grafting             | 15 February   | 46.68 b                 | 43.22 b              | 72.08 a                 | 5.46                      |
|                           | 01 March      | 100.00 a                | 80.00 a              | 56.22 b                 | 5.47                      |
|                           | 15 March      | 80.00 a                 | 80.00 a              | 49.22 c                 | 5.17                      |
|                           | 01 April      | 86.67 a                 | 86.67 a              | 53.33 bc                | 5.04                      |
|                           | 15 April      | 100.00 a                | 93.33 a              | 53.67 bc                | 4.72                      |
|                           | 01 May        | 86.66 a                 | 66.67 a              | 37.11 d                 | 4.64                      |
|                           | 15 May        | 26.66 b                 | 23.33 b              | 26.44 e                 | 4.61                      |
| <i>HSD<sub>T</sub></i>    |               | 29.81                   | 40.35                | 20.28                   | 1.85                      |
| <i>HSD<sub>Chip</sub></i> |               | 25.99                   | 36.51                | 4.75                    | 0.41                      |
| <i>HSD<sub>Whip</sub></i> |               | 32.19                   | 41.71                | 5.24                    | ns                        |

Different letters within columns indicate significant differences by Tukey's at  $p < 0.05$ ; ns: not significant

The mean grafting take and bud sprout ratios, graft shoot length and diameter values were presented in Table 2. The mean grafting take ratio was the highest for chip budding (89.29%). The lowest grafting take ratio was observed in whip grafting (75.24%). However, the mean bud sprout ratio was the highest for whip grafting (71.91%). Actually, some researchers indicated that whip grafting is one of the successfully methods than others due to the callus formation and graft union are better in whip grafting (Miller and Crocker 1994, Bellini 2002).

The highest mean grafting take ratios were found between March 01 and May 01 (from 88.80 to 93.50%). The lowest grafting take and bud sprout ratios for all grafting methods were detected in February 15 (46.11 and 40.00%, respectively). The mean bud sprout ratio was the highest in April 01 (84.44%) and April 15 (77.77%). The climatic data showed that daily mean temperatures between 15 and 25°C at the grafting application time (April) were very successfully for hawthorns. Grafting take and bud sprout ratios were adversely affected by low temperatures in February and March. Similar to our results, low temperatures at the March were showed negative results on grafting success in persimmon (Zenginbal 2015) and kiwi (Öztürk et al. 2012). In addition, April is the stage of speed shoot development on hawthorns in Hatay, Turkey ecological conditions. This time can be perfect time for grafting because of growth hormones are concentrated in the buds and these may be induce differentiation of vascular tissues in the graft union point (Hartmann et al. 2011).

Grafting methods and times had significant effect ( $p < 0.05$ ) on the mean graft shoot length and diameter values (Table 2). The highest mean graft shoot length were found in T budding (55.18 cm), followed by chip budding (50.13 cm) and whip grafting (47.3 cm). The mean graft shoot length was the highest for April 15 and April 01 (67.33 and 59.28 cm, respectively) whereas the lowest mean graft shoot length was found in February 15 (40.92 cm) and May 15 (43.56 cm). T budding had the highest mean graft shoot diameter with 5.57 mm. The lowest mean graft shoot diameter was measured in chip budding (4.18 mm). The mean graft shoot diameter was effected by grafting time. The highest shoot diameter was obtained in April 15, May 01 and May 15 (5.88, 5.65 and 5.45, respectively).

