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CONTENTS

THE EFFECT OF FERTILISERS ON THE QUALITY OF *PETUNIA X HYBRIDA* „DOUBLE PIROUETTE PURPLE“

Margarita Davitkovska[1], Vjekoslav Tanaskovic[1], Zvezda Bogevska[1], Gordana Popsimonova[1], Rukie Agic[1], Boris Dorbic[2]

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Abstract
The survey was conducted in order to determine the quality of the seedlings of *Petunia x hybrida* „Double pirouette purple“. It was produced and treated with different fertilizers, including different concentration. Three different types of liquid fertilizers – Magnicvet with NPK 7-1-5 + ME, Magnihortal with NPK 10-5-5 + ME and Humifulvil, with three different concentrations were used in the experiment. According to the methodology of work and the objective of the examination, following biometric parameters were analysed: height of plants (cm), stem thickness (mm), number of branches, number of flower buds and number of flowers. These biometric parameters were measured 40 days after planting into pots. The highest stem thickness has the plants fertilized with Magnihortal with concentration of 0,3 %. The highest number of flower buds and number of branches has the plants fertilized with Magnihortal with concentration of 0,4 %. The highest plant height has plants fertilized with Magnicvet with concentration of 0,3 %. The highest number of flowers has plants fertilized with Magnicvet with concentration of 0,4 %. On the basis of the results of all the examined biometrical parameters, it is determined that the nutrition with Magnihortal has shown the best results.

Keywords: Magnicvet, Magnihortal, Humifulvil, biometric parameters, concentration.

Introduction

Petunia hybrida hort. belongs to the *Solanaceae* family. There are 35 species in the *Petunia* genus. *Petunia hybrid* hort. was created by cross breeding of *Petunia axillaris* Lam. and *Petunia integrifolia* Hook (Dole and Wilkins, 1999). *Petunia hybrida* is an annual flower, with height of 20-30 cm. Its period of blooming lasts from May to September. In open field it is planted from mid-May, and the distance between the plants should be 20-30 cm (Mayer, 2006). Cultivars of petunias are divided in four main groups or categories based on the flowers and breeding: *grandiflora* (with large flowers, early blooming), *multiflora* (with medium-large flowers, well branched), *floribunda* (with medium-large flowers) and *milliflora* (with small flowers and thick growth). Also there are hanging petunias with many different cultivars (Mayer, 2006). Numerous petunia cultivars are available in a wide range and combinations of colours (Dole and Wilkins, 1999). *Petunia hybrida* L. is used for flower-beds, and it is also very suitable for containers, pots and hanging flower baskets (Hessayon, 2004, Karlović et al., 2005, Paradžiković, 2012). *Petunia hybrida* belongs to semi resistant annual flowers which are being sown in greenhouses and they are replanted in the open field when there is no danger of frost (Hessayon, 1997). Optimal pH is 6,0 and EC 0,5 – 1,0 mS/cm (Hamrick, 2003). Production of seedlings is one of the most significant aspects in flower production, along with protection against diseases and pests. Choosing the most appropriate fertilizer for production of seedlings in protected environment represents one of the most significant activities in the process of production (Davitkovska, 2014). *Petunia hybrida* L. does not require fertilization 7 to 10 days after sowing for better root development (Dimovska, 2008, according to Hamrick, 2003). Fertilization should be performed with liquid fertilizers that contain N, P and K in ratio 20-10-20 and 15-0-15

(Dimovska, 2008, according to Hamrick, 2003). The successfulness of seasonal flowers is in direct dependence with the quality of seedlings. It is known that the quality of seedlings depends on multiple factors like: the quality of the seed, microclimate conditions, production equipment (technical equipment) and the type of protected environment. Next to the above mentioned factors the quality of substrate used in seedlings production is equally relevant (Dimovska, 2008). The goal of this research is to examine the influence of different types of fertilizers on morphological characteristics of petunia seedlings. It is also a goal to establish the most appropriate concentration of fertilizer to get quality seedlings.

Material and methods

The examination was set in the farm "Flower-Garden" in the village Vladevci, Strumica, Republic of Macedonia. The experiment was conducted on *Petunia* x *hybrida* "Double Pirouette Purple". The seed was from a Dutch factory Syngenta. The process of seedlings production from seed was carried out at a private farm "Flower-Garden". Substrate used for seedlings production of *Petunia x hybrida* is known as "Profimix 2 Surfinia" and the manufacturer is JSC "DURPETA" from Lithuania. This substrate is universal for production of flower seedlings. Three different types of liquid fertilizers – Magnicvet with NPK 7-1-5 + ME, Magnihortal with NPK 10-5-5 + ME and Humifulvil, with three different concentrations were used in the experiment. Seedlings of *Petunia hybrida* were grown from seeds which were from Netherlands, factory Syngenta. The seeds were planted in containers and grown in containers up to germination and formation of the first two to three leaves. The seedlings were manually taken out of the container and replanted in plastic pots with 9,5 cm diameter. The experiment contained nine variants. Every variant was consisted of 15 plants or a total of 135 plants in experiment. Fertilization was started when the seedlings had 3 to 4 leaves. 100 ml of solution was applied manually on one plant, i.e. one seedling. They were fertilized once a week, i. e. during the experiment 5 fertilizations were conducted. Types of fertilizers, their concentrations and solution are shown in the following table.

Table 1. Fertilization regime in the experiment

Variant	Type of fertilizer	Concentration	Solution	Number of plants
Variant I	Magnicvet	0,2%	3 ml / 1,5 l	15
Variant II	Magnicvet	0,3 %	4,5 ml / 1,5 l	15
Variant III	Magnicvet	0,4 %	6 ml / 1,5 l	15
Variant IV	Magnihortal	0,2 %	3 ml / 1,5 l	15
Variant V	Magnihortal	0,3 %	4,5 ml / 1,5 l	15
Variant VI	Magnihortal	0,4 %	6 ml / 1,5 l	15
Variant VII	Humifulvil	0,5 %	7,5 ml / 1,5 l	15
Variant VIII	Humifulvil	0,7 %	10,5 ml / 1,5 l	15
Variant IX	Humifulvil	1,0 %	15 ml / 1,5 l	15

When the plants have been replanted from the containers to plastic pots irrigation was immediately carried out. After that irrigation was conducted twice a week. Every plant was irrigated manually with 100 ml of clean water. Measurements of biometric parameters were conducted in the laboratory of Department of vegetable and flower crop production, at the Faculty of Agricultural Sciences and Food in Skopje, University „Sv. Cyril and Methodius"in Skopje. 15 plants of every variant were measured, after 40 days of transplanting in the plastic pots. Following biometric parameters were analysed: plant height (cm), stem thickness (mm), number of branches, number of flower buds and number of flowers. The received results were statistically processed according to the method of analysis of variance and test with LSD (Least Significant Difference) test.

Results and discussion

The highest average value for the height of plants (19,34 cm) was obtained in the plants from variant II. The plants from variant III showed similar results as variant II with an average value of 19,24 cm.

Lowest average value for the height of plants (14,78 cm) was obtained in the variant VIII. Plants from variant V had the most heterogeneous height (CV 27,44%). The height of plants from variants VII and VIII showed significant statistical difference at a level of 0.05 compared with the height of plants from the variant II and variant III. The height of plants from variant VIII showed significant statistical difference at a level of 0.05 compared with height of plants from the variant V. Between the remaining variants there was no statistically significant difference (Table 3).

Table 2. Height of plants (cm)

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	18.87	1.51	7.98	12.8-27.0
II	19.34	4.38	22.66	13.0-28.0
III	19.24	3.91	20.30	12.7-27.7
IV	17.91	3.38	18.90	11.0-22.5
V	18.97	5.21	27.44	13.0-27.7
VI	16.19	4.40	27.20	11.0-26.0
VII	15.01	3.75	24.96	8.9-24.0
VIII	14.78	3.03	20.48	10.0-21.0
IX	17.60	3.57	20.29	9.5-22.0

Table 3. Height of plants (cm) – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	-0.47	-0.37	0.96	-0.1	2.68	3.86	4.09	1.27
II	0.47	Var. II	0.1	1.43	0.37	3.15	4.33	4.56	1.74
III	0.37	-0.1	Var.III	1.33	0.27	3.05	4.23	4.46	1.64
IV	-0.96	-1.43	-1.33	Var. IV	-1.06	1.72	2.9	3.13	0.31
V	0.1	-0.37	-0.27	1.06	Var. V	2.78	3.96	4.19	1.37
VI	-2.68	-3.15	-3.05	-1.72	-2.78	Var.VI	1.18	1.41	-1.41
VII	-3.86	-4.33	-4.23	-2.9	-3.96	-1.18	Var.VII	0.23	-2.59
VIII	-4.09	-4.56	-4.46	-3.13	-4.19	-1.41	-0.23	Var.VIII	-2.82
IX	-1.27	-1.74	-1.64	-0.31	-1.37	1.41	2.59	2.82	Var. IX

LSD 0.05 =4.19; LSD 0.01 =5.77

The average stem thickness ranges from 4,67 mm in the variant III to 5,80 mm in the variant V. Plants from variant I had the most heterogeneous stem thickness (CV 19,52%). The stem thickness from variant II showed significant statistical difference at a level of 0.05 compared with the stem thickness from the variant I. The stem thickness from variant III showed significant statistical difference at a level of 0.01 compared with the stem thickness from the variant I. The stem thickness from variant IX showed significant statistical difference at a level of 0.05 compared with the stem thickness from the variant II. The stem thickness from variants V and VI showed significant statistical difference at a level of 0.01 compared with the stem thickness from the variant II. The stem thickness from variants IV and VIII showed significant statistical difference at a level of 0.05 compared with the stem thickness from the variant III. Variants V, VI and IX showed significant statistical difference at a level of 0.01 compared with variant III. Variant VII showed significant statistical difference at a level of 0.05 compared with variant V. Also, variant VII showed significant statistical difference at a level of 0.05 compared with variant VI. There was statistically significant difference among variant IX and variant VII at the level of 0.05.

Table 4. Stem thickness (mm)

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	5.59	1.09	19.52	5.0-6.5
II	4.91	0.86	17.54	3.0-6.0
III	4.67	0.79	16.84	3.0-5.7
IV	5.25	0.97	18.41	4.0-6.6
V	5.80	0.97	16.73	4.0-7.6
VI	5.72	0.79	13.76	4.5-7.2
VII	5.11	0.82	16.11	4.0-6.6
VIII	5.41	0.80	14.76	4.1-7.0
IX	5.68	0.85	15.04	4.5-7.4

Table 5. Stem thickness (mm) – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	0.68	0.92	0.34	-0.21	-0.13	0.48	0.18	-0.09
II	-0.68	Var. II	0.24	-0.34	-0.89	-0.81	-0.2	-0.5	-0.77
III	-0.92	-0.24	Var.III	-0.58	-1.13	-1.05	-0.44	-0.74	-1.01
IV	-0.34	0.34	0.58	Var. IV	-0.55	-0.47	0.14	-0.16	-0.43
V	0.21	0.89	1.13	0.55	Var. V	0.08	0.69	0.39	0.12
VI	0.13	0.81	1.05	0.47	-0.08	Var.VI	0.61	0.31	0.04
VII	-0.48	0.2	0.44	-0.14	-0.69	-0.61	Var.VII	-0.3	-0.57
VIII	-0.18	0.5	0.74	0.16	-0.39	-0.31	0.3	VarVIII	-0.27
IX	0.09	0.77	1.01	0.43	-0.12	-0.04	0.57	0.27	Var. IX

LSD 0.05 =0.57; LSD 0.01 =0.79

The number of branches was largest in variant VI (8,38 branches). The lowest number of branches had plants of variant III, with the average value of 6,21 branches. The most heterogeneous coefficient of variation had plants from variant I with CV 28,71%.

Table 6. Number of branches

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	7.57	2.17	28.71	3-11
II	7.21	1.93	26.73	5-11
III	6.21	1.31	21.10	4-9
IV	7.00	1.85	26.45	3-10
V	7.73	1.94	25.14	4-13
VI	8.38	1.98	23.62	6-12
VII	6.62	1.85	27.97	3-10
VIII	6.92	0.79	11.46	6-8
IX	6.67	1.83	27.39	3-9

Plants from the variant III showed significant statistical difference at a level of 0.05 in the number of branches compared with plants of variant I. The number of branches from variant V showed significant statistical difference at a level of 0.05 compared with the number of branches from the variant III. Between the variants VI and III there was statistically significant difference at a level of 0.01. Between the variants VI and IV there was statistically significant difference at a level of 0.05. Between the variants VIII and VI there was statistically significant difference at a level of 0.05. Variants VII and IX showed significant statistical difference at a level of 0.01 compared with variant VI (Table 7).

Table 7. Number of branches – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	0.36	1.36	0.57	-0.16	-0.81	0.95	0.65	0.9
II	-0.36	Var. II	1.0	0.21	-0.52	-1.17	0.59	0.29	0.54
III	-1.36	-1.0	Var.III	-0.79	-1.52	-2.17	-0.41	-0.71	-0.46
IV	-0.57	-0.21	0.79	Var. IV	-0.73	-1.38	0.38	0.08	0.33
V	0.16	0.52	1.52	0.73	Var. V	-0.65	1.11	0.81	1.06
VI	0.81	1.17	2.17	1.38	0.65	Var.VI	1.76	1.46	1.71
VII	-0.95	-0.59	0.41	-0.38	-1.11	-1.76	Var.VII	-0.3	-0.05
VIII	-0.65	-0.29	0.71	-0.08	-0.81	-1.46	0.3	Var.VIII	0.25
IX	-0.9	-0.54	0.46	-0.33	-1.06	-1.71	0.05	-0.25	Var. IX

LSD 0.05 =1,23; LSD 0.01 =1,70

The highest average number of inflorescences was obtained in plants from variant VI (13,54 inflorescences). Plants from the variant I had the lowest values, with an average value of 4,5 inflorescences. Plants from variant III had the most heterogeneous number of inflorescences (CV 62,86%).

Table 8. Number of inflorescences

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	4.50	1.51	33.48	2-7
II	6.36	2.65	41.67	4-13
III	5.57	3.50	62.86	2-16
IV	7.73	2.34	30.31	4-12
V	10.07	3.67	36.49	6-18
VI	13.54	4.31	31.86	5-22
VII	9.54	3.95	41.41	3-18
VIII	10.42	2.02	19.40	8-16
IX	8.08	3.90	48.19	3-17

The number of inflorescences in plants from variant IV and IX showed significant statistical difference at a level of 0.05 compared with the number of inflorescences from plants of the variant I. Variants V, VI, VII and VIII showed significant statistical difference at a level of 0.01 compared with variant I. Variants V, VII and VIII showed significant statistical difference at a level of 0.05 compared with variant II. Between the variants VI and II there was statistically significant difference at a level of 0.01. Between the variants VII and III there was statistically significant difference at a level of 0.05. Variants V, VI and VIII showed significant statistical difference at a level of 0.01 compared with variant III. Between the variants VI and IV there was statistically significant difference at a level of 0.01. Between the variants VI and V there was statistically significant difference at a level of 0.05. Variants VII and VIII showed significant statistical difference at a level of 0.05 compared with variant VI. Variant IX showed significant statistical difference at a level of 0.01 compared with variant VI.

Table 9. Number of inflorescences – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	-1.86	-1.07	-3.23	-5.57	-9.04	-5.04	-5.92	-3.58
II	1.86	Var. II	0.79	-1.37	-3.71	-7.18	-3.18	-4.06	-1.72
III	1.07	-0.79	Var.III	-2.16	-4.5	-7.97	-3.97	-4.85	-2.51
IV	3.23	1.37	2.16	Var. IV	-2.34	-5.81	-1.81	-2.69	-0.35
V	5.57	3.71	4.5	2.34	Var. V	-3.47	0.53	-0.35	1.99
VI	9.04	7.18	7.97	5.81	3.47	Var.VI	4.0	3.12	5.46
VII	5.04	3.18	3.97	1.81	-0.53	-4.0	Var.VII	-0.88	1.46
VIII	5.92	4.06	4.85	2.69	0.35	-3.12	0.88	Var.VIII	2.34
IX	3.58	1.72	2.51	0.35	-1.99	-5.46	-1.46	-2.34	Var.IX

LSD 0.05 =2,99; LSD 0.01 =4,12

The highest average value for the number of flowers (2,43 flowers) was obtained in the plants from the variant III. The plants from variant VIII showed similar results as variant III with an average value of 2,33 flowers. Lowest average value for the number of flowers (1,46 flowers) was obtained in the variants VI and VII. Plants from variant VI had the most heterogeneous number of flowers (CV 91,01%).

Table 10. Number of flowers

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	1.50	1.09	72.80	0-3
II	1.57	0.85	54.19	0-3
III	2.43	0.85	35.07	1-4
IV	1.67	1.18	70.51	0-4
V	1.67	1.35	80.71	0-4
VI	1.46	1.33	91.01	0-4
VII	1.46	1.27	86.62	0-4
VIII	2.33	1.56	66.73	1-5
IX	2.00	1.28	63.96	0-5

Results in Table 11 shows that there was no significant statistical difference between all the variants.

Table 11. Number of flowers – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	-0.07	-0.93	-0.17	-0.17	0.04	0.04	-0.83	-0.5
II	0.07	Var. II	-0.86	-0.1	-0.1	0.11	0.11	-0.76	-0.43
III	0.93	0.86	Var.III	0.76	0.76	0.97	0.97	0.1	0.43
IV	0.17	0.1	-0.76	Var. IV	0.0	0.21	0.21	-0.66	-0.33
V	0.17	0.1	-0.76	0.0	Var. V	0.21	0.21	-0.66	-0.33
VI	-0.04	-0.11	-0.97	-0.21	-0.21	Var.VI	0.0	-0.87	-0.54
VII	-0.04	-0.11	-0.97	-0.21	-0.21	0.0	Var.VII	-0.87	-0.54
VIII	0.83	0.76	-0.1	0.66	0.66	0.87	0.87	Var.VIII	0.33
IX	0.5	0.43	-0.43	0.33	0.33	0.54	0.54	-0.33	Var. IX

LSD 0.05 =1,06, LSD 0.01 =1,46

Conclusions

The survey was conducted in order to determine the quality of the seedlings of *Petunia x hybrida* „Double pirouette purple“ which were produced and treated with different fertilizers, including different concentration. Three different types of liquid fertilizers – Magnicvet with NPK 7-1-5 + ME, Magnihortal with NPK 10-5-5 + ME and Humifulvil, with three different concentrations were used in the experiment. The quality of seedlings of *Petunia x hybrida* „Double pirouette purple“ was determined by examination of following biometric parameters: height of plants (cm), stem thickness (mm), number of branches, number of flower buds and number of flowers. The highest average value for the height of plants (19,34 cm) was obtained in the plants fertilized with Magnicvet with concentration of 0,3 %. The highest stem thickness (5,80 mm) has the plants fertilized with Magnihortal with concentration of 0,3 %. The number of branches was largest in the plants fertilized with Magnihortal with concentration of 0,4 %, with 8,38 branches. The highest average number of inflorescences (13,54 inflorescences) was obtained in plants fertilized with Magnihortal with concentration of 0,4 %. The highest average value for the number of flowers (2,43 flowers) was obtained in the plants fertilized with Magnicvet with concentration of 0,4 %.

References

1. Davitkovska, M. (2014). Determination of the Nutritional Regime for Acquirement of Quality Seedling of Flower Species Geranium (Pelargonium x hortorum L. H. Bail.). Doctoral Dissertation, University „St. Cyril and Methodius"–Skopje, Faculty of Agricultural Sciences and Food–Skopje, 3-27.
2. Dimovska, M. (2008). Influence of Substrate Type on the Quality of Seedlings of Petunia hybrida grandiflora hort.. Master Thesis, University „St. Cyril and Methodius"–Skopje, Faculty of Agricultural Sciences and Food–Skopje, 3-36.
3. Dole, M. J., Wilkins, H. F. (1999). Floriculture. Principles and Species, Prentice-Hall, Upper Saddle River, N. J, 464-465.
4. Hamrick, D. (2003). Crop Production Volume 2, Ball Redbook 17th edition. Ball Publishing, 567-574.
5. Hessayon, D. G. (1997). Sve o vrtu. Mozaik knjiga, Zagreb, 28.
6. Hessayon, D. G. (2004). Cvijeće u vrtu. Mozaik knjiga, Zagreb, 33.
7. Karlović, K., Pagliarini, N., Vrdoljak, A., Vršek, I. (2005). Sobno i balkonsko cvijeće. Gospodarski list, 98.
8. Mayer, J. (2006). Cvijeće za balkone i terase. Mozaik knjiga, Grupa mladinska knjiga, 106-107.
9. Paradžiković, N. (2012). Osnove cvjećarstva. Interna skripta, Poljoprivredni fakultet Osijek, 83-86.

THE EFFECT OF FERTILISERS ON THE QUALITY OF *PETUNIA X HYBRIDA* „DUVET PINK“

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Abstract
The research was conducted in order to analyse the effect of different types of fertilizers on morphological parameters of *Petunia x hybrida* „Duvet pink“. Three different types of liquid fertilisers – Magnicvet with NPK 7-1-5 + ME, Magnihortal with NPK 10-5-5 + ME and Humifulvil, with three different concentrations were used in the experiment. According to the methodology of work and the objective of the examination, following biometric parameters were analysed: plant height (cm), stem thickness (mm), number of branches, number of flower buds and number of flowers. These biometric parameters were measured 40 days after planting into pots. The highest stem thickness and number of branches has the plants fertilized with Magnicvet with concentration of 0,4 %. The highest number of inflorescences has the plants fertilized with Magnicvet with concentration of 0,3 %. The highest plant height has plants fertilized with Magnihortal with concentration of 0,4 %. The highest number of flowers has plants fertilized with Humifulvil with concentration of 0,5 %. The analysed biometric parameters have shown that the liquid mineral fertiliser Magnicvet is more appropriate compared with the liquid mineral fertilizers Magnihortal and Humifulvil.

Keywords: biometric parameters, concentration, measurements.