Table 2. The effects of various grafting methods and times on mean grafting take, bud sprout ratio, graft shoot length and shoot diameter in 'Sarı Aliç' hawthorn genotype

| Variable                    | Grafting take ratio (%) | Bud sprout ratio (%) | Graft Shoot length (cm) | Graft Shoot diameter (mm) |
|-----------------------------|-------------------------|----------------------|-------------------------|---------------------------|
| <i>Grafting method</i>      |                         |                      |                         |                           |
| T budding                   | 78.10 b                 | 54.29 b              | 55.18 a                 | 5.57 a                    |
| Chip budding                | 89.29 a                 | 50.48 b              | 50.13 b                 | 4.18 c                    |
| Whip Grafting               | 75.24 c                 | 71.91 a              | 49.73 b                 | 5.02 b                    |
| <i>Grafting time</i>        |                         |                      |                         |                           |
| 15 February                 | 46.11 c                 | 40.00 c              | 40.92 e                 | 2.87 d                    |
| 01 March                    | 93.33 a                 | 66.67 ab             | 46.33 de                | 5.39 ab                   |
| 15 March                    | 88.89 a                 | 48.89 bc             | 50.68 cd                | 4.74 bc                   |
| 01 April                    | 93.35 a                 | 84.44 a              | 59.28 b                 | 4.47 c                    |
| 15 April                    | 93.50 a                 | 77.77 a              | 67.33 a                 | 5.88 a                    |
| 01 May                      | 88.80 a                 | 46.67 bc             | 53.66 bc                | 5.65 a                    |
| 15 May                      | 62.22 bc                | 47.78 bc             | 43.56 e                 | 5.45 a                    |
| <i>HSD<sub>method</sub></i> | 7.91                    | 10.64                | 3.33                    | 0.36                      |
| <i>HSD<sub>time</sub></i>   | 15.40                   | 20.71                | 6.49                    | 0.69                      |

Different letters within columns indicate significant differences by Tukey's at  $p < 0.05$ .

## Conclusions

This study provided the first data indicating the grafting methods and times in hawthorn culture. The results showed that considerable variation in bud sprout percentage, graft shoot length and

diameter values based on grafting methods and times. According the results, we can say that whip grafting can be used successfully from March 01 to April 15 for hawthorn nursery production in Hatay, Turkey ecological conditions. Chip budding may be preferred if there are not enough graft shoots on February 15, March 01 and March 15. T budding can be suggested for high budding success and plant development on April 01 and April 15.

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### References

1. Bellini, E. (2002). Cultural Practices for Persimmon Production. First Mediterranean Symposium on Persimmon. CIHEAM. 23-24 November 2001, Faenza, Italy.
2. Çalışkan, O., Gündüz, K., Serçe, S., Toplu, C., Kamiloğlu, O., Şengül, M. and Ercişli, S. (2012). Phytochemical characterization of several hawthorn (*Crataegus* spp.) species sampled from the Eastern Mediterranean region of Turkey. *Pharmacognosy Magazine*, 8: 16-21.
3. Çalışkan, O. (2015). Mediterranean Hawthorn Fruit (*Crataegus* spp.) Species and Potential Usage. *The Mediterranean Diet. An Evidence-Based Approach* (Eds. Preddy, VR. Watson, RT.)
4. Çalışkan, O., Bayazit, S. and Gunduz, K. (2016). Hawthorn Species from Turkey and Potential usage for Horticulture. VII International Scientific Agriculture Symposium, 6-9 October 2016, Jahorina, Bosnia and Herzegovina.
5. Evans, R.C. and Campbell, C.S. (2002). The origin of the apple subfamily (Maloideae; Rosaceae) is clarified by DNA sequence data from duplicated GBSSI genes. *American Journal of Botany*, 89: 1478–84.
6. Hartmann, H.T., Kester, D.E., Davis, J.R. and Kalantari, S. (2011). *Plant Propagation: Principles and Practices*. Eight Edition. New Jersey.
7. Hummer, K.E. and Janick, J. (2009). *Rosaceae: Taxonomy, Economic Importance, Genomics*. Eds. Foltá, K.M., Gardiner, S.E., *Crops and Models* 1–16.
8. Ljubuncic, P., Portnaya, I., Cogan, U., Azaizeh, H. and Bomzon, A. (2005). Antioxidant activity of *Crataegus aronia* aqueous extract used in traditional Arab medicine in Israel. *J Ethnopharmacol*, 101: 153–61.
9. Miller, E.P. and Crocker, T.E. (1994). *Oriental Persimmons in Florida*. Florida Cooperative Extension Service, Institute of Food and Agricultural Science, University of Florida, 101:1-16.
10. Öztürk, B., Özcan, M. and Öztürk, A. (2012). Effects of different rootstock diameters and budding periods on graft success and plant growth in kiwifruit seedling production. *Journal of Agricultural Sciences*, 17: 261-268.
11. SAS (2005). SAS online doc, version 9.1.3. SAS Inst., Cary, NC, USA.
12. Yilmaz, M. (1992). *Horticultural crops growing techniques*. Cukurova Univ. Publ. Adana, Turkey.
13. Zenginbal, H. (2015). The effects of grafting methods (by hand and with manual grafting unit) and grafting times on persimmon (*Diospyros kaki* L.) propagation. *Acta Sci. Pol. Hortorum Cultus*, 14(4): 39-50.