Introduction

Petunia hybrida hort. was created by cross breeding of *Petunia axillaris* Lam. and *Petunia integrifolia* Hook (Dole and Wilkins, 1999). *Petunia hybrida* is an annual flower. Its period of blooming lasts from May to September. In open field it is planted from mid-May whereas the distance between the plants should be 20-30 cm (Mayer, 2006). *Petunia hybrida* L. is used for flower-beds, and it is also very suitable for containers, pots and hanging flower baskets (Hessayon, 2004, Karlović et al., 2005, Paradžiković, 2012). *Petunia hybrida* belongs to semi resistant annual flowers which are being sown in greenhouses and they are replanted in the open field when there is no danger of frost (Hessayon, 1997). Optimal pH is 6,0 and EC 0,5 – 1,0 mS/cm (Hamrick, 2003). The success of the production of seasonal flowers is in direct dependence of the types of fertilizers. It is known that the quality of seedling depends on several factors such as: the quality of seed, type of substrate, climatic conditions in the region, equipment (technical equipment) and the type of the greenhouse. Besides the above mentioned factors, equally important is the type and concentration of fertilizer which is used in the production. The use of the most appropriate fertilizer has high significance for the obtaining of quality seedlings, which further reflects on growth and development of flower culture. Hence, it is estimated that although it is one of the segments in the production of seedlings, the impact of certain types of fertilizers on the quality of the seedlings of flowers is of great importance. Therefore this research demonstrates that the application of certain fertilizer with certain concentration has a significant role in the production of seedlings.

Material and methods

The examination was made in the farm "Flower-Garden" in the village Vladevci, Strumica, Republic of Macedonia. The experiment was conducted on *Petunia x hybrida* „Duvet pink". The seed was from a Dutch factory Syngenta. The process of seedlings production from seed was carried out at a private farm "Flower-Garden". Substrate used for seedlings production of Petunia x hybrid „Duvet pink". is known as "Profimix 2 Surfinia" and the manufacturer is JSC "DURPETA" from Lithuania. This substrate is universal for production of flower seedlings. Three different types of liquid fertilizers – Magnicvet with NPK 7-1-5 + ME, Magnihortal with NPK 10-5-5 + ME and Humifulvil, with three different concentrations were used in the experiment. Seedlings of *Petunia hybrida* were grown from seeds which were delivered from Netherlands, factory Syngenta. The seeds were planted in containers and grown in containers up to germination and formation of the first two to three leaves. The seedlings were manually taken out of the container and replanted in plastic pots with 9,5 cm diameter. The experiment contained nine variants. Every variant was consisted of 15 plants or a total of 135 plants in experiment. Fertilization was started when the seedlings had 3 to 4 leaves. 100 ml of solution was applied manually on one plant, i.e. one seedling. They were fertilized once a week. During the experiment 5 fertilizations were conducted. Types of fertilizers, their concentrations and solution are displayed in the following table.

Table 1. Fertilization regime in the experiment

Variant	Type of fertilizer	Concentration	Solution	Number of plants
Variant I	Magnicvet	0,2%	3 ml / 1,5 l	15
Variant II	Magnicvet	0,3 %	4,5 ml / 1,5 l	15
Variant III	Magnicvet	0,4 %	6 ml / 1,5 l	15
Variant IV	Magnihortal	0,2 %	3 ml / 1,5 l	15
Variant V	Magnihortal	0,3 %	4,5 ml / 1,5 l	15
Variant VI	Magnihortal	0,4 %	6 ml / 1,5 l	15
Variant VII	Humifulvil	0,5 %	7,5 ml / 1,5 l	15
Variant VIII	Humifulvil	0,7 %	10,5 ml / 1,5 l	15
Variant IX	Humifulvil	1,0 %	15 ml / 1,5 l	15

When the plants have been replanted from the containers to plastic pots irrigation was immediately carried out. After that irrigation was conducted twice a week. Every plant was irrigated manually with 100 ml of clean water. Measurements of biometric parameters were conducted in the laboratory of Department of vegetable and flower crop production, at the Faculty of Agricultural Sciences and Food in Skopje, University „Sv. Cyril and Methodius"in Skopje. 15 plants of every variant were measured, after 40 days of transplanting in the plastic pots. Following biometric parameters were analysed: plant height (cm), stem thickness (mm), number of branches, number of flower buds and number of flowers. The received results were statistically processed according to the method of analysis of variance and test with LSD (Least Significant Difference) test.

Results and discussion

The highest average value for the height of plants (9,30 cm) was obtained in the plants from variant VI. The plants from variant V showed similar results as variant VI with an average value of 9,16 cm. Lowest average value for the height of plants (6,49 cm) was obtained in the variant VIII. Plants from variant IX had the most heterogeneous height (CV 25,91%). The height of plants from variant III showed significant statistical difference at a level of 0.05 compared with the height of plants from the variant I. The height of plants from variants VIII and IX showed significant statistical difference at a level of 0.01 compared with the height of plants from the variant I. The height of plants from variants V, VI and VIII showed significant statistical difference at a level of 0.05 compared with the height of plants from the variant II. Variants V and VI showed significant statistical difference at a level of 0.01 compared with variant III. Variant IX showed significant statistical difference at a level of

0.05 compared with variant IV. Variant VIII showed significant statistical difference at a level of 0.01 compared with variant IV. Also, variant VII showed significant statistical difference at a level of 0.05 compared with variant V. Variants VIII and IX showed significant statistical difference at a level of 0.01 compared with variant V. Variant VII showed significant statistical difference at a level of 0.05 compared with variant VI. Variants VIII and IX showed significant statistical difference at a level of 0.01 compared with variant VI. Variant VIII showed significant statistical difference at a level of 0.05 compared with variant VII. The stem thickness was largest in variant III (4,29 mm). The lowest stem thickness had plants of variant VIII, with the average value of 3,63 mm. The most heterogeneous coefficient of variation had plants from variant VIII with CV 27,66%.

Table 2. Height of plants (cm)

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	8.56	1.09	12.74	7.0-11.0
II	7.88	1.20	15.18	5.8-9.8
III	7.41	0.99	13.31	5.0-9.3
IV	8.19	1.30	15.90	6.0-10.7
V	9.16	1.73	18.85	7.0-12.0
VI	9.30	1.33	14.35	7.1-12.0
VII	7.96	1.23	15.39	6.0-9.5
VIII	6.49	1.33	20.43	4.0-9.7
IX	6.87	1.78	25.91	5.0-12.0

Table 3. Height of plants (cm) – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	0.68	1.15	0.37	-0.6	-0.74	0.6	2.07	1.69
II	-0.68	Var. II	0.47	-0.31	-1.28	-1.42	-0.08	1.39	1.01
III	-1.15	-0.47	Var.III	-0.78	-1.75	-1.89	-0.55	0.92	0.54
IV	-0.37	0.31	0.78	Var. IV	-0.97	-1.11	0.23	1.7	1.32
V	0.6	1.28	1.75	0.97	Var. V	-0.14	1.2	2.67	2.29
VI	0.74	1.42	1.89	1.11	0.14	Var.VI	1.34	2.81	2.43
VII	-0.6	0.08	0.55	-0.23	-1.2	-1.34	Var.VII	1.47	1.09
VIII	-2.07	-1.39	-0.92	-1.7	-2.67	-2.81	-1.47	Var.VIII	-0.38
IX	-1.69	-1.01	-0.54	-1.32	-2.29	-2.43	-1.09	0.38	Var. IX

LSD 0.05 =1,13
LSD 0.01 =1,56

Table 4. Stem thickness (mm)

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	4.16	0.70	16.94	3.0-5.3
II	4.17	1.12	26.80	2.3-6.0
III	4.29	0.82	19.05	3.0-5.4
IV	4.28	0.67	15.67	3.0-5.7
V	3.96	0.75	19.01	2.6-5.3
VI	4.18	0.74	17.68	3.0-5.2
VII	4.01	0.81	20.18	2.0-5.2
VIII	3.63	1.00	27.66	2.0-6.0
IX	3.70	0.62	16.79	3.0-5.0

The stem thickness from variants VIII and IX showed significant statistical difference at a level of 0.05 compared with the stem thickness from the variant III. The stem thickness from variants VIII and IX showed significant statistical difference at a level of 0.05 compared with the stem thickness from the variant IV. Between the remaining variants there was no statistically significant difference (Table 5).

Table 5. Stem thickness (mm) – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	-0.01	-0.13	-0.12	0.2	-0.02	0.15	0.53	0.46
II	0.01	Var. II	-0.12	-0.11	0.21	-0.01	0.16	0.54	0.47
III	0.13	0.12	Var.III	0.01	0.33	0.11	0.28	0.66	0.59
IV	0.12	0.11	-0.01	Var. IV	0.32	0.1	0.27	0.65	0.58
V	-0.2	-0.21	-0.33	-0.32	Var. V	-0.22	-0.05	0.33	0.26
VI	0.02	0.01	-0.11	-0.1	0.22	Var.VI	0.17	0.55	0.48
VII	-0.15	-0.16	-0.28	-0.27	0.05	-0.17	Var.VII	0.38	0.31
VIII	-0.53	-0.54	-0.66	-0.65	-0.33	-0.55	-0.38	Var.VIII	-0.07
IX	-0.46	-0.47	-0.59	-0.58	-0.26	-0.48	-0.31	0.07	Var. IX

LSD 0.05 =0,57

LSD 0.01 =0,79

The number of branches was largest in variant III, with 10,60 branches. The lowest number of branches had plants of variant I, with the average value of 9,33 branches. The most heterogeneous coefficient of variation had plants from variant IV with CV 20,32%.

Table 6. Number of branches

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	9.33	1.59	17.02	6-12
II	10.27	1.58	15.39	8-13
III	10.60	1.30	12.25	9-12
IV	9.93	2.02	20.32	5-12
V	9.73	1.62	16.69	6-12
VI	9.67	1.29	13.36	8-12
VII	9.47	1.55	16.40	7-12
VIII	9.80	1.32	13.47	8-12
IX	9.40	1.88	20.02	7-13

The number of branches from variant III showed significant statistical difference at a level of 0.05 compared with the number of branches from the variant I. Between the remaining variants there was no statistically significant difference (Table 7). The highest average number of inflorescences (13,07 inflorescences) was obtained in plants from variant II. Plants from the variant VII had the lowest values, with an average value of 8,00 inflorescences. Plants from variant V had the most heterogeneous number of inflorescences (CV 31,19%).

Table 7. Number of branches – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	-0.94	-1.27	-0.6	-0.4	-0.34	-0.14	-0.47	-0.07
II	0.94	Var. II	-0.33	0.34	0.54	0.6	0.8	0.47	0.87
III	1.27	0.33	Var.III	0.67	0.87	0.93	1.13	0.8	1.2
IV	0.6	-0.34	-0.67	Var. IV	0.2	0.26	0.46	0.13	0.53
V	0.4	-0.54	-0.87	-0.2	Var. V	0.06	0.26	-0.07	0.33
VI	0.34	-0.6	-0.93	-0.26	-0.06	Var.VI	0.2	-0.13	0.27
VII	0.14	-0.8	-1.13	-0.46	-0.26	-0.2	Var.VII	-0.33	0.07
VIII	0.47	-0.47	-0.8	-0.13	0.07	0.13	0.33	Var.VIII	0.4
IX	0.07	-0.87	-1.2	-0.53	-0.33	-0.27	-0.07	-0.4	Var. IX

LSD 0.05 =1,23, LSD 0.01 =1,70

Table 8. Number of inflorescences

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	11.87	2.20	18.54	8-18
II	13.07	2.40	18.40	10-18
III	12.93	2.37	18.36	7-16
IV	11.57	3.37	29.10	4-17
V	10.73	3.35	31.19	6-15
VI	10.87	3.14	28.86	5-18
VII	8.00	2.20	27.55	5-12
VIII	8.27	2.22	26.84	6-13
IX	9.13	2.00	21.85	5-12

The number of inflorescences in plants from variants VII and VIII showed significant statistical difference at a level of 0.05 compared with the number of inflorescences from plants of the variant I. Variant IX showed significant statistical difference at a level of 0.05 compared with variant II. Variants VII and VIII showed significant statistical difference at a level of 0.01 compared with variant II. Between the variants IX and III there was statistically significant difference at a level of 0.05. Variants VII and VIII showed significant statistical difference at a level of 0.01 compared with variant III. Variants VII and VIII showed significant statistical difference at a level of 0.05 compared with variant IV. Between the remaining variants there was no statistically significant difference (Table 9).

Table 9. Number of inflorescences – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	-1.2	-1.06	0.3	1.14	1.0	3.87	3.6	2.74
II	1.2	Var. II	0.14	1.5	2.34	2.2	5.07	4.8	3.94
III	1.06	-0.14	Var.III	1.36	2.2	2.06	4.93	4.66	3.8
IV	-0.3	-1.5	-1.36	Var. IV	0.84	0.7	3.57	3.3	2.44
V	-1.14	-2.34	-2.2	-0.84	Var. V	-0.14	2.73	2.46	1.6
VI	-1.0	-2.2	-2.06	-0.7	0.14	Var.VI	2.87	2.6	1.74
VII	-3.87	-5.07	-4.93	-3.57	-2.73	-2.87	Var.VII	-0.27	-1.13
VIII	-3.6	-4.8	-4.66	-3.3	-2.46	-2.6	0.27	Var.VIII	-0.86
IX	-2.74	-3.94	-3.8	-2.44	-1.6	-1.74	1.13	0.86	Var. IX

LSD 0.05 =2,99, LSD 0.01 =4,12

The highest average value for the number of flowers was obtained in the plants from the variant VII (9,13 flowers). Lowest average value for the number of flowers (1,27 flowers) was obtained in the variant III. Plants from variant IV had the most heterogeneous height (CV 81,32%).

Table 10. Number of flowers

Variant	Arithmetic Mean	Standard Deviation	Coefficient of Variation	Interval of Variation
I	1.53	0.64	41.74	1-3
II	2.13	1.60	74.89	1-7
III	1.27	0.46	36.14	1-2
IV	4.36	3.54	81.32	1-12
V	7.93	2.94	37.05	3-14
VI	8.27	3.22	38.92	1-13
VII	9.13	2.26	24.78	5-12
VIII	8.53	4.56	53.49	2-15
IX	6.00	3.55	59.09	2-13

The number of flowers from variants IV, V, VI, VII, VIII and IX showed significant statistical difference at a level of 0.01 compared with the number of flowers from the variant I. The number of flowers from variants IV, V, VI, VII, VIII and IX showed significant statistical difference at a level of 0.01 compared with the number of flowers from the variant II. The number of flowers from variants IV, V, VI, VII, VIII and IX showed significant statistical difference at a level of 0.01 compared with the number of flowers from the variant III. The number of flowers from variants V, VI, VII, VIII and IX showed significant statistical difference at a level of 0.01 compared with the number of flowers from the variant IV. The number of flowers from variant VII showed significant statistical difference at a level of 0.05 compared with the the number of flowers from the variant V. The number of flowers from variant IX showed significant statistical difference at a level of 0.01 compared with the number of flowers from the variant V. The number of flowers from variant IX showed significant statistical difference at a level of 0.01 compared with the number of flowers from the variant VI. The number of flowers from variant IX showed significant statistical difference at a level of 0.01 compared with the number of flowers from the variant VII. The number of flowers from variant IX showed significant statistical difference at a level of 0.01 compared with the number of flowers from the variant VIII.

Table 11. Number of flowers – Comparison between variants

Variant	Comparison with Var. I	Comparison with Var. II	Comparison with Var. III	Comparison with Var. IV	Comparison with Var. V	Comparison with Var. VI	Comparison with Var. VII	Comparison with Var.VIII	Comparison with Var. IX
I	Var. I	-0.6	0.26	-2.83	-6.4	-6.74	-7.6	-7.0	-4.47
II	0.6	Var. II	0.86	-2.23	-5.8	-6.14	-7.0	-6.4	-3.87
III	-0.26	-0.86	Var.III	-3.09	-6.66	-7.0	-7.86	-7.26	-4.73
IV	2.83	2.23	3.09	Var. IV	-3.57	-3.91	-4.77	-4.17	-1.64
V	6.4	5.8	6.66	3.57	Var. V	-0.34	-1.2	-0.6	1.93
VI	6.74	6.14	7.0	3.91	0.34	Var.VI	-0.86	-0.26	2.27
VII	7.6	7.0	7.86	4.77	1.2	0.86	Var.VII	0.6	3.13
VIII	7.0	6.4	7.26	4.17	0.6	0.26	-0.6	Var.VIII	2.53
IX	4.47	3.87	4.73	1.64	-1.93	-2.27	-3.13	-2.53	Var. IX

LSD 0.05 =1,06

LSD 0.01 =1,46

Conclusions

The use of the most appropriate fertilizer has high significance for the obtaining of quality seedlings, which further reflects on growth and development of flower culture. Hence, it is estimated that although it is one of the segments in the production of seedlings, the impact of certain types of fertilizers on the quality of the seedlings of flowers is of great importance. Therefore this research demonstrates that the application of certain fertilizer with certain concentration has a significant role in the production of seedlings. Three different types of liquid fertilizers – Magnicvet with NPK 7-1-5 + ME, Magnihortal with NPK 10-5-5 + ME and Humifulvil, with three different concentrations were used in the experiment. The quality of seedlings of *Petunia x hybrida* „Duvet pink" was determined by examination of following biometric parameters: height of plants (cm), stem thickness (mm), number of branches, number of flower buds and number of flowers. The highest average value for the height of plants (9,30 cm) was obtained in the plants fertilized with Magnihortal with concentration of 0,4 %. The highest stem thickness (4,29 mm) and number of branches (10,60 branches) has the plants fertilized with Magnicvet with concentration of 0,4 %. The highest average value for number of inflorescences (13,07 inflorescences) has the plants fertilized with Magnicvet with concentration of 0,3 %. The highest average value for the number of flowers (9,13 flowers) has plants fertilized with Humifulvil with concentration of 0,5 %.

References

1. Dole, M. J., Wilkins, H. F. (1999). Floriculture. Principles and Species, Prentice-Hall, Upper Saddle River, N. J, 464-465.
2. Hamrick, D. (2003). Crop Production Volume 2, Ball Redbook 17th edition. Ball Publishing, 567-574.
3. Hessayon, D. G. (1997). Sve o vrtu. Mozaik knjiga, Zagreb, 28.
4. Hessayon, D. G. (2004). Cvijeće u vrtu. Mozaik knjiga, Zagreb, 33.
5. Karlović, K., Pagliarini, N., Vrdoljak, A., Vršek, I. (2005). Sobno i balkonsko cvijeće. Gospodarski list, 98.
6. Mayer, J. (2006). Cvijeće za balkone i terase. Mozaik knjiga, Grupa mladinska knjiga, 106-107.
7. Paradžiković, N. (2012). Osnove cvjećarstva. Interna skripta, Poljoprivredni fakultet Osijek, 83-86.

COMPARATIVE TRIALS ON KAPIJA TYPE PEPPER CULTIVARS

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Abstract

The experiment was carried out on two locations in the region of Strumica – Boriovo village and Dobrejci village. Four pepper cultivars of the kapija type have been examined: *kaloca*, KP2, KP3 and KP4. All four were compared to the referent variety, well known on the market as *slonovo uvo*. The following traits and characteristics were recorded: duration of different phases of vegetation [days], plant height [cm], number of fruits per plant (percentage of pollination) [%], fruit weight [g], pericarp thickness [mm], consumable fruit portion [%], total yield [t/ha] and chemical composition of the fruit. In general, among the examined cultivars grown in the region of Strumica in comparison to the reference cultivar *slonovo uvo,* the best results were registered in the cultivars *kaloca* and KP2. As a result of the excellent plant structure, good uniformity, the shape and the color of the fruit, these two cultivars can be well established on the markets in the Republic of Macedonia. Both cultivars exhibited better results in comparison with the reference variety regarding the number of fruits per quality class, better characteristics of the fruit, and as the most significant nutritional value, vitamin C content was also higher. The cultivar *κaloca* obtained total yield 75 t/ha, with statistical difference on the level 0,05 in comparison to reference cultivar while KP2 obtained 68,37 t/ha with no statistical differences in comparison to the reference cultivar. It can be concluded that these two cultivars meet all the criteria for a high quality final product. Their placement on the market will result with relevant income for the growers on one hand, but it will also fulfill the expectations of the fresh product consumers and processing industry on the other.

Keywords: varieties, greenhouse production, yield, fruit quality.

Introduction

The Capsicum pepper (*Capsicum annuum* L.) is one of the most widely grown crops in the Republic of Macedonia. The soil and climate conditions for growing peppers in the country are very favorable. According to production area and usage, the pepper dominates among vegetables. Major pepper varieties grown for processing industry are *kurtovska kapija*, amphora, slonovo uvo and others. Pepper variety *kurtovska kapija* is the leading variety, especially in the Strumica region. The pepper production has steady growth, both in terms of area and in terms of total yield. Average yield per hectare is around 16 tones for the period of last ten years 2002-2012. Pepper type kapija produced in the Republic of Macedonia is of high quality and that is why it is needed both on the domestic and foreign market as raw material for processing (Jankulovski, 1997). According to the Macedonian Association of Processors peppers - type kapija accounted for most of the purchased fruits and vegetables, as it is used in preparation of many traditional Macedonian dishes: ajvar, lutenica, djuvec etc. In 2012, this type of pepper accounted for approximately 60% of purchased fruits and vegetables, or around 42000 tons. Sudden climate changes in the recent years which affected the territory of Macedonia lead to the abandonment of the cultivation of pepper in the open field and the growth of obsolete varieties and hybrids of pepper (Tanaskovic et al, 2007). A major factor in abandoning outdoor cultivation is the strong temperature fluctuations during the day and night, which represent a great shock for continued normal function of plants (Bar-Yosef, 1995). The goal is to use high quality seeds of varieties / hybrids that will be able to meet all the needs of the farmer, market and industry (Ivanovska and Popsimonova, 2006). The objective of this research is to place a

variety/ hybrid on the market that will be more tolerant of drastic climate change (such as high temperatures in the summer period and high solar insolation), particularly in the Strumica region, where most of the vegetable production in Macedonia is located.