## **‘SUMMIT’ SWEET CHERRY CULTIVAR ON DIFFERENT ROOTSTOCKS**

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### **Abstract**

The goal of this study was to examine the effect of seven cherry rootstocks ('Gisela 5', 'Gisela 6', 'CAB 6P', 'Alkavo 2', 'F 12/1', 'MaxMa14' and 'MaxMa 60') on the growth and productivity of 'Summit' sweet cherry cultivar. The trees were planted in the spring of 2008 at distances of 4.0 x 3.0 m, shaped in freely growing crown and irrigated by spraying over the crowns. Tree vigour (trunk cross-sectional area and crown volume), yield, yield efficiency and fruit quality were recorded each year in the period 2008-2016. The results clearly showed the influence of rootstocks on the trees growth. Nine years after planting the most vigorous rootstock was 'MaxMa 60' and the weakest were 'Gisela 5' and 'Gisela 6'. The highest 4-year cumulative yield per tree was obtained with 'MaxMa 60', followed by 'CAB 6P' and 'MaxMa 14'. Rootstocks 'Gisela 5' and 'Gisela 6' negatively influenced yield and fruit size.

**Key words:** Canopy dimensions, yield, yield indices, fruit weight.

### **Introduction**

Many improvements have been made in rootstocks used for sweet cherry in the second half of the 20<sup>th</sup> century. Clonal rootstocks selected from *Prunus avium*, *Prunus mahaleb* and *Prunus cerasus* as well as and from interspecific hybridizations have become increasingly popular (Hrotko, 2008; Sansavini and Lugli, 2014). The relationships between the rootstocks and grafted cultivars are important from a horticultural point of view, because they provide a basis for selecting the best scion/rootstock combination, and it is crucial for the production of cherries. Tree vigor continues to be a major concern in cherries production and may be controlled by several ways, which include use of dwarfing rootstocks. These rootstocks have a large effect on precocity and tendency of high tree productivity of sweet cherry, easier application of cultivation procedures, harvest and protection from rainfall and birds, and makes them more suited to high planting density (Grzyb et al., 1998; Bujdosó and Hrotkó, 2005; Fajt et al., 2014; Lang, 2001; Weber, 2001; Whiting et al., 2005). Few research have been done with dwarfing or semi-dwarfing clonal cherry rootstocks in Bulgaria and they are still not sufficient to be choose the best of them for cultivation of sweet cherries (Lichev, 2001; Lichev and Papachatzis, 2011; Sotirov, 2015 a,b).

The main objective of this work was to determine the impact of different rootstocks on tree growth, yield potential and fruit weight of 'Summit' sweet cherry cultivar.