Material and methods

The experiment was conducted in greenhouses at two locations in the Strumica region: Borievo village and village Dobrejci. Four varieties and hybrids of the pepper type kapija were tested: *kaloca*, KP2, KP3 and KP4. All varieties/hybrids were compared with existing varieties on the market – "*slonovo uvo*". Identical agriculture techniques and operations were applied to all varieties in order to assure high quality production. A drip irrigation system was used, which enabled precise irrigation with simultaneous application of fertilizers, or in other words fertigation was applied in both locations. Experimental plots included 50 plants in four repetitions in a randomized block system. Fruit traits were done in technological maturity of 10 fruits from each repetition.

The following characteristics were examined:

1. Stages of growth [days]
2. The height to the first branching and plant height [cm]
3. Number of fruits per plant
4. Fruit set (percentage of pollination) [%]
5. Fruit weight [g]
6. Pericarp thickness [mm]
7. Consumable fruit portion [%]
8. Total yield [t/ha]
9. The chemical composition of the fruit

Measurement of the fruit weight, as well as determining the consumable fruit portion was made using a precision electronic balance to the second decimal and pericarp thickness was measured by caliper tool. The description of the shape and color of the fruit was made by IPGRI descriptor for pepper. The number of marketable fruits per plant and their classification in quality classes was done according to the Rules for the quality of fresh fruit and vegetables for processing according the Law on Quality of Agricultural Products. Prior to descriptive statistics of the investigated traits of varieties/hybrids a t-test was done in order to detect any difference between the results obtained in both experimental locations. Such differences were statistically insignificant and therefore the results presented in the paper are the result of analysis of variance in eight repetitions for each tested property while for total yield an analysis of variance (ANOVA) for one factor (variety/hybrid) was done. Chemical analysis of the fruits was done in the Laboratory of Fruit and Vegetables processing at the Faculty of Agricultural Sciences and Food in Skopje. The following parameters were examined:

a) Percentage of dry matter and water – via the gravimetric method
b) Ash - dry mineralization,
c) Percentage of total acids - by volumetric method and
d) Vitamin C content - method according Tillmans.

Results and discussion

Technological and botanical fruit maturity in different varieties of peppers has a different duration, depending on agro-ecological conditions and agricultural technical practices applicable to their cultivation. Different authors give various lengths of the vegetation period of the peppers. Thus, according Aladzajkov (1966) the vegetation period of pepper to technological maturity is 95-115 days, and the botanical maturity 119-143 days. On the other hand, Jankulovski (1983) states that according to the length of the duration of the vegetation period from sprouting to botanical maturity varieties, peppers can be divided in early varieties with vegetation period from germination to botanical maturity not exceeding 120 days, middle varieties from 121 to 140 days and late varieties with vegetation period with more than 140 days.

Table 1. Length of vegetation period in tested varieties / hybrids

Variety/Hybrid	Transplanting	Flowering	Fruit set	Botanical maturity	Total days
KP 2	17.07.2012	10.08.2012	01.09.2012	20.10.2012	94
KP 3	17.07.2012	12.08.2012	04.09.2012	25.10.2012	99
KP 4	17.07.2012	07.08.2012	28.08.2012	17.10.2012	91
slonovo uvo	17.07.2012	12.08.2012	04.09.2012	27.10.2012	101
caloca	17.07.2012	10.08.2012	02.09.2012	20.10.2012	94

The vegetation period is a time expressed in days from sprouting to first fruits in the botanical maturity. But in production, depending on the type of purpose the pepper is grown for, the term 'earliness' is used to describe the technological and botanical maturity of fruit. Taking into account that pepper-type kapija is used for industrial processing and the fact that examined varieties were grown in protected areas, observation was done on stages of growth from planting to botanical or physiological maturity. For all varieties/hybrids, sowing was carried out on 03.06.2012 and transplanting on 17.07.2012. According to the observed stages of development in the examined varieties/hybrids, shown in Table 1, the earliest flowering and fruit set was observed in KP 4. This hybrid gave the most flowers and fruits set, however, the development of the whole plant and marketable fruits were of lesser quality in comparison to other varieties and hybrids that had longer vegetation periods. According to the height of the plant, Popov (1940) and Jankulovski (1983) grouped pepper varieties in: low (30-40 cm), medium high (46-65 cm), high (66-90 cm) and very high (over 90 cm).

Table 2. Plant high [cm]

Variety	Repetition in location 1 and 2								Mean
	I		II		III		IV		
	1	2	1	2	1	2	1	2	
KP 2	84,60	82,70	89,80	90,20	101,90	103,70	97,70	93,70	93,03
KP 3	81,30	76,30	82,50	81,60	96,30	89,50	95,00	86,80	88,70
KP 4	93,00	76,30	90,00	81,60	88,50	89,30	86,00	85,20	89,30
slonovo uvo	86,00	81,00	91,50	88,50	83,00	98,50	77,50	88,20	84,50
kaloca	97,00	96,00	105,00	104,00	98,00	101,00	101,00	99,70	100,25

Location 1 Borievo
Location 2 Dobrejci

Table 2 shows the values for plant height in both locations. The highest value was observed in hybrid *kaloca* (100,25 cm), followed by the variety/hybrid KP 2 (93,03 cm). According to the categorization of Popov (1940) and Jankulovski (1983), these two genotypes belong to the very high varieties, while other observed varieties/hybrids belong to the group high varieties.

Table3.Percentage of fruit set in examined varieties/hybrids [%]

	KP 2	KP 3	KP 4	*slonovo uvo*	*kaloca*
Number of flower per plant	18,00	18,25	20,00	17,25	17,50
Number of fruit set	13,30	12,45	16,50	14,25	12,00
Percentage of pollination	73,89	68,22	82,50	82,61	68,57
Marketable fruits	9,10	11,00	7,80	9,10	8,80

Tested varieties/hybrids formed relatively uniform flowers high in number (Table 3). Although cultivation in protected areas can disrupt pollination due to high temperatures, the number of fruit

set as a percentage of pollination was extremely high - 68, 57% in *kaloca* to 82.50% in KP 4. These values are somewhat higher than those in the examinations of Trajkovska (2013) where she discovered that androgenic types of *kurtovska kapija* reached approximately 60% pollination. The same table also shows the average number of marketable fruits, where the variety/hybrid KP 4 has the lowest number of marketable fruits in spite of having the highest percentage of pollination. This disadvantage can be interpreted as the weaker habitus of the plant which can't feed all fruit sets.
Regular harvesting of fruits in technological maturity results in the formation of more generative organs or fruits providing reliable reproduction. If the fruits are left to reach botanical maturity, the plant uses assimilates for maturation of fruits, due to the number of fruits per plant which is smaller (Jankulovski, 1983).

Table 4. Number of fruits in quality classes for plant and percentage of proportional representation of quality classes

	I-class		II-class		III-class		Total	
	Number of fruits	%	Number of fruits	%	Number of fruits	%	Number of fruits	%
KP 2	6,00	66	1,60	18	1,50	16	9,10	100
KP 3	4,70	43	3,30	30	3,00	27	11,0	100
KP 4	5,50	71	1,80	23	0,50	6	7,80	100
slonovo uvo	5,30	58	2,00	22	1,80	20	9,10	100
Kaloca	7,30	83	1,00	11	0,50	6	8,80	100

The number of formed fruits per plant is an important economic feature of the pepper, but more important is the percentage of fruit formation which is first class. The difference in purchase price between first and second class is 100%, which determines the profitability of production. According to the results presented in table 4 the highest average number of fruits per plant is found the variety/hybrid KP 3 but only 43% of those fruits are first class quality. As shown in table 4, the highest percentage of first class fruit was registered in *kaloca* (83%), followed by the variety/hybrid KP 4 with a 71% share in first class. Pericarp is a useful part of the pepper fruit. Thickness of pericarp is a variety characteristic, conditioned by the technology of cultivation and agro-ecological conditions. Gvozdenović (2010) and Jankulovski (1997) classified peppers according the thickness of the pericarp as such:
- Very thin (0,5 mm), poorly fleshy peppers
- Thin (1-2 mm), slightly thin peppers
- Mean (2-4 mm), intermediate fleshy peppers
- Thick (4-6 mm), fleshy peppers
- Very thick (6-10 mm), very fleshy peppers.

This characteristic is related to the size of the fruit. Most often, small size peppers have thinner pericarp and large size peppers have thicker pericarp. Varieties with thinner pericarp are characterized by a smaller percentage of usable parts of the total weight of the fruit, but when used for processing in the dry state, such as red pepper powder, usable value is higher in varieties with thinner pericarp. The purpose of the production of certain pepper varieties depends on the thickness of the pericarp (Jankulovski, 1983). The thickness of the pericarp was relatively uniform within studied varieties/ hybrids, and there were no big difference among the tested varieties as shown in Table 5. The highest value for the thickness of the pericarp was determined in the variety/hybrid KP 2 (6, 24mm) which is slightly higher than the control variant *slonovo uvo* (6,16mm). The dimensions of the fruit, shape and weight of the fruit of the pepper by Andrews (1995) are highly variable and mainly determined by genetic and environmental factors. Less data has been acquired for the pepper regarding the genetic causes of these fluctuations compared with the findings on the tomato as a representative of the family Solanaceae (Russo, 2012). The formation of the fruit and its shape is determined by several external factors, especially temperature. More authors (Rylski, 1973, Rylski and Spigelman, 1982, Olareweju, 1988, Aloni et al., 1999) stated that if the temperature in the phase

of fruit formation is lower than 16°C, fruits are deformed, atypical for the variety and damaged. According to Gvozdenović (2010), the size of the fruit of the pepper can be: very large (weighing over 150 g), large (weighing 40-150 g), medium large (weighing 10-40 g), small (with weight of 4-10 g), very small (less than 4 g). According to the values of fruit mass, shown in Table 6, the largest mass of the fruit was achieved by the hybrid *kaloca* (120,43g). In comparison to the referent variety *slonovo uvo* (103,35g), KP 2 (108,05g) and *kaloca* (120,43g) had higher values of fruit mass.

Table 5. The thickness of the pericarp in tested cultivars / hybrids [mm]

	KP 2	KP 3	KP 4	*slonovo uvo*	*κaloca*
I	5,86	6,36	6,00	6,14	5,86
II	6,87	5,14	5,54	6,14	5,00
III	6,14	5,14	6,00	6,14	6,04
IV	6,14	6,00	5,80	7,01	5,14
V	6,73	5,20	6,00	6,00	5,39
VI	6,14	6,00	6,87	6,00	5,88
VII	6,14	5,86	5,60	5,86	5,12
VIII	5,88	5,14	4,90	6,00	6,36
Mean	6,24	5,61	5,84	6,16	5,60
Standard error	0,13	0,18	0,20	0,13	0,18
Standard deviation	0,37	0,50	0,56	0,36	0,50
Variance Width	1,01	1,22	1,97	1,15	1,36

Table 6. Fruit mass in tested varieties / hybrids[g]

	KP 2	KP 3	KP 4	*slonovo uvo*	*kaloca*
I	112,00	87,00	73,20	94,00	111,00
II	97,50	92,70	90,70	97,20	109,50
III	114,70	72,20	64,50	114,20	125,70
IV	96,00	116,00	68,90	106,40	130,60
V	114,70	102,60	80,10	111,00	113,20
VI	102,30	104,50	85,00	107,00	110,20
VII	126,50	84,50	83,50	96,00	130,80
VIII	100,70	104,50	97,20	101,00	132,40
Mean	108,05	95,50	80,39	103,35	120,43
Standard error	3,75	4,96	3,91	2,62	3,65
Standard deviation	10,61	14,02	11,06	7,40	10,33
Variance Width	30,50	43,80	32,70	20,20	22,90

Consumable fruit portions are calculated as the ratio of fruit mass without seeds and placenta and whole fruit mass. In large pepper types, such as type *kurtovska kapija*, stem and seeds placenta and seeds amounting to 40% of the total weight of the fruit (Gvozdenović, 2010).

Consumable fruit portions for tested varieties/hybrids varied from 84.13% in the variety/hybrid KP 4 to 90.29% in the variety *slonovo uvo* which is a slightly higher percentage than the results of Gvozdenović (2010) who reports 82.6% consumable fruit portion in the variety *kurtovska kapija*. The yield per unit area of pepper depends on the variety type and producers are interested in varieties that will ensure high and quality yield. The yield is determined by several characteristics of peppers that are in particular dependence and correlation. The yield of pepper is positively correlated to the

number of fruits per plant and the weight of the fruit, which means that plants with more and larger fruits will have a higher yield per plant (Gvozdenović and Cvejić, 2009).

Table 7. Consumable fruit portionin tested varieties/ hybrids [%]

Variety/ Hybrid	Repetition per location 1 and 2								Mean
	I		II		III		IV		
	1	2	1	2	1	2	1	2	
KP 2	87,50	86,70	89,80	88,70	85,60	84,20	90,30	89,40	87,78
KP 3	86,70	83,00	85,20	78,10	85,30	82,40	88,30	84,00	84,13
KP 4	88,50	86,20	89,30	83,10	88,80	82,50	90,40	84,30	86,64
slonovo uvo	91,30	87,90	90,60	90,60	92,40	88,60	91,20	89,70	90,29
Kaloca	85,00	87,20	86,00	85,10	92,00	90,20	87,00	86,00	87,31

Location 1 Borievo
Location 2 Dobrejci

For high yield of pepper with good quality of the fruits, it is important to fulfill certain external conditions, such as an average temperature of 21-23^{o}C during vegetative growth and 21°C during fruit growth (Bakker and van Uffelen, 1988). The yield is higher when daily ambient temperature during flowering is 18-31^{o}C (Aloni et al., 1999). The yield is limited by the adverse impact of high temperatures during flowering and harmful effects of low temperatures on the shape of the fruit (Rylski and Spiglman, 1982).

Table 8. Total yield in tested varieties/hybrids [t/ha]

	KP 2	KP 3	KP 4	*slonovo uvo*	*kaloca*
I	62,37	55,04	50,65	67,42	69,79
II	64,90	62,78	56,43	71,67	73,70
III	68,42	50,58	57,78	74,76	79,94
IV	64,24	70,30	58,90	61,51	78,33
V	77,64	72,93	60,20	66,85	64,44
VI	68,53	64,02	64,75	65,32	79,07
VII	70,60	64,42	63,77	72,80	74,32
VIII	70,28	63,97	60,57	63,35	81,20
Mean	68,37	63,01	59,13**	67,96	75,10*
Diference	+0,41	-4,96	-8,83	0,00	+7,14
LDS 0,05	5,55				
LSD 0,01	7,43				

Jankulovski (1983) pointed out that, depending on the variety, purpose for which the variety is grown, agro-technical measures and climate conditions, the yield of pepper can be: for early production of 40 to 50 t/ha, for later production of 20 to 25 t/ha and production of pepper varieties for red powder of 12 to 20 t/ha. The same author groups pepper varieties for early, middle and late production according to yield per hectare as the following:

1. Varieties with low yield - 20 t/ha;
2. Middle yield varieties - from 21 to 35 t/ha;
3. High yield varieties - from 36 to 50 t/ha and
4. Very high yield varieties - with over 50 t/ha.

According to the above categorization, all tested hybrids/varieties belong to the group of very high yield varieties, due to the intensive nature of farming. The highest yield was observed in the *kaloca*

hybrid with 75,10 t/ha and in comparison to the control variety *slonovo uvo* the difference in yield was statistically significant at the level of probability of 0.05. The lowest yield gave variety/hybrid KP 4 (59,13 t/ha) with statistically high significant difference at level of 0.01 (Table 8).
Results of chemical composition of pepper fruits of different tested varieties/hybrids are described in table 9. The highest value of water content was observed in the variety/hybrid *kaloca* (91,928 %) while the lowest in the variety/hybrid KP2 (90,691%). The rest of the varieties/hybrids had lower water content in comparison to the control variety *slonovo uvo* (91,652%).

Table 9. Chemical composition of pepper fruits in tested varieties/hybrids

Variety/Hybrid	Water (%)	Dry matter (%)	Ash (%)	Total acids (%)	Vitamin C (mg/100g)
slonovo uvo	91,652	8,348	0,301	0,292	125, 004
kaloca	91, 928	8, 072	0,328	0,284	147, 548
KP 2	90,691	9,309	0,273	0,289	140,466
KP 3	91,126	8,874	0,294	0,273	149,443
KP 4	90,882	9,051	0,374	0,225	133,397

In terms of dry matter content, the control variety/hybrid contained 8,348% dry matter while the least dry matter content was in the *kaloca* (8,072%). The variety/hybrid KP2 had the highest percentage of dry matter (9,309%). In the research of Babamovska-Milenkovska et al. (2016) the dry-matter content in the variety *kurtovska kapija* (9,58%) was similar within the varieties/hybrids KP2 (9,309%) and KP4 (9,051). The largest deviation in ash content occurred in the variety/hybrid KP4, where the ash content was highest and amounted to 0.374%, while the lowest percentage of ash content occurred in the variety/hybrid KP2. The content of total acids in all tested varieties/hybrids was lower than referent variety/hybrid *slonovo uvo* (0,292%). The variety/hybrid KP4 had the lowest percentage of total acids (0,225%). According to the results of Babamovska-Milenkovska et al. (2016), the content of total acids in fresh fruits of *kurtovska kapija* variety was 0,333% which is higher in comparison to tested varieties/hybrids. The content of vitamin C was lowest in the control variety/hybrid *slonovo uvo* (125,004 mg/100g) while the highest content of vitamin C was found in the variety/hybrid KP3 (149,443 mg/100 g).

Conclusions

In general, all tested varieties/hybrids of pepper grown in the Strumica region exhibited good results. The best results in comparison with the control variety/hybrid *slonovo uvo* were achieved in *kaloca* and KP 2. Because of the excellent vigorous plants, uniformity in shape and color of fruits and their nutritional value, varieties/hybrids KP 2 and *kaloca* can reach a high status on the market in The Republic of Macedonia. Both mentioned varieties/hybrids compared with the control variety/hybrid showed a higher number of fruits per plant in all three classes, better and more typical form of the fruit, and as a significant trait in terms of nutritional value, these two varieties/hybrids had higher vitamin C content compared to the control variety/hybrid. Their placement on the market will result in relevant income for the growers, on one hand, but it will also fulfill the expectations of the fresh product consumers and processing industry, on the other.

References

1. Aladzajkov, L. (1966). Specijalno gradinarstvo. Univerzitet vo Skopje: 465.
2. Aloni, B., Pressman, E., Karni, L. (1999). The effect of fruit load, defoliation and night temperature on the morphology of pepper flowers and on fruit shape. Annals of Botany, 83: 529-534.
3. Andrews, J.(1995). Peppers: The Domesticated Capsicums. New ed. University of Texas Press, Austin. 186.
4. Babanovska-Milenkovska, F., Karakasova, Lj., Petanovska-Ilievska, B., Manasievska-Simic, S., Miskoska-Milevska, E., Velkoska-Markovska L., Jankulovska, M. (2016). Change in the quality

properties of two different pepper varieties in fresh and dried condition. Agriculture & Food ISSN 1314-8591, Volume 4: 250-259.
5. Bakker, J.C., van Uffelen, J.A.M.(1988). The effects of diurnal temperature regimes on growth and yield of sweet pepper. Netherlands Journal of Agricultural Science. 36: 201-208.
6. Bar-Yosef, B. (1995). Fertirigated vegetables in arid and semi arid zones. IPI-Bull. No. 13: 54-104.
7. FAOSTAT, www.faostat.fao.org (accessed on 15.07.2015)
8. Gvozdenović, Đ., Cvejić, S. (2009). Oplemenjivanje paprike. Institut za ratarstvo i povrtarstvo, Novi Sad:164.
9. Gvozdenović, Đ.(2010). Paprika. Institut za ratarstvo i povrtarstvo. Novi Sad.
10. Ivanovska, S., Popsimonova, G. (2006). Konzervacija na rastitelen agrobiodiverzitet. Skopje: BIGOSS:229.
11. Jankulovski, D., Cirkova , M., Martinovski, Gj., Petrevska, J.K. (1997). Evaluacija na avtohton genofond piperki vo Makedonija. Zborik na simpozium "Novi tehnologii vo gradinarstvoto i cvekjarstvoto", Ohrid:65-172.
12. Jankulovski, D. (1983). Proucuvanje na bioloskite, morfoloskite i kvalitetnite svojstva na povaznite populacii dolgi piperki vo SR. Makedonija. Doktorska disertacija, Zemjodelski fakultet, Skopje: 174.
13. Olareweju, J.D. (1988). Effect of night temperature on fruit set and development in sweet pepper (*Capsicum annuum*L.). Haryana Journal of Horticultural Sciences, 18: 285-288.
14. Попов, П. (1940). Принос към проучването на псоространетите в Бъгария пипери (Capsicum annuum L.), Пловдив.
15. Russo, M.V. (2012). Peppers: Botany, Production and Uses. Trade Cloth: 432.
16. Rylski, I. (1973). Effect of night temperature on shape and size of sweet pepper (*Capsicum annuum*L.). Journal of the American Society for Horticultural Science, 98: 149-152.
17. Rylski, I., Spigelman, M. (1982). Effects of different diurnal temperature combinations on fruit set of sweet peppers. Scientia Horticulturae, 17: 101-106.
18. Tanaskovic, V., Cukaliev, O., Iljovski, I. (2007). Vlijanie na nacinot i rezimot na navodnuvanje i prihrana vrz prinosot na domatot. Jubileen godisen zbornik na Fakultetot za zemjodelski nauiki i hrana "60 godini Fakultet za zemjodelski nauki i hrana", Skopje, R. Makedonija, godina 53: 137-149.
19. Trajkova, F. (2013). Karakterizacija i agronomska evaluacija na nekoi linii piperka (*Capsicum annuum* L.) dobieni so metodot na androgeneza, (doktorska diseratacija). Univerzitet "Sv. Kiril i Metodij" – Skopje. Fakultet za zemjodelski nauki i hrana vo Skopje.

AGRONOMIC EVALUATION OF TOMATO HYBRIDS FOR GLASS-HOUSE PRODUCTION

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Abstract

The tomato is the most widely cultivated vegetable crop, both in glasshouses and walk-in tunnels. The objective of the trial is to make a recommendation for tomato hybrids intended for protected crops production in the Republic of Macedonia. It was carried out in the region of Sv. Nikole (Central Macedonia) in glasshouses. The following newly introduced tomato hybrids, made by four different producers, have been examined: Fito - *Meriva* F_1, Enza Zaden - *Lezoforta* F_1 and *Amaneta* F_1, Syngenta - *Zouk* F_1, Clause - *Loreli* F_1. The applied experimental design was a randomized block system in five replications. Aside from the dynamics of germination, flowering, and ripening and harvesting, the productive traits of each hybrid were analyzed using ANOVA. It was confirmed that the hybrids Zouk F_1 and Loreli F_1 had the earliest germination and the latest germination was exhibited by the Amaneta F_1. The shortest period between germination and flowering was recorded in the hybrid Loreli F_1, and the longest in Lezoforte F_1. The period between flowering and fruit ripening was shortest in the hybrid Zouk F_1, and the longest one in Amaneta F_1. The shortest period starting from germination to the beginning of ripening was recorded in the hybrid Amaneta F_1 and the longest in Zouk F_1. In reference to the productive traits, it was recorded that the largest number of flowers per cluster had the hybrid Loreli F_1. The hybrid Loreli F_1 exhibited the largest percentage of fruit sets. The lowest percentage of fruit sets per plant was found in the hybrid Lezoforta F_1. The largest average fruit mass was observed in the hybrid Meriva F_1. The largest yield per plant was recorded in the tomato hybrid Meriva F_1. Finally, the highest yield was recorded in the hybrid Meriva F_1(173,73 t/ha).

Keywords: protected crops, vegetable production, yield.

Introduction

The tomato (*Lycopersicon esculentum* Mill.) is the leading vegetable crop in the country and in the world. In the production structure in the EU the tomato accounted for 25% while in The Republic of Macedonia it accounted for three percent less (Lazic et al., 2007). For its production in protected houses it is necessary to gain knowledge derived from experience and research on morphology, biology and production technology. On the market there are many different varieties and hybrids of tomatoes. In The Republic of Macedonia, according to the studies of Bogevska et al. (2010), there are 150 recognized varieties of which 126 are foreign, 18 are local and 6 are newly created domestic varieties (Trejsi, 1997, Joldzhik, 1997, Ace, 2001, Asmara, 2001, Longina, 2004, Laguna, 2004), while the number of indigenous landraces is 156. In the area belonging to The Republic of Croatia, 31 varities of tomatoes had been recognized during the period between 1997 and 2000, of which 17 proved suitable for greenhouse production and outdoors production (Borošić et al., 2001). Milutinović and Đukić (1996) studied 12 varieties of tomatoes. The highest yield were achieved by the hybrids *luna* (67t/ha) and *kazanova* (61t/ha). The largest number of fruits per plant was realized in the hybrid *luna*, 5 fruits per flower cluster, while the highest weight of the fruit of 240 g was achieved in the hybrid *kazanova*. Hybrids *luna*, *lido*, *kazanova*, and the *narvik* variety were recommended for production. Demirovska et al.(1990) examined the yield of five varieties of tomato grown in plastic tunnels intended for autumn production in two production years: 1986 to 1987. The examined varieties were as follows: luka F_1 reference hybrid, balka F_1, duple F_1, caramelo F_1 and

ruders Sk. The highest average yield per plant gave balka F1 - 1,48 kg, then the variety luka F1-1,23 kg following the variety duple F1 - 1,01 kg. The total yield was highest in variety balka F_1 - 54,64t/ha followed by the variety luca F_1 - 46,85 t/ha and the variety duple F_1 37,10 t/ha which is expected for this kind of production. Jankulovski et al. (2002) examined new tomato hybrids for greenhouse production over a period of two years: Sinatra F_1, Alcudia F_1, Gessa F_1, Ibiya F_1 and Q-75-48 F_1. These varieties were compared to the leading variety for greenhouse production, Marfa F_1. During the vegetation several parameters were examined: the vegetation period from sprouting to first harvest, percentage of fertilization, characteristics of the fruit, total yield and index of earliness. Based on the analyzed parameters authors recommended hybrids Gessa F_1, Sinatra F_1 and Alcudia F_1. Ibraim (2012) characterized 14 indigenous tomato landraces originating from other regions in Macedonia grown on various altitudes. Regarding the length of the vegetation period 5 landraces were characterized by a relatively short period of 103-105 days, while the other 9 have a longer vegetation period of up to 115 days. In terms of morphological characteristics, there were very significant differences in the tested landraces. For example, fruit weight ranged from 119 g in landrace 1 to 225 g in landrace 5. In reference to production traits the author stated that the yields achieved per plant and per hectare differ significantly in terms of the average of all tested landraces. In 7 landraces the yield is lower, while in the other 7 the yield increased from 24 to 27%. With many varieties and hybrids producers are asking themselves which ones are suitable for production. This means that the choice depends on the necessary information available for morphological, biological and most importantly, the production features. Hence, the aim of this research is to study the advantages and potentials of several tomato hybrids in glasshouse production through comparative study of biological, morphological and commercial properties. The differences in the results of the survey will be used for identification and recommendation of these hybrids that will guarantee stable production in glasshouses in Sveti Nikole.

Material and methods

In order to achieve the objective, according to the methodology, research was done in 2012 on 5 tomato hybrids from four seed companies (Fito -Meriva F_1, Enza Zaden - Lezoforta F_1, Amaneta F_1, Syngenta - Zouk F_1, Clause- Loreli F_1). Meriva F_1 is an indeterminate medium-early maturing tomato hybrid intended for production in protected houses. The stem is robust with vigorous leaf mass and a well-developed root system. The fruits are large, weighing 200-220 g, spherical in shape, slightly flattened, with good toughness. The fruit is red. Resistant to diseases Verticillium. Fusarium, Tobaco mosaic virus and Cladosporium. Lezoforta F_1 is a medium-early maturing tomato hybrid used for protected houses as the first crop. The stem is robust with vigorous leaf mass and a well-developed root system. Fruits are round, slightly elongated in size and weigh from 200 to 220 g. The color is dark red. Resistant to diseases Verticillium, Fusarium, Tobaco mosaic virus and Cladosporium. Amaneta F_1 is an early indeterminate tomato hybrid intended for production in protected houses as the first crop. The stem is robust with strong leaf mass and strongly developed root system. The fruits are round, slightly flattened with a size of 220-250 g. The color of the fruit is dark red. Resistant to diseases Verticillium. Fusarium, Tobaco mosaic virus and Cladosporium. Zouk F_1 is an early indeterminate hybrid with high production potential. It can be grown throughout the year. The stem is robust with strong leaf mass and a strongly developed root system. Fruits are round, dark red with short sepal. The fruit size is 170-180 g, it is resistant to cracking and the following diseases: Verticillium. Fusarium, Tobaco mosaic virus and Cladosporium. Loreli F_1 is an early indeterminate tomato hybrid intended for production in protected houses as well as in the open field. The plant is robust with strong leaf mass and a strongly developed root system compared to the other hybrids of the breeding company Clause. It is recommended to be grown as the first crop, because it is characterized by high tolerance to changing production conditions. The fruits are arranged in the form of a fish bone, weighing 180-220 g with a beautiful red color and excellent flavor properties. It is resistant to the following diseases: Verticillium. Fusarium, Tobaco mosaic virus and Cladosporium. The hybrids were tested in glasshouses in Sveti Nikole, where there are favorable conditions for

cultivation. The experiment was set by a method of randomized blocks with five repetitions. In order to perform characterization during the vegetation more biological and morphological parameters were analyzed. The technology of production was common for tomato growing. Hybrids were grown with previous production of seedlings. During the vegetation period the following phenological stages of growth were registered: days from sowing to sprouting, days from sprouting to flowering, days from flowering to early maturing, growing season (vegetation period) in order to determine the earliness of examined hybrids. Morphological characteristics were observed through the following parameters: number of leaves to the first flower branch, length of internodes and number of leaves between flowering branches, number of flowers per flower cluster, number of fruits per cluster, fruit shape, fruit mass, length of the peduncle, thickness of pericarp, cracking of tomato fruit, outbreak of cat face, number of chambers in the fruit, the color of the fruit. The number and weight of fruits per plant, yield per unit area, dynamics of yield per harvests contributed to obtain fertility of hybrids or to evaluate production properties. During the vegetation period, standard technology of tomato production in glasshouses was applied. The obtained results from examined parameters were statistically processed by calculating the mean value, standard deviation, coefficient of variation, error of the mean value. The yield and yield components were statistically processed by analysis of variance (ANOVA) and LSD test at the level of 0,05 and 0,01.

Results and discussion

In Table 1, stages of growth in days are shown. The number of days from sowing to sprouting ranged from 5 to 7 days, where the hybrid Amaneta F_1 germinated last for 7 days, compared to hybrids Zouk F_1 and Loreli F_1, who sprouted for 5 days. Unlike sprouting, the hybrid Amaneta F_1 flourished first for 81 days, while the hybrid Lezoforta F_1 flourished last for 88 days. In this survey days from flowering to ripening ranged from 46 to 65 days. The shortest time from flowering to ripening had the hybrid Zouk F_1 (46 days), unlike hybrid Amaneta F_1 which began ripening for 65 days.

Table 1. Stage of growth in days

Hybrid	Date of sowing	Date of transplanting	Days from sowing to sprouting	Days from sprouting to flowering	Days from flowering to ripening	Vegetation period
MerivaF_1	03.12.2011	24.02.2012	6	86	47	133
Lezoforta F_1	03.12.2011	24.02.2012	6	88	54	142
Amaneta F_1	03.12.2011	24.02.2012	7	81	65	146
ZoukF_1	05.12.2011	24.02.2012	5	85	46	131
Loreli F_1	08.12.2011	24.02.2012	5	83	49	132

The vegetation period for all hybrids ranges from 131 to 146 days where the hybrid Zouk F_1 had the shortest vegetation period (131 days), compared to the hybrid Amaneta F_1 where vegetation period was 146 days. Hybrids Meriva F_1 and Loreli F_1 very much alike to Zouk F_1, had the shortest period of 133 days and 132 days respectively, unlike the hybrid Lezoforta F_1 which had 142 days. Together with the hybrid Amaneta F_1, it belongs to hybrids with the longest growing season. The characteristics of the whole plant and the fruit of the tested hybrids are given in Table 2.

Table 2. Characteristic of plant and fruit

Hybrid	Number of leaves to first flower cluster	Length between nodes (cm)	Number of leaves between flower cluster	Fruit shape	Cracking Yes/No	Cat face	Number of locular cavity	Fruit color
Meriva F_1	6	21	3	round to slightly flattened	No	no	6	red
Lezoforta F_1	6	17	3	round to slightly elongated	No	no	7	red
Amaneta F_1	7	20	3	round to slightly flattened	No	no	5	red
Zouk F_1	6	15	3	round	No	no	6	red
Loreli F_1	7	18	3	round	No	no	7	red

According to the measured parameters, the number of leaves to the first flower branch for all hybrids is similar. Hybrids Amaneta F_1 and Loreli F_1 (7) had the highest number of leaves, while the lowest number was registered in hybrids Meriva F_1, Lezoforta F_1 and Zouk F_1 (6). Length between nodes (flower branch) ranged from 21cm in hybrid Meriva F_1 to 15 cm in hybrid Zouk F_1. The number of leaves between flower clusters is the same in all examined hybrids (3). The shape of the fruit is round to slightly flattened in hybrids Meriva F_1 and Amaneta F_1, round to slightly elongated in hybrid Lezoforta F_1 and round in hybrids Zouk F_1 and Loreli F_1. Cracking as common phenomena depends on the specifics of the hybrid and the conditions and method of cultivation. In this research cracking was not expressed in tested hybrids. Also, there is no appearance of a high degree of deformity of the fruit commonly known as cat face in examined hybrids. The number of locular cavity as part of the fruit structure ranges from 5 in Amaneta F_1 to 7 in LezofortaF_1 and Loreli F_1. Fruit color in all tested tomato hybrids was red.

Table 3. Number of flowers in cluster

Hybrid		Meriva F_1	Lezoforta F_1	Amaneta F_1	Zouk F_1	Loreli F_1
Repetition	I	5,1	4,4	5,4	5,1	5,8
	II	5,6	4,8	5,4	4,6	5,6
	III	5,4	4,8	5,5	4,5	6,1
	IV	5,2	4,7	5,4	4,5	6,0
	V	5,2	4,6	5,4	4,5	5,9
Mean		5,3	4,7	5,4	4,6	5,9
STDEV		0,21	0,18	0,04	0,25	0,22
CV		3,91	3,91	0,78	5,41	3,68
sx		0,09	0,08	0,02	0,11	0,10

The average number of formed flowers of five hybrids ranges from 4,6 to 5,9, where the largest number of flowers had Loreli F_1(5,9) while the lowest number of formed flowers in a flower cluster had Zouk F_1 (4,6) (Table 3). In the research of Lekshmi and Celine (2015) the number of flowers per cluster in different tomato hybrids was from 5,65 to 7,16 which is higher in comparison to our findings. The coefficient of variation was calculated. The lowest coefficient of variation was observed in Amaneta F_1 (0,78%) while the highest was recorded in Zouk F_1 (5,41%). More important in the analysis of hybrid traits is the number of fertilized flowers. The number of fertilized flowers is presented in Table 4.

Table 4. Number of fertilized flowers in flower cluster

Hybrid		MerivaF$_1$	LezofortaF$_1$	AmanetaF$_1$	ZoukF$_1$	LoreliF$_1$
Repetition	I	4,1	4,2	4,3	4,6	5,1
	II	4,9	4,3	4,8	4,2	5,3
	III	4,9	4,0	4,8	4,2	5,4
	IV	4,8	4,2	4,4	4,3	5,6
	V	4,7	4,1	4,9	4,2	5,4
Mean		4,7	4,2	4,6	4,3	5,4
sx		0,15	0,05	0,12	0,08	0,09
STDEV		0,33	0,12	0,26	0,18	0,21
CV		7,17	2,82	5,63	4,16	3,84
LSD	0,05	0,32				
	0,01	0,44				

The analysis of the number of fertilized flowers (Table 4) showed that the highest average number of fertilized flowers had the hybrid Loreli F_1 (5,4), while the hybrid Lezoforta F_1 had lowest number of fertilized flowers (4,2). Concerning the coefficient of variation the highest coefficient was determined in the hybrid Meriva F_1 (7,17%) while the lowest in the hybrid Lezoforta F_1 (2,82%)

Table 5. Fruit mass (g)

Hybrid		Meriva F_1	Lezoforta F_1	Amaneta F_1	Zouk F_1	Loreli F_1
Repetiton	I	261,0	227,0	252,0	182,0	216,0
	II	241,0	206,0	229,0	196,0	209,0
	III	237,0	226,0	225,0	207,0	209,0
	IV	245,0	225,0	249,0	181,0	188,0
	V	256,0	221,0	220,0	209,0	203,0
Mean		248,0	221,0	235,0	195,0	205,0
STDEV		10,2	8,7	14,5	13,3	10,6
CV		4,1	3,9	6,2	6,8	5,5
sx		4,5	3,9	6,5	5,9	4,7

The mass of the fruit ranged from 195g in Zouk F_1 to 248g in Meriva F_1. According to the coefficient of variation the highest value had the hybrid Zouk F_1 (6,8%) while the lowest hybrid Lezoforta (3,9%). The coefficient of variation showed that fruits of the hybrid Lezoforta F_1 were more uniform by weight. The mass of fruit is given in Table 5. The mass of the flower cluster was highest in Meriva F_1 (1158 g), while the lowest mass of flower cluster was determined in Zouk F_1 (837 g). The lowest value of the coefficient of variation was calculated in Amaneta F_1 (0,88%) while the highest was recorded for Zouk F_1 (4,77%). The mass of one flower cluster is shown in Table 6. The lowest yield per plant of 6 flowering clusters was obtained in Zouk F_1 (5,02 kg), while the highest yield was achieved by Meriva F_1(6,95kg). The coefficient of variation was lowest in hybrid Amaneta F1 (0,88%), while highest in hybrid Zouk F_1 (4,77%). One important parameter of all tested hybrids is the obtain yield per unit area. The yield of this survey is calculated by the usual production structure within the 25,000 plants, which confirmed the results obtained in the optimal range for the tested hybrids. Values of yields for one year research are presented in Table 8. The yield in tested hybrids ranged from 125,6t/ha in hybrid Zouk F_1 to 173,7 t/ha in hybrid Meriva F_1. The lowest coefficient of variation was determined in hybrid Amaneta F_1 (0,9%) while the highest was determined in hybrid Zouk F_1 (4,8%). Shrestha and Sah (2014) made an evaluation of tomato cultivars for Central Tarai of Nepal. Results showed that cv. HRDTOM-005XHRDTOM-010 (30.64 t/ha) followed by Makis (28.90 t/ha) and cv. Srijana (28.87 t/ha) gave higher yield among the tested cultivars which does not correspond with our findings.

Table 6. Mass of one flower cluster (g)

Hybrid		MerivaF$_1$	LezofortaF$_1$	AmanetaF$_1$	ZoukF$_1$	LoreliF$_1$
Repetition	I	1070,0	953,0	1084,0	837,0	1102,0
	II	1181,0	886,0	1099,0	823,0	1108,0
	III	1161,0	904,0	1080,0	869,0	1129,0
	IV	1176,0	945,0	1096,0	778,0	1053,0
	V	1203,0	947,0	1078,0	878,0	1096,0
Mean		1158,2	927,0	1087,4	837,0	1097,6
STDEV		51,55	30,04	9,53	39,94	27,86
CV		4,45	3,24	0,88	4,77	2,54
sx		23,06	13,44	4,26	17,86	12,46

Table 7. Yield per plant from 6 flower clusters (kg)

Hybrid		MerivaF$_1$	LezofortaF$_1$	AmanetaF$_1$	ZoukF$_1$	LoreliF$_1$
Repetition	I	6,420	5,718	6,504	5,022	6,612
	II	7,086	5,316	6,594	4,938	6,648
	III	6,966	5,424	6,480	5,214	6,778
	IV	7,056	5,670	6,576	4,668	6,318
	V	7,218	5,682	6,468	5,268	6,576
Mean		6,95	5,56	6,52	5,02	6,59
STDEV		0,31	0,18	0,06	0,24	0,17
CV		4,45	3,24	0,88	4,77	2,56
sx		0,14	0,08	0,03	0,11	0,08
LSD	0,05	0,29				
	0,01	0,40				

Table 8. Yields in t/ha

Hybrid		Meriva F$_1$	Lezoforta F$_1$	Amaneta F$_1$	Zouk F$_1$	Loreli F$_1$
Repetition	I	160,50	142,95	162,60	125,55	165,30
	II	177,15	132,90	164,85	123,45	166,20
	III	174,15	135,60	162,00	130,35	169,35
	IV	176,40	141,75	164,40	116,70	164,40
	V	180,45	142,50	161,70	131,70	164,65
Mean		173,7	139,1	163,1	125,6	165,98
STDEV		7,7	4,6	1,4	6,0	2,0
CV		4,6	3,3	0,9	4,8	1,2
Sx		3,5	2,1	0,6	2,7	0,9
LSD	0,05	6,9				
	0,01	9,5				

Conclusions

Taking into consideration all examined traits, the hybrids Meriva F$_1$, Loreli F$_1$ and Amaneta F$_1$ can be recommended for production in glasshouses.

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We would like to dedicate this paper to our late and appreciated colleague Aco Ivanov who worked in the glasshouses in Sveti Nikole.

References

1. Bogevska, Z., Davitkovska, M., Iljovski, I.,Ristovska, B. (2010). Potencijalot na Republika Makedonija vo sozdavanje na brend sorti od zelenchukovi kulturi. Godisen zbornik na Fakultetot za zemjodelski nauki I hrana vo Skopje, Godina/Volume 55, str. 23-29.
2. Borošić, J., Lovoković-Milinković, J., Matotan, Z., Marušić, V., Čermak-Horbec, K. (2001) Novo introducirane sorte rajčiće, paprike i salate u Republici Hrvatskoj. Znastveni skup hrvatskih agronoma s međunarodnim sudjelovanjem. Zbornik sažetaka.
3. Demirovska, V., Čirkova, M., Jankulovski, D., Jovančev, P. (1990) Jesenja proizvodnja paradajza u plastenicima u skopskom region. Jugoslovenski simpozijum intenzivno gajenje povrća i proizvodnja u zaštićenom prostoru. Zbornik radova. pp. 369-374
4. Ibraim, J. (2012). Karakterizacija na avtohtoni populacii domat (*Lycopersicon esculentum Mill.*) od tipot jabucar vo Republika Makedonija (Magisterski trud). Univerzitet "Sv. Kiril I Metodij" – Skopje. Fakultet za zemjodelski nauki i hrana vo Skopje.
5. Jankulovski, D., Martinovski, Gj.,Petrvska, J.K., Jankuloski, Lj. (2002). Characteristics of new tomato hybrids (*Lycopersicon esculentum* Mill.) for greenhouse production. First Symposium on Horticulture, Symposium proceedings, Skopje. pp. 186-190.
6. Lazic, B., Markovic, V., Gjurovka M., Ilin Z., Jankulovski, D., Martinovski, Gj. (2007). Specijalno gradinarstvo (avtorizirani predavanja). Univerzitet „Sv. Kiril i Metodij" – Skopje. Fakultet za zemjodelski nauki i hrana vo Skopje.
7. Lekshmi, S. L., Celine, V. A. (2015). Evaluation of tomato hybrids for fruit, yield and quality traits under polyhouse conditions. International Journal of Applied and Pure Science and Agriculture (IJAPSA). Volume 01, Issue 7. pp. 58-64
8. Milutinović, S., Đukić, Ž. (1996). The yield and yield components at tomato cultivars and hybrids. First Balkan Symposium Vegetables & Potatoes. The Book of Abstracts. p. 134
9. Shrestha,S. L., Sah R. L. (2014). Evaluation of Tomato Cultivars for Central Tarai of Nepal. Nepal Journal of Science and Technology Vol. 15, No.2.pp. 11-16.