### **Material and methods**

The present study was carried out at the Institute of Agriculture in Kyustendil (Bulgaria) during the period 2008-2016 with sweet cherry cultivar 'Summit' grafted on 'Gisela 5', 'Gisela 6', 'CAB 6P', 'Alkavo 2', 'F 12/1', 'MaxMa14' and 'MaxMa 60' rootstocks. The experimental orchard was established in the spring of 2008. The trees were planted at distances of 4.0x3.0 m and grown on Chromic Luvisols soil with a light sandy loamy texture, weakly acidic (pH 5.0-5.2 in KCL). 'F 12/1' rootstock was chosen as a standard for comparison. The trees were trained in freely growing crowns. The only pruning was done after planting to cut the tree stems at a height of 80 cm and then the trees were left to freely manifest their growth and reproductive characteristics. Standard management practices (soil cultivation, weed, pest and disease control) were followed in the experimental orchard. Irrigation was applied by spraying upon the tree crowns in July and August.

Five representative trees from each cultivar-rootstock combination were selected for data collection. At the end of the growing seasons were measured and calculated the parameters: trunk cross-sectional area (TCSA) - 30 cm above the grafting zone; canopy dimensions (height, width, volume and horizontal projection area); average and cumulative yields per tree; yield indices (kg cumulative yield/cm<sup>2</sup> of TCSA), kg/m<sup>3</sup> of canopy volume and kg/m<sup>2</sup> of the canopy horizontal area) and average fruit weight - determined on random samples of 30 fruit from each cultivar-rootstock combination. Formation of root suckers of the trees was also recorded.

Data were statistically evaluated by analysis of variance (ANOVA) and means were separated by Duncan's multiple range test at  $p \leq 0.05$ .

### Results and discuss

The results obtained showed that rootstock affects significantly the growth of experimental sweet cherry trees concerning the trunk cross-sectional area (Table 1). 'MaxMa 60' was the most vigorous rootstock, exceeding the control 'F 12/1' by 31.6% at the end of 9<sup>th</sup> growing season. 'Gisela 6' and 'Gisela 5' were the most vigor-controlling rootstocks, reducing TCSA to 49.5 and 52.1% of 'F 12/1'. The trees on 'Alkavo 2' had about 36% less TCSA than trees on 'F 12/1' but they not differed significantly than those on 'Gisela 5' and 'Gisela 6'. 'CAB 6P' and 'MaxMa 14' were intermediate in their vigor control, reducing TCSA to 71.9 and 95.2% of 'F 12/1', respectively.

The dimensions of tree crowns are important indicators for determining the mutual influence of rootstock and scion. The results for height, width, volume and horizontal projection area of the crowns at studied scion/rootstock combinations confirmed the expressed differentiation among the rootstocks manifested in TCSA.

Table 1. Rootstock effect on trunk cross-sectional area (cm<sup>2</sup>) of 'Summit' sweet cherry trees in the period 6<sup>th</sup>-9<sup>th</sup> growing season

| Rootstock  | 2013  | 2014  | 2015  | 2016     | % of F 12/1 |
|------------|-------|-------|-------|----------|-------------|
| F 12/1 (c) | 78.2  | 128.2 | 145.0 | 184.8 c  | 100         |
| CAB 6P     | 65.0  | 114.0 | 125.4 | 132.9 ab | 71.9        |
| Alkavo 2   | 60.8  | 90.6  | 101.5 | 118.4 a  | 64.1        |
| Gisela 5   | 56.3  | 84.1  | 91.6  | 96.2 a   | 52.1        |
| Gisela 6   | 50.7  | 80.2  | 87.4  | 91.4 a   | 49.5        |
| MaxMa 14   | 83.3  | 155.4 | 168.5 | 175.9 bc | 95.2        |
| MaxMa 60   | 110.4 | 179.7 | 206.0 | 243.2 d  | 131.6       |

Means followed by the same letter in columns are not significantly different at  $p \leq 0.05$  (Duncan's test).

Nine years after planting, with the exception of the trees on the two 'Gisela' rootstocks, the crowns height was about 4.0 m and along with the stem the trees reached about 4.80- 5.0 m height. The width of the crowns varied from 3.0 to 4.0 m (Table 2). This led to overlapping and poor lighting of the trees at these planting distances, and necessitates limiting the size of the crowns by pruning.

Differences in the height were more pronounced than those of the crown width, but for both indicators there were significant differences between most variants. 'MaxMa 60', 'CAB 6P' and 'MaxMa 14' induced larger tree sizes and their crown volume exceeded that of 'F 12/1' with 48.8, 42.1 and 24.8%, respectively. 'Gisela 5', 'Gisela 6' and 'Alkavo' 2 had a reducing effect. On these rootstocks the crown volume was reduced by 47.1, 29.8 and 12.4%, compared to 'F 12/1'. The differences between 'F 12/1' and the other rootstocks were statistically proven. The results of the crown horizontal projection areas showed that the scion/rootstock combinations followed the trend of the crown diameter increase.

'CAB 6P' rootstock showed the highest root suckering ability - average 6.0 – 12.2 number per tree in the row lines (width 1.50 m), average for the period 6<sup>th</sup>-9<sup>th</sup> leaf. In combinations with 'F 12/1', 'MaxMa 60', 'MaxMa 14' and 'Alkavo 2', were also recorded root suckers but to a much lesser extent – 2.5-4.8 suckers per tree. No root suckers were recorded in variants with 'Gisella 5' and

‘Gisella 6’ rootstocks.

Table 2. Canopy dimensions of ‘Summit’ sweet cherry trees on different rootstocks, at the end of 9<sup>th</sup> growing season

| Rootstock  | Height (m) | Width (m) | Volume (m <sup>3</sup> ) | Volume (%) of F 12/1 | Projection area (m <sup>2</sup> ) |
|------------|------------|-----------|--------------------------|----------------------|-----------------------------------|
| F 12/1 (c) | 4.0 c      | 3.4 b     | 12.1 d                   | 100                  | 9,1 b                             |
| CAB 6P     | 4.1 cd     | 4.0 d     | 17.2 f                   | 142.1                | 12.6 d                            |
| Alkavo 2   | 4.2 de     | 3.1 a     | 10.6 c                   | 87.6                 | 7.5 a                             |
| Gisela 5   | 2.7 a      | 3.0 a     | 6.4 a                    | 52.9                 | 7.1 a                             |
| Gisela 6   | 3.0 b      | 3.3 b     | 8.5 b                    | 70.2                 | 8.5 b                             |
| MaxMa 14   | 4.0 c      | 3.8 c     | 15.1 e                   | 124.8                | 11.3 c                            |
| MaxMa 60   | 4.3 e      | 4.0 d     | 18.0 f                   | 148.8                | 12.6 d                            |

Means followed by the same letter in columns are not significantly different at  $p \leq 0.05$  (Duncan’s test).

During the studied period, the rootstocks had a tremendous effect on trees productivity, and most of the differences between the different variants were very well expressed and statistically proven (Table 3). Good yields were recorded only in the 8<sup>th</sup> year (2015), and in the remaining years they were severely reduced as a result of late spring frosts. With higher productivity in the individual years and total for the period stand out the trees grafted on ‘MaxMa 60’, ‘CAB 6P’ and ‘MaxMa 14’. On these rootstocks, the cumulative yield was between 20 and 30% higher than that of ‘F 12/1’. Good cumulative yield was also recorded on ‘Alkavo 2’, which was only 5% lower and does not differ significantly from that of F 12/1. The least productive were the trees on about Gisela rootstocks. Cumulative yields on ‘Gisela 5’ and ‘Gisela 6’ were around 62 and 73% than that of ‘F 12/1’.