EXAMINATION OF SOIL FERTILIZERS IN *SPATHIPHYLLUM WALLISII* REGEL PRODUCTION

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Abstract

During our work, three kinds of soil fertilizers Austrian Volldünger® Linz, Hungarian FitoHorm® Complex Plus and FitoHorm® for potted plants were applied in *Spathiphyllum wallisii* Regel production. Two experiments were set up. First the effects of the two FitoHorm agents were examined on the leaf size. In the second experiment, FitoHorm agents and Volldünger were compared, leaf size, chlorophyll content, fresh and dry weight were measured. The FitoHorm agents did not significant difference regarding the leaf size, but the groups treated with FitoHorm were bushier. In the second experiment, Volldünger and FitoHorm Complex Plus significantly increased the chlorophyll content comparing to the control. None of the treatments showed significant difference regarding the leaf length and width, fresh and dry weight comparison to the control or each other. Best dry weight rate was measured by Volldünger treatment. In conclusion, Volldünger® Linz, FitoHorm® Complex Plus and FitoHorm® for potted plants had positive effects on vegetative growth and chlorophyll content, therefore these agents can be recommended in ornamental plant production.

Keywords: *S. wallisii*, soil fertilizers, potted plants.

Introduction

The sustainability of agricultural systems is an important global issue (CHENG ET AL, 2010). Fertilizers represent a wide array of materials added to soils to improve plant growth and able to supply nutrients. Plants use these nutrients to make components such as proteins or carbohydrates. Fertilizers are applied to root zones or spray directly on foliage (TRAUNFELD AND NIBALI, 2013, GELLINGS AND PARMENTER, 2016).They can include manures, plant residues and also essential elements (ALLEY AND VANLAUWE, 2009).The quality and quantity of applied fertilizers are key factors affecting the growth and quality of flowers (CHENG ET AL, 2010). Using manures and chemical fertilizers as a supplementary and complementary nutrition additions remain the alternative solution for growers to protect the soil health (KHANAM ET AL, 2017). In the last decades, there were several researches with using soil and foliar fertilizers in ornamental plant production. Fertilizers are applied to tropical potted plants at high rates (BROSCHAT, 1995). Soil and foliar fertilizers significantly increased the plant height, leaf area, plant health, vigor and colour on *Heliconia psittacorum* L.f. (LINARES-GABRIEL ET AL, 2016). Sulphur fertilizer had positive effect on *Aloë vera* (L.) Burm.f. (EISA ET AL, 2014). Sangral foliar fertilizer showed significant increase in the growth parameters and also stimulated the flowering parameters on *Dianthus caryophyllus* L. (EL-NAGGAR, 2009). Nitrogen fertilizer significantly increased the chlorophyll content and leaf size of *Philodendron andreanum* Dwansaye (BO ET AL, 2010). Chemical fertilizers (high concentration of nitrogen) found suitable for growth and productivity in *Chlorophytum tuberosum* (Roxb.) Baker (SHARMA AND KUMAR, 2011). Biofertilizers had positive effects on number of leaves, size of spadix and chlorophyll content on *Spathiphyllum* 'Illusion' (ABBASNIAYZARE ET AL, 2012). The main aim of this study was to examine the positive effects of FitoHorm® Complex Plus, FitoHorm® on potted plants and Volldünger® Linz on *Spathiphyllum wallisii* Regel.

Material and methods

The examination was carried out in the greenhouse of the Department of Floriculture and Dendrology, Corvinus University, Budapest (BCE), in autumn 2013. The processing of samples was performed in the labour of the Department of Floriculture and Dendrology, Corvinus University, Budapest. *Spathiphyllum wallisii* Regel is a very popular foliar plant (SARDOEI, 2014). It is shade tolerant with dark foliage and white spathes (HENRY ET AL, 2004). During this work, three kinds of soil fertilizers such as Hungarian FitoHorm® Complex Plus, FitoHorm® for potted plants and Austrian Volldünger® Linz, were compared. FitoHorm® Complex Plus is a chlorine-free, water-soluble soil conditioner. It is enriched with microelements, applied in ornamental plants and vegetable production. It can also be spread through the foliage and soil (www.fitohorm.hu). FitoHorm® for potted plants is also chlorine-free and liquid nutrient solution. It is enriched with microelements, specially developed for potted plants. It helps to eliminate the leaf chlorosis and fall of leaves (www.fitohorm.hu). Volldünger® Linz is a water-soluble solid soil fertilizer. It is enriched with microelements, applied in viticulture, ornamental plants, fruit and vegetable production. It could be a starter and foliar fertilizer (www.kwizda.hu). The first experiment was started on 2nd October 2013 and the second experiment was started on 6th February 2015 with the division of shoots from stock plants in the greenhouse of the Department of Floriculture and Dendrology, BCE. Shoots were planted into ASB Greenworld® Potting Soil (pH 5.0-6.5, 50-300 mg/l N, 40-300 mg/l P_2O_5 and 80-500 mg/l K_2O) and 12 cm diameter containers. The treatment groups can be seen in *Table 1*. In the first experiment every treatment groups contained 5 plants in 4 repetitions and in the second experiment every treatment groups contained 5 plants in 3 repetitions. The plants were treated once a week with 150 ml mixture of fertilizers and water.

Table 1.The treatment groups

First experiment			Second experiment		
Treatment groups		Concentration	Treatment groups		Concentration
1.	Control (CØ)	-	1.	Control (CØ)	-
2.	FitoHorm Complex Plus (FHC)	1 g/l	2.	FitoHorm Complex Plus (FHC)	1 g/l
3.	FitoHormfor potted plants (FHP)	9 ml/l	3.	FitoHorm for potted plants (FHP)	9 ml/l
			4.	Volldünger (V)	1 g/l

In the first experiment, the morphological characteristics such as leaf length and leaf width were measured. In the second experiment additional parameters: fresh and dry leaf weight was also measured. With the chlorophyll content the physiological conditions were examined. For determination of fresh and dry leaf weight, 3 plants were separated from each treatment group. The root system was cut by the root neck; the whole green plant was measured with Mettler Toledo J 1502G scale. Green organs were dried out at 80 °C temperature in dry heat oven (Binder) for 24 hours and after that they were measured again with the same scale. The relative dry content was calculated based on the ratio of these values. To determine chlorophyll content, 100 mg of leaf sample were collected. The samples were homogenized with quartz sand and completed with 80 % acetone solution to 10 ml. After 24 hour rest the light absorbance of the solution was measured on 663, 644 and 480 nm wave length with spectrophotometer (Genesys™ 10 Vis) (HORVÁTH AND ERDEI, 2003). Statistical analysis was performed by IBM® SPSS STATISTICS (Version 23) by 95% significance level. One-way ANOVA model was used to compare the varieties. The assumptions of homogeneity of variance were hold. Normality of residuals was proved by Kolmogorov-Smirnov test. Pairwise comparisons were run by Tukey's Post Hoc test (TABACHNICK AND FIELDS, 2013).

Results and discussion

First experiment

In the first experiment, the morphological characteristics with leaf length and leaf width measurements were examined. We were not able to detect significant differences between the treatments groups in leaf length [F(2;57)=0.619; p=0.542]and leaf width [F(2;57)=1.988; p=0.146]at the beginning and also at the end of the experiment (leaf length [F(2;57)=0.759; p=0.473], leaf width [F(2;57)=1.226; p=0.301]) *(Figure 1 and 2.)*. The longest leaves were obtained in group which treated with FitoHorm for potted plants. At the last measurement, the control group became 0.9 cm longer, while the FHC group 1 cm and FHP group 1.1 cm longer. The widest leaves were obtained in control group. In practical aspect these results are not mentionable, do not improve the quality.

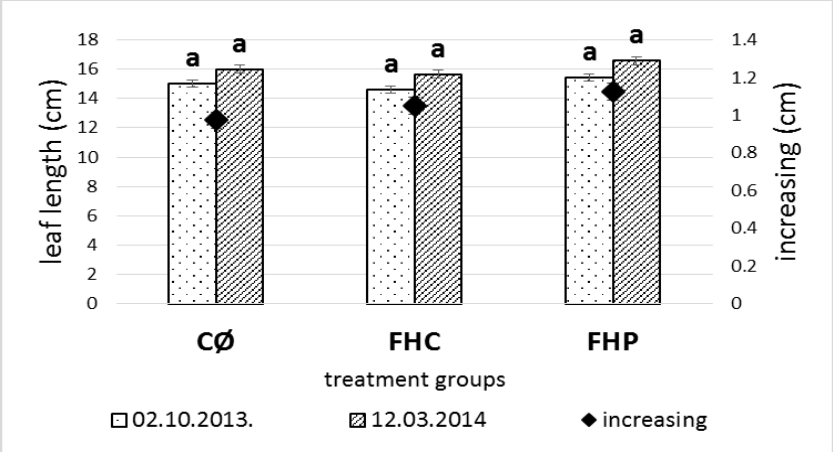

Figure 1. The leaf length of Spathiphyllum wallisii Regel

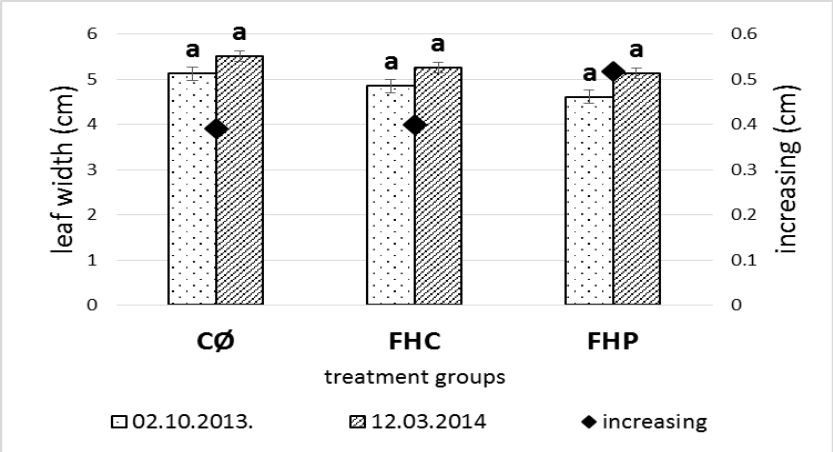

Figure 2. The leaf width of Spathiphyllum wallisii Regel

Despite of the fact than there were no significant differences between treatments groups, foliage development was observed in groups which treated with FitoHorm. Those groups were bushier than the control plants (*Figure 3.*).

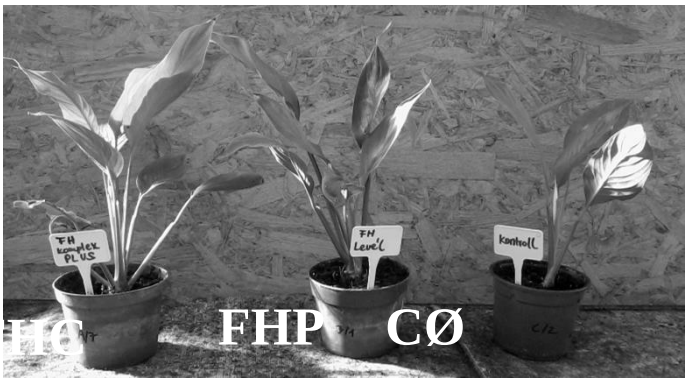

Figure 3.The most typical plants of the treatment groups.

Development of flower initiations was not statistically evaluable, but some trends were observed in the treatment groups. The flower initiations were developed at similar rates in groups which were treated with FitoHorm. In these groups five flowers were developed during five months. Deformation and pigmentation were observed on these flowers. There was no abnormality in the control group.

Second experiment

In the second experiment, in addition to leaf length and width, root mass, fresh and dry leaf weight and chlorophyll content were also measured. None of the treatments groups showed significant differences regarding leaf length [F(2;57)=0.519; p=0.545] and width [F(2;57)=1.958; p=0.148] *(Figure 4. and 5.)* at the beginning and at the end of the experiment (leaf length [F(2;57)=0.779; p=0.476], leaf width [F(2;57)=1.236; p=0.303]). Group treated with FitoHorm Complex Plus gave the best results at both of the measurement time on leaf length and width.

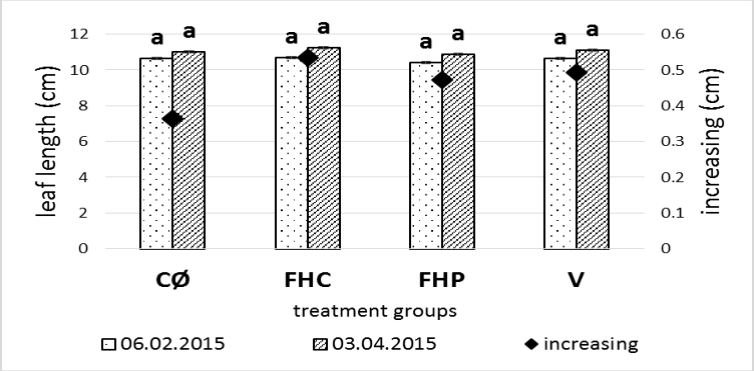

Figure 4. The leaf length of Spathiphyllum wallisii Regel.

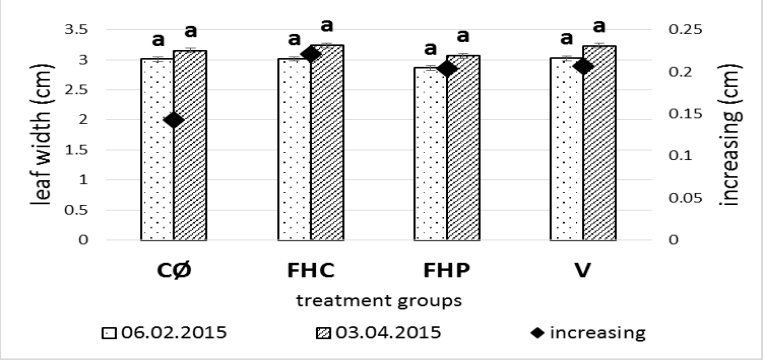

Figure 5. The leaf width of Spathiphyllum wallisii Regel.

All treatments increased the chlorophyll content in the leaves of *S. wallisii* (*Figure 6.*). FHC and V groups showed significant differences in comparison to the control group [F(3;16)=6.660; p=0.004]. Chlorophyll content had 50% increase in groups treated with FitoHorm Complex Plus and Volldünger. From the practical aspect these results are worth mentioning and the may improve quality and physiological conditions.

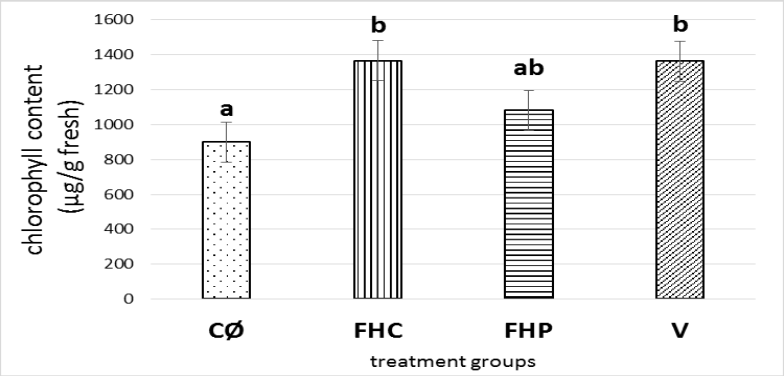

Figure 6. The chlorophyll content of Spathiphyllum wallisii Regel.

As Figure 7. demonstrates there were no significant differences between treatment groups regarding fresh [F(3;50)=2.306; p=0.088] and dry leaf weight [F(3;47)=2.908; p=0.044]. The differences of fresh weight were quite minor, only a tenth of grams. The control group had the best result. The trends were the same for dry weight, but there were some deviations in the ratios. Differences between the two FitoHorm agents decreased. While examining the rate of fresh and dry weight, the lowest rate was observed in the group treated with FitoHorm for potted plants and the highest rate was in the group treated with Volldünger. Plants treated with Volldünger contained the lowest amount of water.

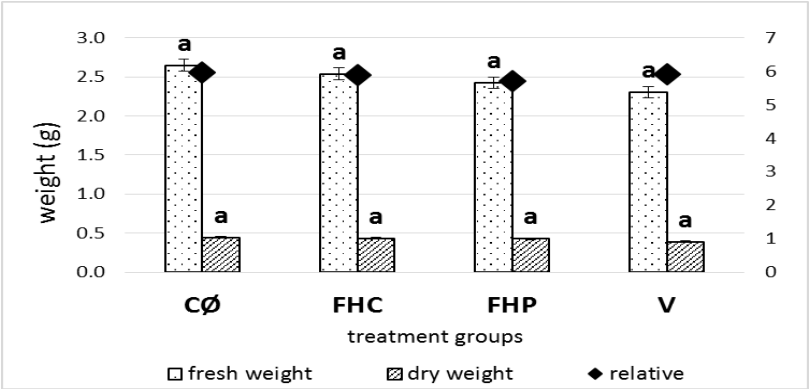

Figure 7. The fresh weight, dry weight and fresh/dry of Spathiphyllum wallisii Regel.

Conclusions

In the first experiment, the groups, treated with two kinds of FitoHorm agents, were bushier than the control group in spite of the results of the statistical analysis. Trends were observed in flower formation. Flowers of the treated groups developed at same rates, but deformation, discoloration and scorching occurred.The control group had the largest number of flowers and there was no deformation. These are important aesthetic aspect of ornamental plants by which the plants are classified in quality category during sale. The treatments did not have a positive effect on generative development. Appropriate treating volume could play a role in the experiment. Probably, the weekly treated prescribe concentrations were too undiluted. To summarize the first experiment, in which the FitoHorm agents, where used beneficial effect on the vegetative growth were noticed. A lower concentration is recommended for healthy flowering. In the second experiment, the leaf growth was

similar, but the change was not considerable in the first experiment. FitoHorm Complex Plus and Volldünger had significantly differences on chlorophyll content. Our results are important in practical aspect because of the more active photosynthesis increases the plant productivity. There was no significantly differences between treatment groups of fresh and dry weight.Summarizing our measured parameters for bushier stand, it is worthy to using one of these agents. Based on the second experiment, the best is the Volldünger or FitoHorm Complex Plus. These agents had beneficial effects on intensive vegetative growth and chlorophyll content. Nevertheless, a lower concentration or water treatment between treatments are recommended. Further experiments suggested for determine of the optimal concentration.

References

1. ABBASNIAYZARE, S. K., SEDAGHATHOOR, S., DAHKAEI, M. N. P. 2012. Effect of biofertilizer application on growth parameters of Spathiphyllum illusion. *Amer-Eurasian J Agric Environ Sci*, *12*(5): 669-673.
2. ALLEY, M. M., VANLAUWE, B. 2009. The Role of Fertilizers in Integrated Plant Nutrient Magamenet. International Fertilizer Industry Association and Tropical Soil Biology and Fertility Institute of the International Centre for Tropical Agriculture. Paris. p. 15.
3. BO, S., RUIXIA, L., JILI, Z. 2010. Studies on Effects of Foliar Fertilizer on the Ornamental Characteristics and Electrolyte Leakage of *Philodendron andreanum*. Ch. Agric. Sci. Bulletin, 4, 49.
4. BROSCHAT, T. K. 1995. Nitrate, Phosphate, and Potassium Leaching from Container-grown Plants Fertilized by Several Methods. HORTSCIENCE 30(1): 74–77.
5. CHENG, K. H., WU, R. Y., CHUANG, K. C., HSIEH, T. F., CHUNG, R. S. 2010. Effects of chemical and organic fertilizers on the growth, flower quality and nutrient uptake of *Anthurium andreanum*, cultivated for cut flower production.Scientia Horticulturae 125 (2010): 434–441.
6. EISA, E. M., IDRIS, T. I. M., WARRAG, M. O. 2014. Influence of Sulfur Fertilizer on Growth and yield of *Aloe vera* plants. Sudan Journal of Science and Technology (2014) 15(1): 65-73.
7. EL-NAGGAR, A. H. 2009. Response of *Dianthus caryophyllus* L. plants to foliar nutrition. *World Journal of Agricultural Sciences*, *5*(5): 622-630.
8. GELLINGS, C. W., PARMENTER, K. E. 2016. Energy efficiency in fertilizer production and use. Efficient Use and Conservation of Energy, Vol II. p: 123-136.
9. HENNY, R. J., NORMAN D. J., CHEN, J. 2004. Progress in ornamental aroid breeding research. Annals of the Missouri Botanical Garden 91: 465-473.
10. HORVÁTH G., ERDEI S. 2003. Növénybiokémiai és növényélettani gyakorlatok [Plant biochemical and plant physiological practice]. Budapesti Közgazdaságtudományi és Államigazgatási Egyetem, Kertészettudományi Kar, University notes. p: 26.
11. KHANAM, R., KUNDU, D., PATRA, S. K. 2017. Integrated Nutrient Management on Growth, Quality, Yield and Soil Fertility of Gladiolus in Lower Gangetic Plain of India. International Journal of Current Microbiology and Applied Sciences (2017) 6(4): 453-459.
12. LINARES-GABRIEL, A., LÓPEZ-COLLADO, C. J., TINOCO-ALFARO, C. A., VELASCO-VELASCO, J., LÓPEZ-ROMERO, G. 2016. Application of biol, inorganic fertilizer and superabsorbent polymers in the growth of heliconia (*Heliconia psittacorum* cv. Tropica). Revista Chapingo Serie Horticultura, 23 (1): 35-48.
13. SARDOEI, S. A. 2014. Evaluation chlorophyll contents assessment on *Spathiphyllum wallisii* Regel with plant growth regulators. International Journal of Biological Sciences 01: 35-39.
14. SHARMA, H., KUMAR, A. 2011. Effect of plant growth regulators and chemical fertilizers on growth and productivity of *Chlorophytum tuberosum* and *Pergularia daemia*. Journal of Medicinal Plants Research, 5(13): 2647-2651.
15. TABACHNICK, B. G., FIDELL, L. S. 2013. Using Multivariate Statistics. 6th edition. Boston: Pearson.
16. TRAUNFELD, J., NIBALI, E. 2013. Soil Amendments and Fertilizers - Fertilizing Guidelines Included by Plant Group. Home & Garden Information Center. University of Maryland. 2013 43: 1-8.
17. http://fitohorm.hu/termekek/fitohorm-komplex-plusz-mutragya/ (21.06.2017)
18. http://fitohorm.hu/termekek/hobbi/fitohorm-leveles-disznovenyekre/ (21.06.2017)
19. http://www.kwizda.hu/volldunger_linz_classic (21.06.2017)

POSSIBLE DEVELOPMENTS ISSUES IN ORGANIC VEGETABLE PRODUCTION IN R. OF MACEDONIA

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Abstract

Due to the presence of rural regions clean from industry and pollution, the usage of own inputs in the crop production, Republic of Macedonia has favorable pre-conditions for development of organic agriculture. As a result of that the certified area under organic production increased from 226 ha in 2005 to 3239.88 ha in 2016, while the number of farms from 50 increased to 562, for the same period. But despite satisfactory conditions for the development and increase of the organic production in RM, very experienced vegetable growers with long tradition of cultivating several crops there are still a number of issues that obstruct its development and need careful attention and effort by all stakeholders in the process of organic production in order to overcome them. Among these limitations primarily are included: lack of organic seeds and other inputs, lack of machines for cultivation of the soil, lack of markets for selling the products, inappropriate application of agro-technical measures (cultivation, crop rotation, plant protection, fertilizing, irrigation, harvest), disconnection of farmers in rural areas, poor infrastructure to the city markets, failure to meet market and consumer requirements regarding quality, quantity and continuous supply of organic plant products, lack of information for the new technology in the organic production, inadequate transportation, lack of storage facilities and very important package, economic crisis, etc. Although some of the plant protection products and fertilizers for organic production are present on the market, the reproductive material (seeds) still remains to be the largest constrain for organic vegetable production. These limitations are probably the reason for low participation of organic vegetable production in the overall organic scheme. In 2016 there are only 93.17 ha (in conversion and organic), that is only 2.87% of the total organic production. As future steps for accelerating the development of organic production in Macedonia must be considered: connecting of the farmers that have a common interest in associations, cooperatives, in order to supply cheaper input materials, group certification, support of trainings, information technology and so on.