Table 3. Rootstock effect on average and cumulative yield, and fruit weight of ‘Summit’ sweet cherry trees in the period 6<sup>th</sup>-9<sup>th</sup> growing season

| Rootstock | Yield (kg/tree) |        |        |       |            | Fruit weight (g) |      |      |      |         |
|-----------|-----------------|--------|--------|-------|------------|------------------|------|------|------|---------|
|           | 2013            | 2014   | 2015   | 2016  | Cumulative | 2013             | 2014 | 2015 | 2016 | Average |
| F 12/1    | 4,0cd           | 9,7bc  | 36,3ab | 3,7c  | 53,7bc     | 10,5             | 9,7  | 7,9  | 9,7  | 9,5a    |
| CAB 6P    | 8,0e            | 16,3e  | 40,3b  | 1,9b  | 66,5d      | 7,3              | 7,5  | 7,1  | 10,1 | 8,0a    |
| Alkavo 2  | 1,9b            | 12,1cd | 36,1ab | 1,0ab | 51,1b      | 10,2             | 12,2 | 6,8  | 10,5 | 9,9b    |
| Gisela 5  | 0,2a            | 3,8a   | 28,5a  | 0,6a  | 33,1a      | 12,1             | 7,7  | 5,7  | 9,9  | 8,9a    |
| Gisela 6  | 2,8bc           | 8,5b   | 26,8a  | 1,1ab | 39,2a      | 10,2             | 8,1  | 5,6  | 7,8  | 7,9a    |
| MaxMa14   | 5,0d            | 14,8de | 42,9b  | 1,6ab | 64,3cd     | 8,8              | 9,0  | 6,5  | 11,1 | 8,9a    |
| MaxMa60   | 5,5d            | 9,8bc  | 50,5b  | 4,0c  | 69,8d      | 8,9              | 8,7  | 8,4  | 10,8 | 9,2a    |

The correlations between the yield and the trunk-cross sectional area (Figure 1) and the yield and canopy volume (Figure 2) showed a direct link between these components and give some advantage to the more vigorous rootstocks. These results confirm other our studies, in which also was found that the yield from more vigorous cultivar/rootstock combinations was higher than the lesser ones (Sotirov, 2015 a,b) and differed from the opinion of other authors who established that trees on ‘Gisela 6’ and ‘Gisela 5’ were more productive than vigorous Mazzard (Whiting et al., 2005).

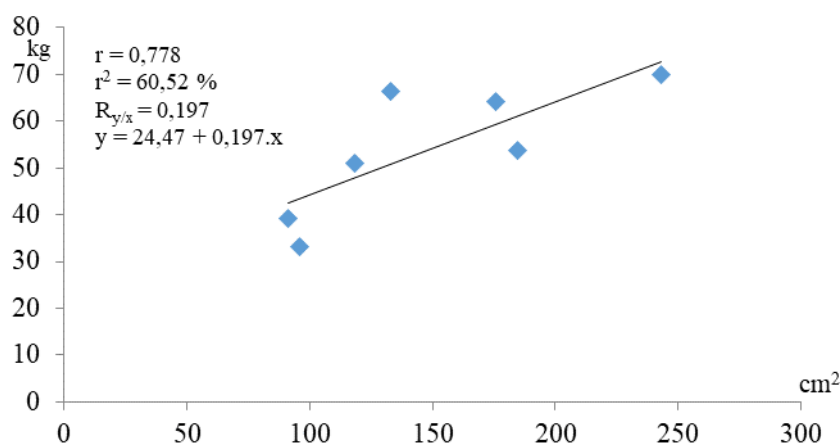


Figure 1. Correlation between the trunk-cross sectional area (cm<sup>2</sup>) and the yield (kg/tree) of 'Summit' sweet cherry trees on different rootstocks

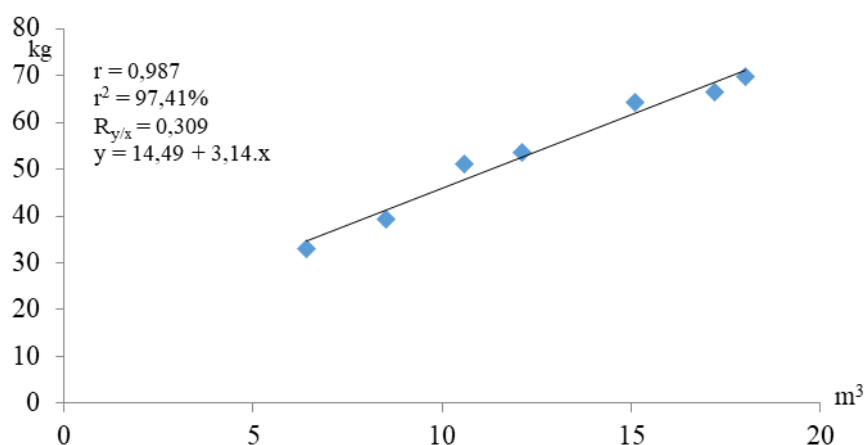


Figure 2. Correlation between the canopy volume (m<sup>3</sup>) and the yield (kg/tree) of 'Summit' sweet cherry trees on different rootstocks

The highest cumulative yield efficiency was calculated for trees on 'CAB 6P' (0.50 kg/cm<sup>2</sup>) and the lowest on 'F 12/1' and 'MaxMa 60' (0.29 kg/cm<sup>2</sup>). On the remaining rootstocks was achieved medium yield efficiency (0.34-0.43 kg/cm<sup>2</sup>) and the differences among them were not significant (Table 4). 'Gisela 5' had the highest cumulative yield per unit crown volume due primarily to its weaker vigour. The lowest were the values for 'CAB 6P' and 'MaxMa 60', but the differences between the different variants were not significant. The best result in regard to yield per unit canopy horizontal projection area was obtained for 'Alkavo 2'. 'Gisela 5' and 'Gisela 6' exhibited the lowest values of this parameter. Trees on the other rootstocks also had a good specific yield.

The average fruit weight varied considerably over the years, with some differences between the rootstocks, but there was no one-sidedness of the results (Table 3). In the first and last years, the fruit weight was the greatest, because of the lowest yields, which is logical. During the year with a normal yield (2015), the weight of the fruits was higher on 'MaxMa 60', 'F12/1' and 'CAB 6P'. On these three rootstocks there was a tendency to keep their fruit weight relatively constant at a substantial variation in the yields. At the same time, 'Gisela 5' and 'Gisela 6' showed a tendency to reduce the fruit weight when the trees were overloaded. The decrease in the fruit weight under their influence in 2015 is about ½ compared to 2013. On average, for the period of the study, the fruit of the trees on 'Alkavo 2' were proven to be larger, while the differences between the other rootstocks were not significant.

Table 4. Yield indices of 'Summit' sweet cherry trees on different rootstocks, at the end of 9<sup>th</sup> growing season

| Rootstock  | Cumulative yield per:         |                                       |                                     |
|------------|-------------------------------|---------------------------------------|-------------------------------------|
|            | TCSA<br>(kg/cm <sup>2</sup> ) | canopy volume<br>(kg/m <sup>3</sup> ) | canopy area<br>(kg/m <sup>2</sup> ) |
| F 12/1 (c) | 0,29 a                        | 4,44 a                                | 5,90 ab                             |
| CAB 6P     | 0,50 b                        | 3,87 a                                | 5,28 ab                             |
| Alkavo 2   | 0,43 ab                       | 4,82 a                                | 6,81 b                              |
| Gisela 5   | 0,34 ab                       | 5,17 a                                | 4,66 a                              |
| Gisela 6   | 0,43 ab                       | 4,61 a                                | 4,61 a                              |
| MaxMa 14   | 0,37 ab                       | 4,26 a                                | 5,69 ab                             |
| MaxMa 60   | 0,29 a                        | 3,88 a                                | 5,54 ab                             |