Keywords: organic vegetable seeds, distribution channels, inputs, limitations, potential.

Introduction

The concept of organic agriculture developed in the early twentieth century, first in Europe and then in the United States (Tomaš-Simin and Glavaš-Trbić, 2016). Organic production is gaining an increasing share of the vegetable market since it is promoted and per-ceived by consumers as healthier and safer for the environment (Orsini et al, 2016, Sobieralski et al., 2013). Total area under organic vegetable production (almost 354000 ha) is 0,6% of the total area of vegetables grown in the world (58 million hectares) (FAOSTAT, 2013). The countries with the largest vegetable areas are the USA, China, Poland and Italy (each with areas over 20000ha). The USA reported 100000 ha of organic vegetables (Wiler and Lernoud, 2017). Conditions for vegetable and flower crop production- the comparative advantages of the territory of the Republic of Macedonia significantly cause vegetable production to be one of the most important area in agriculture. In terms of area of total 420000ha under arable land and gardens, vegetables are grown on more than 50000ha (12%) and after cereals (43%) is the largest area of the particular plant group. However, in the value of crop production, vegetable crops have the largest share (37,6%), while wheat covers 14,5% (Jankulovski et al., 2013).

Republic of Macedonia has favorable pre-conditions for development of organic agriculture such as having regions clean from industry and pollution and use of own inputs in production. The country has high potentials for recognition and utilization of natural resources appropriate for further development of organic farming The organic sector is the fastest growing agricultural sector in Macedonia. The certified area under organic production increased from 226 ha in 2005 to 3,239.88 ha in 2016 and the number of farms from 50 increased to 562, for the same period. But despite favorable conditions for the development and intensification of the organic production in RM, there are still a number of constraints that hinder its development, that need careful attention and effort by all stakeholders in the process of organic production in order to overcome them. Among these limitations primarily are included: lack of organic seeds and other inputs, lack of machines for cultivation of the soil, lack of markets for selling the products, disconnection of farmers in rural areas, poor infrastructure to the city markets, lack of information for the new technology in the organic production, inadequate transportation, lack of storage facilities and very important package. These limitations and shortcomings in details will be discussed in the following sections of this paper.

Material and methods

In this paper are given tables with data for organic production in R. Macedonia taken from Ministry of Agriculture, Forestry and Water Economy in R. Macedonia. The paper presents current situation of organic plant production in R. Macedonia with special focus on vegetables, major constraints and limitations following with recommendation what should be done in order to promote organic vegetable production in R. Macedonia.

Results and discussion

Due to the evidence for the good environmental performance of organic farming and the trust in organic foods, there are high expectations of organic farming for the future in the Republic of Macedonia. The analysis was done for organic production development in Republic of Macedonia for the period 2005-2016 presented in the following tables.

Table 1. Capacities under organic production in RM

Year	Total certified production area/ha	Number of operators
2005	266.00	50
2006	509.42	102
2007	714.47	150
2008	1029.00	226
2009	1373.83	321
2010	5228.00	562
2011	6580.92	780
2012	4663.08	576
2013	3167.85	400
2014	2359.49	344
2015	2631.96	481
2016	3239.88	533

Source: Ministry of Agriculture, Forestry and Water Economy in R. Macedonia

In table 1 are given data for capacity of organic production in R. Macedonia. The total certified production area (ha) and numbers of operators are increasing from 2005 (266.00 ha, 50 operators) till 2011 where total certified area and number of operators reaches highest peak 6580.92 ha and 780 operators. After that period the production area and operators decline and raise reaching 3239.88 ha and 533 operators in 2016. This is partly due to public awareness for organic know-how and undeveloped domestic market for organic products.

Table 2. Organic plant production in RM for 2014, 2015 and 2016 in ha

Organic plant production in RM									
	2014			2015			2016		
Crop	In conversion	Organic	Total	In conversion	Organic	Total	In conversion	Organic	Total
Wheat	193.90	702.50	896.40	187.29	417.13	604.42	358.82	579.58	938.40
Feed	69.48	454.51	523.99	231.65	745.68	977.33	209.60	539.38	748.98
Industrial	-	-	-	-	-	-	-	-	-
Oilseeds	24.02	95.51	119.53	82.7	20.86	103.56	10.00	32.84	42.84
Fruits	317.97	109.15	427.12	220.28	179.91	400.19	178.01	244.13	422.14
Vineyards	71.05	25.49	96.54	49.71	26.68	76.39	11.29	6.25	17.54
Vegetables	30.24	22.17	52.41	42.36	41.52	83.88	53.23	39.94	93.17
Fallow land	204.22	38.98	243.20	103.28	539.01	642.29	171.69	230.45	402.14

Source: Ministry of Agriculture, Forestry and Water Economy in R. Macedonia

Organic plant production in Republic of Macedonia for 2014, 2015 and 2016 is given in table 2. According to the data the highest area in conversion, organic and total in the three years is under wheat production while there is no organic production for industrial crops. The vegetables cover small area in conversion, organic and total in comparison with other crops. Although the area with vegetables organic production is small there is a trend of increasing the area with vegetables within the years. In 2014 the area under vegetables in conversion, organic and total is 30.24 ha, 22.17 ha and 52.41 ha respectively. The following year 2015 the area increase and reaches 42.36 ha in conversion, 41.52 ha organic and 83.88 ha total. In the last year 2016 the total area under vegetables in conversion (53.23 ha) and organic (39.94 ha) is 93.17 ha which is only 2.87% (both organic and conversion to organic) of the total organic production (table 3).

Table 3. Organic vegetable production in the Republic of Macedonia in the period of 2010-2016

Year	Total certified organic area (ha)	Total vegetable area organic + conversion(ha)	% of the total organic area
2010	5.228,00	199,9 ha	3,82
2011	6.580,92	263, 19 ha	3,99
2012	4.663,06	57, 68 ha	1,23
2013	3.167,85	121,37 ha	3,83
2014	2359,49	52,41 ha	2,21
2015	2.631,96	83.88 ha	3,18
2016	3.239,88	92.18 ha	2,84

Despite the increase of total organic plant production area, vegetables cover very small portion in the total organic area which is not comparable with the total conventional vegetable area. The major constraints are: still small operational farms, lack of organic seeds and other inputs, lack of machines for cultivation of the soil, inappropriate application of agro-technical measures (cultivation, crop rotation, plant protection, fertilizing, irrigation, harvest), poor infrastructure to the city markets, lack of continuous supply of organic plant products, lack of information for the new technology in the organic production, lack of storage facilities, inadequate transportation and very important package. As regarding limitations there is still lack of information regarding organic production at all levels, limited availability of approved inputs, still lack of trained advisors, lack of contract farming for organic production. These limitations are probably the reason for low participation of organic vegetable production in the overall organic scheme. The organic seeds supply is one of the biggest

problem. Farmers use their own seeds from landraces and non-treated seeds from well-established varieties. They are cultivated in a traditional way in the garden, in a very small space in a rural areas without the use of pesticides (Agic et al., 2013). The same authors stated that in collection missions (2005-2010) in R. Macedonia, 507 accessions from 29 vegetable crops were inventoried, collected, described and documented. This landraces are valuable material and suitable for small scale organic production in rural areas and for the local market. But the quantities are not sufficient to cover demand for larger organic vegetable producers. From 2017, there is only one Macedonian company representative of organic seed from Swiss company that is available for import of organic seeds, but the prices are much too high for the local producers. Local producers can purchase seed from this company only on order for certain variety or cultivar mainly intended for export. However there is a wide range of available input materials such as organic fertilizers and plant protection products on the market in R. Macedonia. But there is still insufficient knowledge and expertise of organic producers about possibilities and methods of use of allowed inputs during production. So, know-how training of organic producers is necessary and is a very good tool for overcoming any production obstacles. The level of formal and non-formal education and research in this area still insufficient. The main role of education in organic farming is to increase the level of public awareness, knowledge and information of organic issues among experts, farmers, and other stakeholders in the organic chain. In order to have proper development of this sector, research activities should be initiated for organic farming techniques, availability of natural resources and the national and international market demands (Agic et al., 2012). Another problem, is that there is no marketing strategy for selling organic products. Organic producers sell their product by themselves on open-air markets, supermarkets or organic shops in bigger cities where the demand is very high in comparison to rural areas. Here is important to mention that in healthy food stores there is lacking of fresh organic produce such as vegetables and fruits although the demand is very high. This can be result in disconnection of farmers in rural areas and poor infrastructure to the city markets. So, there is a need of overcoming these obstacles in distribution channels with networking a small scale suppliers which could further boost and channel organic production in the future. Another limitation is improper packaging of organic vegetables. The package should protect the produce from foreign matter and give all necessary information on the label concerning identification, nature of the produce, origin of produce, commercial specifications, official control mark and logo for organic produce. Assumed the fact that the demand for organic products increases in our country (especially in large cities) and in the European countries, which is a good challenge, it is necessary to take appropriate measures to overcome the above constraints for accelerating the development of organic production in Macedonia. As future steps must be considered: connecting of the farmers that have a common interest in associations, cooperatives, in order to supply cheaper and improvement of availability of organic farming inputs, possibilities for group certification, support of trainings, information technology, revitalization of landraces, production of sufficient quantities and assortment of organic products. Also, major wholesalers/traders/consolidators should be involved in organizing and selling (exporting) of organic products. They could serve as valuable source of information regarding current product features such as product packaging, instruct the producers on type and quantities of products needed by the retailers. Also in R. Macedonia there is an organization IME (Increasing Market Employability) with following activities: increasing market transparency, increasing public awareness and recognition and visibility for Macedonian organic products, stimulate ecotourism development with organic products, increasing sales of Macedonian organic products on export markets and increasing the knowledge on export markets and competitiveness of Macedonian organic products. IME also support promotion of organic products at the world leading organic fair at BIOFACH. According to their market research for fresh organic products as most demanded at the consumer level are: organic potatoes, broccoli, carrots, beetroot, spinach, tomato, pepper and from fruits: apples, pears and cherries. Macedonia has a developed sector of processing fruits and vegetables. However, only a small number of processing entities are involved or are considering to get involved in organic production. There is a need for closer

cooperation of organic production and processing companies. Today, despite growing trends for organic products, the supply of processed organic products is almost insignificant. The reasons for this situation are following:
- lack of knowledge of processing technology of organic products;
- insufficient quantities of certified raw material for processing entities;
- processing entities are not involved in planning the primary production and there is no practice of contractual farming;
- lack of certified/allowed products for processing organic products;
- ignorance of processing entities about the existing support for organic product processing.
- lack of information to processing entities about access to organic product markets;
- insufficient involvement of the state in promotion of organic processed products;
- insufficient support for processing organic products.

Additional reason for this may be the fact that when processing entities get interested for organic products, organic farmers raise the prices enormously in spite of the subsidies they receive. This further discourages the processing entities. Unfortunately, in Macedonia there is no tradition of contractual farming between primary producers and processing companies, but contrary to this, business is done opportunistically, each hoping the other one will find themselves in unenviable situation to be forced to sell the products by low prices or to buy the products by high prices. Market potential for European investors in the agriculture and particularly the organic agriculture sector, as far as demand in Europe and especially in Germany is concerned, is mostly in sourcing products, raw materials and processed goods. Berries, selected vegetables, soy and cereal products might therefor prove to be of highest interest (Simic, 2017). Taking into consideration that Europe has widely open doors only for organic products, it would be necessary to build more serious approach towards the organic agriculture in Macedonia.

Conclusions

Given the fact that interest in organic products increases in our country (especially in large cities) and in the European countries, which is a good challenge, it is necessary to take appropriate measures to overcome the above constraints to accelerate the development of organic production in Macedonia. Future steps must be connecting of the farmers that have a common interest in associations, cooperatives, in order to supply cheaper input materials, group certification, support of trainings, information technology and so on. Efforts should be done for increasing education among experts, farmers and other stakeholders in organic chain. Also there is a need for upgrading the extension services, strenthening univerisity partnerships, increasing the exchange of academic staff and promoting membership in various international associations for organic production. This would help to develop the market and the supply chains by stronger promotion of modern technologies, processing and market systems. With such assistance of appropriate measures Macedonia may hope speedily and efficiently reach national, regional and global organic markets.

References

1. Agic, R., Popsimonova, G. (2013). Current status and future perspectives of vegetable genetic resources in R. Macedonia. Symposium proceedings. International Symposium for agriculture and food. 12-14 December, Volume I, Skopje, 522-529
2. Agic, R., Popsimonova, G., Ibusoska, A., Bogevska, Z., Davitkovska, M., Ristovska, B., Iljovski, I. (2012). Perspective of the Organic Production in Macedonia. Acta Horticulturae 960, 263-268.
3. FAOSTAT, 2013. http://www.fao.org/faostat/en/#data/QC
4. Jankulovski, D., Hadji-Pecova, S., Martinovski, Gj., Popsimonova, G., Rukie, A. (2013). Recent trends in vegetable and flower crop production in Republic of Macedonia. Symposium Proceedings. International Symposium for agriculture and food. 12-14 December, Volume I, Skopje, 512-520
5. Orsinia, F., Maggiob, A., Rouphaelb, Y., De Pascaleb, S. (2016). "Physiological quality" of organically grown vegetables. Scientia Horticulturae 208: 131–139

6. Simic, I. (2017). Organic agriculture in Serbia at a glance 2017. National Assosiation Serbia Organica. ISBN 978-86-88997-09-6.
7. Sobieralski, K., Siwulski, M., Sas-Golak, I. (2013). Nutritive and health-promoting value of organic vegetables. Acta Sci. Pol., Technol. Aliment. 12(1): 113-123.
8. Tomaš-Simin, M., Glavaš-Trbić, D. (2016). Historical development of organic production. Economics of Agriculture, (63) 3: 1083-1099.
9. Wiler, H., Lernoud, J., 2017. The world of organic agriculture statistics and emerging trends. IFOAM – Organics international and FIBL. ISBN 978-3-03736-040-8., 126

THE POSSIBILITY OF IMPROVING THE PRODUCTION OF FLOWERS IN THE REPUBLIC OF SERBIA

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Abstract
Flower production is highly intense production, which does not require large areas, but requires knowledge and organization of the overall production and sales. In recent years there has been growing interest of individuals, in the context of their family households, on small estates, organize the production of flowers. Production is mainly concentrated around the major cities and it deals with around 2,115 farms on a total area of 382, 24 hectares. In terms of production structure dominates production of seedlings of seasonal flowers, and the manufacture of some types of suitable for cut flower (rose, chrysantemum, lilies, gladiolus, kala). The volume of annual production of 2500 t does not meet the needs of the domestic market. For these reasons, the import of flowers is several times higher than exports. Starting from the fact that the Republic of Serbia not only has significant land resources, but also scientific institutions in the field of agriculture which have made are significant scientific results, improvement of the total production of flowers, can significantly reduce imports but at the same time significantly increase the export of flowers to other countries.

Keywords: agriculture, production, floriculture, Serbia.

Production potentials of Serbia

Serbia is located in the southeastern part of Europe and occupies the central part of the Balkan Peninsula. It covers an area of 88361 km². It borders with eight neighboring states and with a total length of 2397 km, of which 1717 km of subway borders and 680 km of rivers. According to the administrative-territorial division, Serbia has 174 municipalities deployed in 29 administrative districts. Based on the results of the last 2011 population census, 7186862 inhabitants live in Serbia, of which 59.44% live in urban settlements, and 40.56% in other settlements, among which the largest number of rural areas. The population density is about 101 inhabitants / km². The moderate-continental climate dominates. It is characterized by an average annual rainfall of 500-700 mm and a average annual temperature of 10-12°C. In terms of lend characteristics Serbia is rich in lands that are suitable for the production of agricultural crops in their physical and chemical properties. The most important types of soil are: black earth (carbonate, meadow, sandy), rattan black and alluvial deposits. By positioning favorable natural conditions, further development and improvement of agricultural production, Serbia can be the main lever of economic development in general, in which the production of flowers could find its significant place.

Areas under flowers in Serbia

According to the Statistical Office of the Republic of Serbia for 2016, the total area under arable land is 3861477 ha, while the number of registered agricultural holdings is 631552. The most developed branches of agriculture are cattle breeding (43%), field crops (42%), fruit growing and viticulture (12%). Thanks to demand throughout the year, the production of floral and decorative planting material has been showing a marked development in recent years. The production of flowers and ornamental plants in Serbia deals with 2125 farms (Tab. 1) on an area of 382.24 ha. Since the production of flowers is mainly concerned with small family farms, the main problem that hinders the further development of this branch of production is the inability to more efficiently use the workforce as well as the inability to rational use of modern means of production.

Table 1. Number of households and areas under flowers and ornamental plants in Serbia

	Total	
	Area, ha	Number of holdings
Flowers and ornamental plants: Number of farms and areas by size of flowers and ornamental plants area		
REPUBLIC OF SERBIA [1]		
Total	382.34	2125
Family	334.61	2010
Legal entity	28.19	44
Entrepreneurs	19.54	71

Flowering production is also organized in the open field and in a protected area. In 2016, the area of open space where flowers were grown was 263 ha, and a protected area of 119 ha (Tab. 2), which makes less than 10% of the total area per protected area in Serbia (2083 ha).

Table 2. Areas under flowers in Serbia (open field and protected area)

	≤ 1 ha	1,01 – 2 ha	2,01 – 5 ha	5,01 – 10 ha	10,01 – 50 ha	≥ 50 ha	Total
Total area (ha)	106	63	90	60	59	4	382
Protected place	42	18	24	23	10	2	119
Open field	64	45	66	37	49	2	263

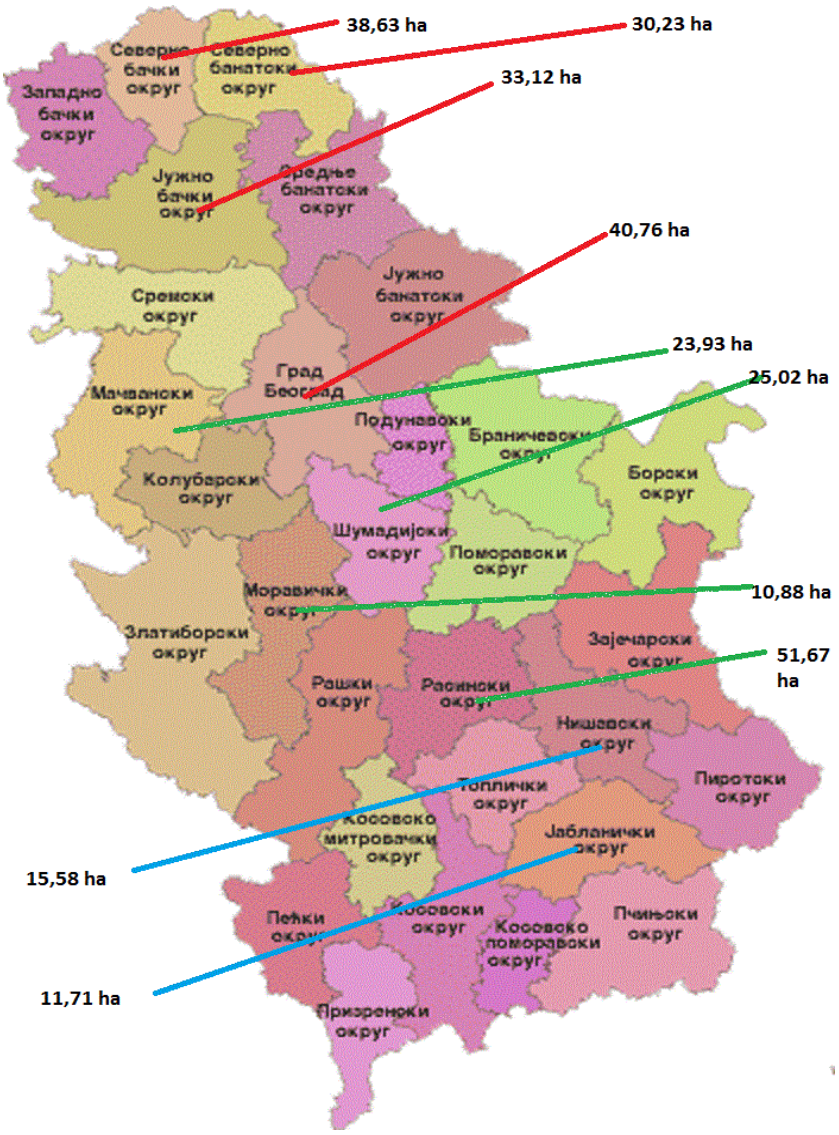
Data source: Republic Institute for Statistics, January 2016.

In protected areas, the production of flowers is done in small batches because they are small objects. The number of flower-producing objects in the last decade has been gradually decreasing, not due to the consequence of reducing the need for flowers, but because of the high construction and maintenance costs. Flowering production is concentrated in several major areas (Fig. 1). The main production centers are: Belgrade region with 40.76 ha; Macvan region (Sabac with 19.44 ha); Moravian region (Čačak with 9.88 ha); Rasin's region (Varvarin with 18,96 ha, Krusevac with 12,45 ha) and Sumadija region (Kragujevac with 11,58 ha).

In terms of production structure, the production of seasonal flower plants dominates in the protected area. In the open field, the most prevalent is the production of Rose, Gladiolus, Chrysanthemum, Calla and Lily. The areas under these cultures vary from year to year. The largest areas occupy roses, while other cultures are represented in a much smaller percentage. In relation to the countries of the region, the production of flowers in Serbia is organized on larger areas in relation to Croatia (300 ha) and Bosnia and Herzegovina (there is no accurate data), and half of the area in relation to Macedonia (691 ha) and Hungary (670 ha).

Foreign trade of flowers

According to statistics from the World Trade Organization, more than sixty countries in the world are participating in international flower trade, which exceeds $ 7 billion annually. Half of these revenues come from the placement of cut flowers, and the rest of nursery production, flower pots and seeds. Since the 1990s, the volume of foreign trade of flowers in the world has started to grow.



Flowers worth EUR 4 million are exported from Serbia annually, while the value of imports is tripled. Therefore, a foreign trade deficit is expressed in this economic branch. A similar situation exists in countries in the region. In the structure of imports, according to the Serbian Chamber of Commerce, the largest share in 2016 has a group of cut flowers (roses, carnations, chrysanthemums and orchids), with 44% of total imports (Tab. 3 and 4), followed by seed and fruit material and flower pots. Importing dried flowers is negligible. Compared to the structure of imports from 2006 and 2007, there are no major changes either in assortment or in value (43%). Most flowers are imported from EU countries (Netherlands, Italy, Belgium, Hungary), then from Kenya, Turkey, Ecuador and Colombia.

Table 3. The quantity of imported flowers in Serbia (in tones)

Product	2012.	2013.	2014.	2015.	2016.
Fresh cut flowers and buds for bouquets	1968	2000	2031	1907	2244
Bulbs, tubers, rhizomes	139	125	189	189	225

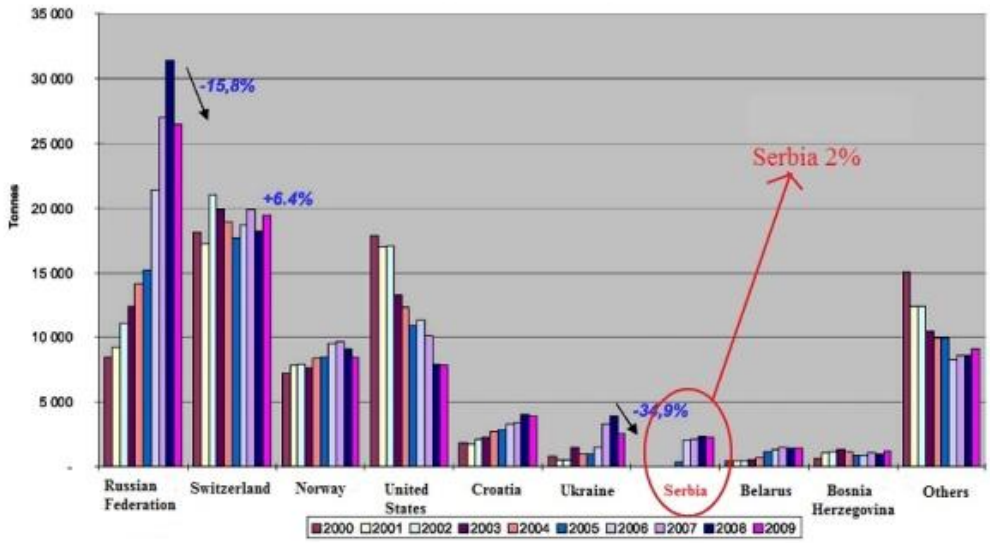
Data source: Republic Institute for Statistics, January 2016.

Table 4. Imports of flowers in Serbia (000 USD)

Product	2012.	2013.	2014.	2015.	2016.
Fresh cut flowers and buds for bouquets	4070	3835	3638	3000	2979
Bulbs, tubers, rhizomes	526	514	718	640	971

Data source: Republic Institute for Statistics, January 2016.

The most important foreign trade partners of Serbia in the trade of flowers and ornamental plants are the countries of the European Union (EU) and the Free Trade Zone in Central Europe (CEFTA), which account for almost 80% of the total exchange of these products (Graph 1). Of the CEFTA countries, most flowers are exported to Bosnia and Herzegovina and Croatia (391t) and from the EU countries to the Netherlands, Poland and Germany. Serbia also exports flowers to Russia, Belarus, Switzerland, the United States.



Graph1. Exports of cut flowers and greenery by countries in tones

A similar situation exists in countries in the region. Croatia, with nearly the same production area, imports as much as 90% of its flowers. Macedonia imports 80%, although it has larger areas under flowers than Serbia. In Hungary, there is also a higher rate of imports compared to exports, which also indicates higher consumption.

Conclusions

Starting from the fact that the Republic of Serbia does not only have significant land resources, but also scientific institutions in the fields of agriculture that have given significant scientific results, improving the overall production of flowers, can significantly reduce imports, but at the same time significantly increase the export of flowers to other countries. The main lever for the improvement of flower production is the strategy of the country's economic development and the strategies of foreign trade relations. Associated manufacturers have great chances to improve their production, provide a greater quantity of products that will enable them to increase their competitiveness in both the domestic and foreign markets. The education programs of producers represent a very important segment of the promotion of flower production in Serbia. Because only in this way manufacturers can be introduced to the potentials that they possess and which can be used and thus participate in the development of their environment and society as a whole. For a better foreign trade, it is necessary to organize a stock market for the flow of flowers, which will ensure the

continuity of supply, trade and demand. In conceiving the export strategy to the international market, priority should be given to the countries in the environment. And to the countries of CEFT (Bosnia and Herzegovina, Macedonia, Croatia and Albania), which now exceeds 50% of exports. Also, we should strive for greater participation in the market of the Russian Federation, Ukraine and other countries of the former USSR. In this way, domestic placement would be relieved, and production and thus revenues would increase.

References

1. RSZ (2016): Statistički godišnjak Republike Srbije, Republički zavod za Statistiku, 2016
2. DZH (2016): Statistički godišnjak Republike Hrvatske, Državni zavod za Statistiku 2016
3. DZM (2016): Drzaven zavod za statistika(MAKSTAT) 2016.
4. Cvijanović,D., Bukvić, P., Lazarević, S., Popović, V, Simonović V., Vujošević, A.(2005): Revitalizacija i unapređenje proizvodnje cveća , Monografija. Institut za ekonomiku poljoprivrede-Beograd; Šumarski fakultet-Beograd, Poljoprivredni fakultet-Beograd
5. Vujošević, A., Stevanetić, S., Zarić, V. (2008): Proizvodnja I spoljnotrgovinska razmena cveća i ukrasnog bilja u Srbiji, Agroekonomska nauka I struka u tranziciji obrazovanja I agroprivrede, Tematski zbornik, 24-25-okt., Beograd, 223-235.
6. Vujošević, A., Stevanetić, S.,(2007): Izvozne mogućnosti cvećarske i rasadničarske proizvodnje, Naučno-stručno savetovanje agronoma Republike Srpske, Agroznanje vol.8, br.3., 111-119.
7. Munćan M., Todorović, S., Ivkov I. (2008): Model porodičnog gazdinstva kao osnova za eksperimentisanje u agroekonomskim istraživanjima, Agroekonomska nauka I struka u tranziciji obrazovanja I agroprivrede, Tematski zbornik, 24-25-okt., Beograd, 211-221.

THE EFFECT OF SUBSTRATE TYPE ON THE QUALITY OF WATERMELON SEEDLINGS

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Abstract
Watermelon is one of the leading vegetable crops grown in R. Macedonia. It is regularly produced by seedlings and very seldom by seed. The objective of the research was to determine the influence of growing substrate type on the quality of watermelon seedlings, variety Fantasy F_1. Three different substrates were tested as follows: homemade substrate mixture of organic cattle manure and soil in ratio 1:1 (Reference treatment), commercial Brill substrate with Trichoderma (Treatment 1) and mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1 (Treatment 2). According to research methodology several biometrical parameters were measured in order to determine quality of the watermelon seedlings as follows: seedling length (cm), mass of the aerial seedling part (g), stem thickness (mm), leaves number, leaves mass (g), root length (cm) and root mass (g). The seedlings were measured 18 days after sprouting. The results showed that seedlings grown in Brill substrate with Trichoderma had the best parameters for mass of the aerial seedling part (5.10cm), stem thickness (3.30mm), leaves number (6.27), leaves mass (9.04g), root length (17.17cm) and root length (13.95g). Only seedlings from treatment 2 were longer (16.23cm) in comparison to the Reference treatment (14.6cm) and Treatment 1 (14.73cm).

Keywords: hybrid, growing medium, biometrical parameters.

Introduction

Watermelon and melon are one of the widely grown vegetables in R. Macedonia. According to State Statistical Office of the Republic of Macedonia (2016) watermelon and melon are grown on 5509 ha with total production of 131039 t and average yield of 23786 kg^{-ha}. Watermelon is grown either with direct sowing or more commonly with seedling production. Major factors that influence the quality of seedling production are: high quality and healthy seeds with good biological values (high germination rate and germination energy), optimal microclimate conditions (heat, light, humidity), optimal space between plants, rich growing (soil, soil mixes, organic and inorganic substrates), timely irrigation, proper nutrition with macro and micro elements and adequate plant protection (Bogevska, 2008). According to Yaping and Diankui (2005) the best maturity stage for watermelon seedling is between 20 and 25 days, when they are grown in a plug cell size of 78.4 cm^3 - 82.9 cm^3. Same authors recommend that ultimately a seedling maturity of 30 days with a cell size of 131.6 cm^3 can be acceptable. More often watermelon is produced by grafted seedlings. It is very important to choose the right rootstock as it influences on the plant growth as well as yield and quality of scion fruit, suggesting an important consideration in the potential use of grafting applications in watermelon (Yetisir and Sari, 2003). When seedlings are not grafted the choice of growing substrate is crucial. It includes its composition and irrigation water quality (Silva et al., 2015). That was the thesis of this research - to determine the quality of watermelon seedlings grown on different substrates.

Material and methods

For this trial the watermelon hybrid Fantasy F_1 was chosen. It is a very attractive hybrid of Allsweet type. This variety produces uniform, elongated fruit weighing 10 to 12 kg. The rind has dark green

stripes on a medium-green background. Its flesh is bright red and very sweet. The seedlings were sown in trays containing three different substrates as follows:

- Homemade substrate mixture of organic cattle manure and soil in ratio 1:1 (Reference treatment),
- Commercial brill substrate with Trichoderma (Treatment 1) and
- Mixture of homemade and brill substrate with Trichoderma in ratio 1:1 (Treatment 2).

Chemical composition of the organic cattle manure is: 18.74% $CaCO_3$, total nitrogen 0.246% phosphorous 76.20 mg/100 g, potassium 80.10 mg/100g, humus 3.17 % and pH-7.99. The composition of the commercial brill substrate with Trichoderma is as follows: 80% white peat, 20% frozen black peat, 500g NPK fertilizer (14+10+18)/m^3, 500g MikroTR/m^3, 100g trace elements/m^3, 100ml wetting agent/m^3, pH-value ($CaCl_2$): 5.7 with fine structure (0-10mm). The chemical properties content of the commercial substrate is: nitrogen (N) 70 mg/L, phosphorus (P_2O_5) 50 mg/l, potassium (K_2O) 90mg/L and salt content approximately 0.5g/L. Sowing date for all treatments was March 20th 2017. The plants sprouted after seven days. During the seedling production standard technology was applied. Just before transplanting, several biometrical parameters were measured in order to determine the quality of the watermelon seedlings as follows: seedling length (cm), mass of the aerial seedling part (cm), stem thickness (mm), leaves number, leaves mass (g), root length (cm) and root mass (g). The stem thickness was measured with caliper tool, the aerial seedling part, the leaves root mass were measured with digital balance. The seedling and root length were measured with a ruler. The seedlings were measured 18 days after sprouting. For each treatment 30 plants were recorded. Recorded measurement were statistically processed according to the method of Analysis of Variance and tested with LSD test at the level 0.05 and 0.01.

Results and discussion

According to the results the seedling grown in mixture of homemade substrate and brill substrate with Trichoderma in ratio 1:1 resulted in longest seedlings (16.23cm) which was statistically significant at probability level of 0.01 in comparison to the seedlings from Reference treatment that were 14.60cm long in average (Table 1). The seedlings grown in mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1 showed significant statistical difference in length of 1.63cm at a level of 0.01 compared to the length of the seedlings from commercial Brill substrate with Trichoderma (Treatment 1) with seedlings of 14.73cm length in average. The most heterogeneous measurements about this trait were observed in the homemade substrate (Reference treatment) with coefficient of variation 4.62.

Table 1. Seedling length (cm)

Treatment	Arithmetic Mean	Standard Deviation	Standard error of the mean	Coefficient of Variation
Reference	14.60	0.67	0.12	4.62
Treatment 1	14.73	0.45	0.08	3.05
Treatment 2	16.23	0.57	0.10	3.50
Comparison between treatments				
Reference	14.60	-0.13	-1.63	
Treatment 1	0.13	14.73	- 1.5	
Treatment 2	1.63 [ab]	1.50 [ab]	16.23	
	Reference	Treatment 1	Treatment 2	

LSD 0.01 = 0.66[a] LSD 0.05 = 0.40[b]

Table 2. Aerial seedling mass (g)

Treatment	Arithmetic Mean	Standard Deviation	Standard error of the mean	Coefficient of Variation
Reference	4.13	0.26	0.05	6.24
Treatment 1	5.10	0.27	0.05	5.21
Treatment 2	3.72	0.19	0.03	5.02
Comparison between treatments				
Reference	4.13	-0.97	0.41 [ab]	
Treatment 1	0.97 [ab]	5.10	1.38 [ab]	
Treatment 2	-0.41	-1.38	3.72	
	Reference	Treatment 1	Treatment 2	

LSD 0.01 = 0.33[a] LSD 0.05 = 0.20 [b]

Seedlings with highest mass (5.10g) of the aerial part were recorded in commercial Brill substrate with Trichoderma, as presented in Table 2. The lowest aerial seedling mass of 3.72g was observed in the seedlings grown mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1 (Treatment 2). The seedlings from both treatments had statistically higher aerial mass at a level of 0.01 compared to the aerial seedling mass from the reference treatment. The aerial seedling mass from Treatment 1 (commercial Brill substrate with Trichoderma) showed significant statistical difference at a level of 0.01 compared to the one from Treatment 2 (mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1). The least uniform seedlings regarding the seedling mass had the homemade substrate (Reference treatment) with the coefficient of variation 6.24%. The highest value for stem thickness (3.30mm) was observed in the seedlings grown commercial Brill substrate with Trichoderma (Treatment 1) while the lowest one (3.03mm) was recorded in seedlings grown in homemade substrate and Brill substrate with Trichoderma in ratio 1:1 (Treatment 2), as presented in Table 5. According to the research of Kokalis-Burelle et al. (2003) thickness of the watermelon stem varied from 3.6 to 4.6 mm which was little bit higher in comparison to our findings. The thickness of stem from commercial Brill substrate with Trichoderma showed significant statistical difference at a level of 0.05 compared to the Reference treatment. The stem thickness of seedlings grown in the commercial Brill substrate with Trichoderma was significantly larger at a probability level of 0.01 compared to the thickness of stem from mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1 (Treatment 2). The most homogenous stem thickness was observed in the seedlings grown on mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1 with coefficient of variation 6.02.

Table 3. Stem thickness (mm)

Treatment	Arithmetic Mean	Standard Deviation	Standard error of the mean	Coefficient of Variation
Reference	3.13	0.35	0.06	11.03
Treatment 1	3.30	0.47	0.09	14.12
Treatment 2	3.03	0.18	0.03	6.02
Comparison between treatments				
Reference	**3.13**	-0.17	0.1	
Treatment 1	0.17 [b]	**3.30**	0.27 [ab]	
Treatment 2	-0.1	-0.27	**3.03**	
	Reference	Treatment 1	Treatment 2	

LSD 0.01 = 0.25[a] LSD 0.05 = 0.15[b]

According to the biometric parameter number of leaves the highest value of 6.27 in average had the seedling grown on commercial Brill substrate with Trichoderma (Treatment 1) followed by the seedlings grown on mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1 (Treatment 2) that had 6,23leaves in average and the lowest number of leaves (6.0) was recorded in the Reference treatment as presented in Table 4. There was statistical difference at a level of 0.05 compared with the Reference treatment in number of leaves from commercial Brill substrate with Trichoderma and mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1. There are no statistical differences between Treatments 1 and 2 (Table 4). The number of leaves was the same in all measured seedling grown on homemade substrate Reference treatment which can be concluded form calculated standard deviation and coefficient of variation. The average values for the leaves mass per seedling are given in table 5. The highest leaves mass of 9.04g was recorded for the seedlings grown in commercial Brill substrate with Trichoderma (Treatment 1) with a significant statistical difference at a level of 0.01 compared with the mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1 (Treatment 2) and the Reference treatment. The most heterogeneous seedlings in regards to leaves mass were observed in homemade substrate with 7.47 coefficient of variation.

Table 4. Number of leaves per seedling

Treatment	Arithmetic Mean	Standard Deviation	Standard error of the mean	Coefficient of Variation
Reference	6.00	0.00	0.00	0.00
Treatment 1	6.27	0.45	0.08	7.18
Treatment 2	6.23	0.43	0.08	6.90
Comparison between treatments				
Reference	**6.00**	-0.27	-0.23	
Treatment 1	0.27 [b]	**6.27**	0.04	
Treatment 2	0.23 [b]	-0.04	**6.23**	
	Reference	Treatment 1	Treatment 2	

LSD 0.01 = 0.31[a] LSD 0.05 = 0.19[b]

Table 5. Leaves mass (g)

Treatment	Arithmetic Mean	Standard Deviation	Standard error of the mean	Coefficient of Variation
Reference	4.73	0.35	0.06	7.47
Treatment 1	9.04	0.18	0.03	2.01
Treatment 2	7.13	0.31	0.06	4.28
Comparison between treatments				
Reference	**4.73**	-4.31	-2.4	
Treatment 1	4.31 [ab]	**9.04**	1.91 [ab]	
Treatment 2	2.40 [ab]	-1.91	**7.13**	
	Reference	Treatment 1	Treatment 2	

LSD 0.01 = 0.29[a] LSD 0.05 = 0.18[b]

The root length of 17.17cm was largest in the seedlings grown in commercial Brill substrate with Trichoderma followed by the mixture of homemade substrate and Brill substrate with Trichoderma with seedlings root length of 15.97cm in average and 15.23cm for the ones grown in homemade substrate. As presented in Table 6 the roots length in mixture of homemade substrate and Brill substrate with Trichoderma in ratio 1:1 (Treatment 2) was significantly larger at statistical a level of

0.05 compared to the ones grown in homemade substrate. The seedlings grown in commercial Brill substrate with Trichoderma (Treatment 1) had better developed roots at a level of 0.01 compared to the ones in Reference substrate. A difference with same statistical significance was observed between both Treatment 1 and 2. The highest value of variation of 5.74 for root length was calculated for the seedlings grown on commercial Brill substrate with Trichoderma.

Table 6. Root length (cm)

Treatment	Arithmetic Mean	Standard Deviation	Standard error of the mean	Coefficient of Variation
Reference	15.23	0.43	0.08	2.82
Treatment 1	17.17	0.99	0.18	5.74
Treatment 2	15.97	0.89	0.16	5.57
Comparison between treatments				
Reference	15.23	-1.94	-0.74	
Treatment 1	1.94 [ab]	17.17	1.20 [ab]	
Treatment 2	0.74 [b]	-1.20	15.97	
	Reference	Treatment 1	Treatment 2	

LSD 0.01 = 0.98[a] LSD 0.05 = 0.59[b]

Average values for the root mass per seedling and comparison between treatments are given in Table 7. The highest root mass of 13.95g in average was measured in the seedlings grown in commercial Brill substrate with Trichoderma (Treatment 1) while lowest one of 8.84g in average was recorded in the seedlings grown in the Reference substrate. There is a significant statistical difference at a level of 0.01 between and among the both Treatments 1 and 2, compared to the Reference substrate, as presented in Table 7. The most heterogeneous seedlings in regards to root mass with coefficient of variation 8.54 were the ones grown in homemade substrate, the Reference treatment.

Table 7. Root mass (g)

Treatment	Arithmetic Mean	Standard Deviation	Standard error of the mean	Coefficient of Variation
Reference	8.84	0.76	0.14	8.54
Treatment 1	13.95	0.17	0.03	1.19
Treatment 2	10.59	0.49	0.09	4.62
Comparison between treatments				
Reference	8.84	-5.11	-1.75	
Treatment 1	5.11[ab]	13.95	3.36 [ab]	
Treatment 2	1.75 [ab]	-3.36	10.59	
	Reference	Treatment 1	Treatment 2	

LSD 0.01 = 0.86[a] LSD 0.05 = 0.52[b]

Similar results were gained by Balicevic et al. (2008) in tomato seedling planted with two substrates: specialized substrate for production of seedlings by German manufacturer Gebr. Brill Substrate GmbH & Co (substrate I), and randomly taken mixture of garden mould (substrate II). Tomato plants planted in substrate I Brill substrate had better state of health and higher plant number if compared to the seedlings planted in substrate II.