### Conclusions

The results of this study showed that the rootstock has a significant impact on the growth and productive manifestations of 'Summit' sweet cherry cultivar. According to trunk-cross sectional area and the canopy volume, 'MaxMa 60', 'MaxMa 14' and 'CAB 6P' were the most vigorous, and 'Gisela 5' and 'Gisela 6' - the most dwarfing rootstocks. Higher yields were obtained from the trees on more vigorous rootstocks, as confirmed by the high correlation coefficients -  $r = 0.778$  between the yield and the trunk-cross sectional area and  $r = 0.987$  between the yield and the canopy volume. The highest cumulative yield efficiency (kg/cm<sup>2</sup> of TCSA) had the trees on 'CAB 6P' and the lowest on 'F 12/1' and 'MaxMa 60'. The highest yield per 1 m<sup>3</sup> of the crown volume was calculated for 'Gisela 5' due to its smallest volume. The trees of 'Alkavo 2' had the highest yield per 1 m<sup>2</sup> of the crown projection area. The differences on these three yield indices were insignificant between the other rootstocks.

Except for 'Alkavo 2', no significant differences were found in average fruit weight between the tested rootstocks, although in years of higher load, 'Gisela 5' and 'Gisela 6' lead to a reduction in the fruit weight, probably due to a disturbed balance between the leaf area and the yield. The results of this study are not yet sufficient to conclude which of these rootstocks is the best suited for Summit sweet cherry cultivar for the specific conditions of the experiment, so the researches should continue.

### References

1. Bujdosó, G., Hrotkó, K. (2005). Rootstock-scion interactions on dwarfing cherry rootstocks in Hungary. *Hortic. Sci. (Prague)*, 32: 129–137.
2. Fajt, N., Bassi, G., Folini, L. and Siegler, H. (2014). 'Lapins' on ten rootstocks in the Alpe Adria region. *Acta Hortic.* 1020: 371-376.
3. Grzyb, Z.S., Sitarek, M., Omiecińska, B. (1998). Growth and fruiting of five sweet cherry cultivars on dwarfing and vigorous rootstocks. *Acta Hortic.* 468: 333-338.
4. Hrotko, K. (2008). Progress in cherry rootstock research. *Acta Hortic.* 795: 171-177.
5. Lang, G.A. (2001). Intensive sweet cherry orchard systems-rootstocks, vigor, precocity, productivity and management. *Compact Fruit Tree*, 34(1): 23-26.
6. Lichev, V. (2001). First results from testing cherry clonal rootstocks Gisela and Weiroot in Bulgaria. 9<sup>th</sup> International conference of horticulture, fruit growing and viticulture, Lednice, Czech Republic, Vol.1: 111-115.
7. Lichev, V. and Papachatzis, A. (2011). Results from the 11-year examining of rootstocks of the series Gisela and Weiroot in Bulgaria. *Acta Hortic.* 903: 529-534.
8. Sansavini, S. and Lugli, S. (2014). New rootstocks for intensive sweet cherry plantations. *Acta Hortic.* 1020: 411-434.
9. Sotirov, D. (2015 a). Performance of the sweet cherry cultivars 'Van' and 'Kozerska' on clonal rootstocks. *Acta Hortic.* 1099: 727-733.
10. Sotirov, D. (2015 b). Growth and productive manifestations of Lapins sweet cherry cultivar on different rootstocks. *Journal of Mountain Agriculture on the Balkans of Mountain Stockbreeding and*

Agriculture, Troyan. Vol. 18, 4: 740-749.

11. Weber, M. (2001). Sweet cherry orchard management with dwarfing rootstocks in Germany. Compact Fruit Tree, 34(1): 20-22.

12. Whiting, M., Lang, G., Ophardt, D. (2005). Rootstock and training system affected sweet cherry growth, yield, and fruit quality. Hort. Sci. 40(3): 582-586.