Conclusions

According to the results obtained from a field trial with watermelon seedlings grown on different substrates it can be concluded that the best quality of seedlings can be achieved by growing watermelon seedlings on commercial Brill substrate mixed with Trichoderma (Treatment 1).

References

1. Belicevic, R., Parađiković, N., Cosić, J., Jurković, D., Samota, D. (2008). Influence of substrate in biological control of tomato seedlings against Rhizoctonia solani and Pythium debaryanum. Cereal Research Communications 36:1499-1502.
2. Bogevska, Z. (2008). Influence of substrate type on the pepper (*Capsicum annuum* L.) seedlings quality growth and development, Master thesis, Faculty of Agricultural Sciences and Food, University "Ss. Cyril and Methodius" – Skopje.
3. Ceglie, F. G., Bustamante, M. A., Ben Amara, M., & Tittarelli, F. (2015). The Challenge of Peat Substitution in Organic Seedling Production: Optimization of Growing Media Formulation through Mixture Design and Response Surface Analysis. PLoS ONE, 10(6), 0128600.
4. Kokalis-Burelle, N., Vavrina, C. S., Reddy, M. S., Kloepper, J. W. (2003). Amendment of Muskmelon and Watermelon Transplant Media with Plant Growth-Promoting Rhizobacteria: Effects on Seedling Quality, Disease, and Nematode Resistance. HortTechnology, 13 (3): 476-481.
5. Silva Júnior, Eugênio Gonçalves da, Silva, Anselmo Ferreira da, Lima, Jucelino de Sousa, Silva, Maria de Fátima Caetano da, & Maia, Josemir Moura. (2017). Vegetative development and content of calcium, potassium, and sodium in watermelon under salinity stress on organic substrates. Pesquisa Agropecuária Brasileira, 52(12), 1149-1157.
6. Silva, José E. S. B. da, Matias, Janete R., Guirra, Keylan S., Aragão, Carlos A., Araujo, Gherman G. L. de, & Dantas, Bárbara F. (2015). Development of seedlings of watermelon cv. Crimson Sweet irrigated with biosaline water. Revista Brasileira de Engenharia Agrícola e Ambiental, 19(9), 835-840.
7. State Statistical Office of Republic of Macedonia (2016). Statistical Yearbook. Chapter Agriculture.
8. Yaping, S., Diankui, C. (2005). Influence of Plug Cell Size for Root Vigor and Seedling Quality on Graft Watermelon Seedling. International Conference on Sustainable Greenhouse Systems - Greensys2004. Acta Hort. (ISHS) 691:107-112.
9. Yetisir, H., Sari, N. (2003). Effect of different rootstock on plant growth, yield and quality of watermelon. Australian Journal of Experimental Agriculture, 43, 1269–1274.

IN VITRO EVALUATION OF THE EFFECTS OF ESSENTIAL OILS ON *ASCOSPHAERA APIS*, THE CAUSATIVE AGENT OF CHALKBROOD DISEASE

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Abstract

Honeybee diseases cause considerable expenses to beekeepers for cost of maintaining apiary's inspection, colonies damaged or destroyed and drugs fed to prevent bee's infections. One of them is *Ascosphaera apis,* a fungal pathogen causing Chalkbrood disease in honey bee larvae. Chalkbrood is most frequent during damp conditions. Infected larvae turn chalky white color, become hard, and then turn black. It can be regarded as "the most widespread infectious disease" and this has led to economic loss in apiculture. We aim to find an alternative approach by using essential oils from medicinal plants in combating and controlling the disease.Therefore, current article compiled the effects of ten essential oils (citral, geraniol, citronelol, cinamaldehid, thymol, eucalyptus oil, lemongrass oil, fennel oil, clove oil and cedar oil) on *Ascosphaera apis* in the laboratory with an agar diffusion method and the method of serial dilutions in microdilution plate. *A. apis* strains were isolated from the dead honey bee larvae and the fungal strains were identified using a light microscopy. Various concentrations (0.08% - 10%) of the essential oils were experimented for determination of minimal fungistatic and also fungicide concentration. After incubation, the zones of inhibition were determined with a magnifying lens. The minimal inhibitory concentration was in range of 0.3125% - 1.25% for tested essential oils (MIC- values), and the minimal fungicide concentration was in range of 1.25% - 5%. All tested substances shows *in vitro* fungistatic and fungicide potential and can be considered as potential alternative active agents for prevention and control of chalkbrood disease without the use of antibiotics.

Keywords: fungus, pathogen, fungicide inhibitory concentration, agar diffusion test, method of serial dilutions, honeybees.

Introduction

Insect pathogenic fungi can be found throughout the fungal kingdom (Humber, 2008), all being capable of invading their hosts and overcoming their immune systems. Chalkbrood of honeybees (*Apis mellifera*) is a fungal disease caused by the spore-forming ascomycete fungus *Ascosphaera apis* (Spiltoir, 1955) affecting the honeybee larvae, producing severe damages in apiarian producers. The bee larvae ingest spores of *A. apis* with the food. The spores germinate in the lumen of the gut, probably activated by CO_2 from tissue (Heath and Gaze, 1987). It only infects larvae that are three to four days old, which are most susceptible to the fungus, especially if these are chilled after ingesting spores of *A. apis*. The larvae mostly die in the L5 developmental stage. Worker, drone and queen larvae all are susceptible to chalkbrood disease. At first, dead larvae inside recently capped cells are covered by a fluffy white mold, and later on these dry and become black or white mummies, and at the peak of the disease, mummies are easily detected at the entrance to the hive as nurse bees remove them from their cell. This color variation is due to the presence or absence of the black fruiting bodies that are formed on the outside of the larvae. The disease may become severe in some colonies, but is not expected to be a serious problem for beekeepers. The fungus causing chalkbrood in honey bees has a narrow host range and a unique infection route, it relies solely on sexual reproduction and has many host-specific adaptations. The field diagnosis of chalkbrood is based on visual detection of diseased, mummified brood, commonly known as "chalkbrood

mummies". Chalkbrood can reduce colony productivity by lowering the number of newly emerged bees, and in some cases may lead to colony losses (Jensen et al., 2013). The disease is found infecting honey bee brood in most regions of the world, including warm and dry climates. Clinical symptoms of chalkbrood often appear for only a short time, typically under cold and damp weather conditions (Aronstein and Murray, 2010). The chalkbrood spores are very resistant to environmental conditions and can remain viable and infective for more than 15 years. Treatments for the disease involve a variety of chemicals, which have been applied in a continuous and excessive way, so the fungus may develop resistance to antibiotics, besides generating residues in honey, thus affecting quality and commercialization. Therefore, during the last years it has been appealed to natural substances, such as essential oils, to treat infected beehives (Colin et al., 1989; Floris and Carta, 1990). Essential oils are the result of a vapour hydrodistillation of plant species, which are thus separated because of being immiscible in water. They are complex mixtures in whose composition there are mainly terpenic compounds, bencenic compounds, and phenols, which are being continuously studied, e.g., as natural biocide agents. In this work *in vitro* behaviour of ten essential oils has been studied against *Ascosphaera apis*, the causative agent of chalkbrood disease.

Material and methods

Chemicals

Citral, geraniol, citronelol, cinamaldehid, thymol, eucalyptus oil, lemongrass oil, fennel oil, clove oil and cedar oil were derived from the commercial chemical company Sigma-Aldrich®.

Preparation of the inoculum

Isolation and cultivation of *A. apis* was conducted by use of a culture. Three chalkbrood-infected, white and dark spore bearing larvae (freshly removed from the colony) were crushed and suspended in 5mL of distilled water. The suspension was filtrated using a membrane filter to separate larger larval particles. The suspension (10 µL) was then transferred onto Sabouraud Dextrose Agar (SDA) plates by three sector streaks and incubated for 48 hours at 30°C (Borchert, 1974). Identification of *A. apis* was conducted by light microscopy, and by comparison with Aronstein and Murray (2010). Five single colonies were removed from the agar plate and suspended in 2 mL 0.9% NaCl followed by 30 sec in a Vortex-mixing device. The confirmation of spore density was carried out with a hemocytometer in a dilution of 1:100. The start inoculum was $5x10^6$ colony forming units (CFU) per mL.

Agar diffusion test

The antifungal oil activity was determined using agar diffusion method with paper disks. The agar diffusion test was conducted with four concentrations of essential oils (0.5%, 1.25%, 5.0%, and 7.5%) in three replicates. The essential oils were diluted in 50% sugar syrup (w/w). The start inoculum (200 µL) was spread area-wide onto the Sabouraud Dextrose Agar. Each concentration of test substance (20 µL) was pipetted onto filter paper (ø 0.8 cm); the negative control papers were treated with a 50% sugar syrup; the positive control papers were treated with a Chlorimidazole (1 mg/ml). Incubation took place at 30°C in an incubator. After 48 hours, the zones of inhibition were determined with a magnifying lens. All experiments were carried out in triplicate.

Microdilution method

Also, the antifungal activities of the essential oils were assessed using a modified version of the microdilution techniques described by Drummond and Waigh (2000). The antifungal assay was performed by using a sterile 96-well plate and the Minimal Inhibitory Concentration (MIC) and the Minimal Fungicidal Concentration (MFC) values were determined. All the assays were prepared using aseptic conditions. The growth of *A.apis* was inspected visually. Sterile Sabouraud Dextrose Broth (SDB) was used as a growth medium in the assay. A positive and a negative control were used in each plate in order to ascertain the viability of the fungal culture and the sterility of the working

conditions and solutions. Each essential oil was subjected to serial dilutions in descending concentrations starting from a concentration of 10% and finishing with a concentration of 0.08%. The microtiter plates were wrapped in sterile tinfoil in order to prevent contamination and then were incubated at 30°C for 7 days. The assays were inspected visually: a clear solution indicated absence of growth, while visual indication of mycelia indicated microbial growth or absence of inhibition. The MIC was determined to be the lowest concentration of essential oils that resulted in the inhibition of the visible growth of *A. apis* after a seven days incubation, compared with the growth control. All the essential oils that demonstrated inhibitory activities were further tested for fungicidal activity. Namely, a sample from each well that tested positive for inhibitory activity was inoculated on fresh sterile Sabouraud Dextrose Agar plates and incubated an additional 5-7 days. Absence of colonies/mycelia was regarded as positive for fungicidal activity, while growth of colonies/mycelia was regarded as negative. All the tests were performed in triplicate.

Results and discussion

Agar diffusion test

The lack of any effective control agent for chalkbrood has resulted in an increased interest in the investigation of alternative control strategies. Essential oils and oleoresins of many plants are known to exhibit significant antimicrobial activity against a wide spectrum of microorganisms. Out of the ten essential oils tested, all exhibited antifungal activity against *A. apis*, with zone of inhibition (ZI) ranging from 6.3 mm to 0.3 mm (Table 1). All essential oils proved to have a fungistatic effect (Table 1). The minimal inhibitory concentration of all tested essential oils were 1.25%. These were the lowest concentrations showing a repression of fungi growth. Higher concentrations of tested essential oils dried faster and had insufficient time in the agar to inhibit the growth of *A. apis*. This effect could be the explanation for the smaller inhibitory areas at higher concentrations of all essential oils in the experiment. The zone of inhibition produced by the Chlorimidazole was 8.0 mm, which was little more than to the zone of inhibition produced by citronelol (6.3 mm). Overall, this study shows that citronelol has the best results against *A. apis* with zone of inhibition of 6.3±0.8 mm (1.25 % of citronelol).

Table 1. Antifungal activity (inhibition zone, mm) of essential oils against *A. apis* by the disc diffusion method. Data are presented as mean±SD.

concentration [%]	essential oils									
	citral	geraniol	citronelol	cinamaldehid	thymol	eucalyptus oil	lemongrass oil	fennel oil	clove oil	cedar oil
0.5	2.2±0.2	1.9±0.3	3.2±0.5	0.9±0.1	3.6±0.6	3.0±0.8	4.1±0.7	0.7±0.2	2.9±0.3	3.1±0.6
1.25	5.9±0.6	4.6±0.4	6.3±0.8	2.8±0.6	6.1±0.8	5.7±0.6	6.0±0.3	2.7±0.6	5.5±0.5	5.9±0.7
5	3.4±0.4	3.0±0.8	4.2±0.9	1.1±0.2	3.2±0.7	3.5±0.6	4.2±0.4	1.1±0.3	3.2±0.8	3.0±0.6
7.5	2.0±0.8	2.1±0.7	3.2±0.5	0.5±0.2	2.5±0.4	2.1±0.6	3.3±0.5	0.3±0.1	2.1±0.7	2.4±0.8
Negative control (50% sugar syrup)	0.0±0.0									
Positive control (Chlorimidazole 1 mg/ml)	8.0±1.4									

Clove oil has been found to effectively inhibit the growth of many Gram-negative bacteria, several species of *Penicillium* (Azzouz and Bullerman, 1982), as well as the spore-forming *Clostridium botulinum* (Ismaiel and Pierson, 1990). Cinamaldehid exhibits activity against mycotoxigenic moulds, *Penicillium* spp. and *C. botulinum*. Thymol, a major component of thyme oil, is highly active against *Aspergillus parasiticus* (Buchanan and Shepherd, 1981), *Staphylococcus aureus* (Karapinar and Aktug,

1987) and *C. botulinum*. The literature cites references which have investigated the susceptibility of a broad range of fungi to natural products, including citral in lemon peel (Rodov *et al.,* 1995), sorbic acid and essential oils (Kubo and Lee, 1998).

Microdilution method

Recently, the scientific interest into biological properties of essential oils and natural products in general has been increased as a series of molecules with antimicrobial activity have been found in plants. Active research on the use of the biologically active secondary metabolites present in essential oils of plants such as phenols, flavonoids, alkaloids, terpenes, tannins and others, has been seen as a potential alternative to the conventionally used antifungal agents, and as means to control pathogenic fungi and fungal contamination. The response of different essential oils usually depends on the fungal species tested and may include ranges from resistant to various degrees of susceptibility (Amini et al., 2012). *In vitro* antimicrobial activity was investigated by the microdilution method and MIC and MFC was determined. Each essential oil was subjected to serial dilutions in descending concentrations starting from a concentration of 10% and finishing with a concentration of 0.08%. The data relating to these activity is summarized in Table 2. Antimicrobial activity (MIC and MFC) was observed in all essential oils included in the study. Our findings showed that citronelol has the best resultst against A. apis, with MIC of 0.3125% and MFC of 1.25%, following with thymol, with MIC of 0.625% and MFC of 1.25%.

Table 2. The antifungal assay performed by using a sterile 96-well plate and the Minimal Inhibitory Concentration (MIC) (concentration, %) and the Minimal Fungicidal Concentration (MFC) (concentration, %) of ten essential oils tested in this study.

essential oils	MICs (%)	MFCs (%)
citral	0.625	2.5
geraniol	1.25	2.5
citronelol	0.3125	1.25
cinamaldehid	1.25	5
thymol	0.625	1.25
eucalyptus oil	0.625	0.5
lemongrass oil	0.625	2.5
fennel oil	1.25	5
clove oil	1.25	2.5
cedar oil	0.625	2.5
Negative control (50% sugar syrup)	0	0
Positive control (Chlorimidazole 1 mg/ml)	0.5	1

Since ancient times, essential oils have been used for domestic and therapeutic purposes; these oils possess broad-spectrum antimicrobial properties. Essential oils have been selectively used to treat various microbial infections (Deans, 1991; Hammer et al., 1998; Hili et al., 1997). The antimicrobial properties of essential oils suggest that these substances could be used to control *A. apis*. Recently, a few studies have demonstrated the activity of essential oils against honeybee pathogens. Natural antibiotics based on essential oils may represent alternatives to chemically synthesized antibiotics. It is important to control honeybee diseases with natural antibiotics because most honeybee by-products, such as honey, must be free from contaminants. We studied the *in vitro* efficacy of ten essential oils against *A. apis.* Out of the ten selected essential oils, all were found to be effective. Our findings demonstrate that these essential oils might not only have the potential to control *A. apis* infection but may also represent possible alternatives to the use of control Chalkbrood. Our data (Table 2) clearly demonstrate that all of the essential oils used inhibited the growth of *A. apis*, which

is in contrast to the results o earlier studies on essential oils by Colin et al., 1989; Calderone et al., 1994; Larran et al., 2001.

Conclusions

All tested substances shows *in vitro* fungistatic and fungicide potential and can be considered as potential alternative active agents for prevention and control of chalkbrood disease without the use of antibiotics. In conclusion, the results presented in this paper demonstrate that most of the selected essential oils have potential antifungal activities. The use of non-toxic natural compounds could represent a natural alternative to the use of synthetic antibiotics in the control of Chalkbrood, which should therefore reduce antibiotic resistance and the levels of antibiotic residues. Further research must be conducted on these essential oils to isolate the active ingredients that kill *A. apis*.

References

1. Amini, M., Safaie, N., Salmani, M. J., Shams – Bakhsh, M. (2012). Antifungal Activity of Three Medicinal Plant Essential Oils Against Some Phytopathogeic Fungi. Trakia Journal of Sciences, 10 (1): 1–8.
2. Aronstein, K., Murray, P. (2010). Chalkbrood disease in honey bees. Journal of Invertebrate Pathology, 103(Supplement): 20-29.
3. Azzouz, M.A. and Bullerman, L.B. (1982). Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. Journal of Food Protection, 45:1298-1301.
4. Buchanan, R.L. and Shepherd, A.J. (1981). Inhibition of *Aspergillus parasiticus* by thymol. Journal of Food Science, 46:976-977.
5. Calderone, N.W., Shimanuki, H., Allen-Wardell, G. (1994). An *in vitro* evaluation of botanical compounds for the control of the honeybee pathogens *Bacillus larvae* and *Ascosphaera apis*, and the secondary invader *B. alvei*. Journal of Essential Oil Research, 6: 279–287.
6. Colin, M.E., Ducos de Lahitte, J., Larribau, E., Boue, T. (1989). Activity of essential oils of *Lamiaceae* on *Ascosphaera apis* and treatment of an apiary. Apidologie, 20: 221-228.
7. Deans, S.G. (1991). Evaluation of antimicrobial activity of essential (volatile) oils. In Linskens, H.F., Jackson, J.F. (Eds.), Modern Methods of Plant Analysis, Essential Oils and Waxes: Springer-Verlag, Berlin, 310–320.
8. Drummond, A.J. and Waigh, R.D. (2000). Recent Research Development. Phytochemistry, 4:143–152.
9. Floris, I. and Carta, C. (1990). *In vivo* activity of *Cinnamomum zeylanicum* Nees essential oil against *Bacillus larvae* White. Apicoltura, 6: 57-61.
10. Hammer, K.A., Carson, C.F., Riley, T.V. (1998). *In vitro* activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products, against *Candida* spp. Journal of Antimicrobial Chemotherapy, 42: 591–595.
11. Heath, L. A. F. and Gaze, B. M. (1987). Carbon dioxide activation of spores of the chalkbrood fungus *Ascospheara apis*. Journal of Apicultural Research, 26(4): 243-246.
12. Hili, P., Evans, C.S., Veness, R.G. (1997). Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. Letters of Applied Microbiology, 24: 269–275
13. Humber, R.A. (2008). Evolution of entomopathogenicity in fungi. Journal of Invertebrate Pathology, 98(3): 262-266.
14. Ismaiel, A. and Pierson, M.D. (1990). Inhibition of germination, outgrowth and vegetative growth of *Clostridium botulinum* 67B by spice oils. Journal of Food Protection, 53:755-758.
15. Jensen, A. B., Aronstein, K., Flores, M. F., Vojvodic, S., Palacio, M. A., Spivak, M. (2013). Standard methods for fungal brood disease research. Journal of Apicultural Research, 52(1): 1-20.
16. Karapinar, M. and Aktug, S.E. (1987). Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethone. International Journal of Food Microbiology, 4:161-166.

17. Kubo, I. and Lee, S.H. (1998). Potentiation of antifungal activity of sorbic acid. Journal of Agricultural and Food Chemistry, 46(10):4052-4055.
18. Larran, S., Monaco, C., Alippi, H. (2001). Endophytic fungi in leaves of *Lycopersicon esculentum* Mill. World Journal of Microbiology and Biotechnology, 17: 181–184.
19. Rodov, V., Ben-Yehoshua, S., Fang, D.Q., Kim, J.J., Ashkenazi, R. (1995). Preformed antifungal compounds of lemon fruit: Citral and its relation to disease resistance. Journal of Agricultural and Food Chemistry, 43:1057-1061.
20. Spiltoir, C. F. (1955). Life Cycle of *Ascosphaera apis* (Pericystis apis). American Journal of Botany, 42(6): 501-508.

CHARACTERISTICS OF *ERWINIA AMYLOVORA* STRAINS ISOLATED FROM ORNAMENTAL SHRUBS

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Abstract
This paper presents the characteristics of *Erwinia amylovora* strains isolated from *Cotoneaster horizontalis* and *Pyracantha coccinea* on the territory of Nis District in Serbia, as well as biochemical and physiological characteristics of these strains. Identification of *Erwinia amylovora* strains was made according to internationally recognized methods, such as: Biolog test, immunofluorescence, ELISA test and agglutination test, and biochemical and physiological characteristics of the isolates of *Erwinia amylovora* were determined by levan production, oxidase and catalase activity, hydrolysis of gelatin and esculin, VP - test, MR - test, and glucose metabolism. Characteristics of the isolated strains fully coincide with the control strains which were isolated from pome fruits.

Key words: *Erwinia amylovora*, fire blight, *Cotoneaster horizontalis*, *Pyracantha coccinea*.

Introduction

Erwinia amylovora is the causative agent of fire blight of pome fruit trees and other rosaceous plants. The most important bacteria hosts in Serbia are: pear, apple, medlar and quince, and other plants of the species *Sorbus* (Vojinović 2006, Gavrilović et al. 2008) *Cotoneaster* (Balaz et al. 2004), *Crategus* (Vojinović et al. 2009), *Pyracantha*, *Chaenomeless* and *Photinia*. This bacterium is on the international list of quarantine organisms (34 Bulettin OEPP / EPPO, 2008). It is also on the A2 list of quarantine harmful organisms in Serbia, and it seriously affects the production of pome fruits in each country where it is registered (Gavrilović et al., 2009). A number of ornamental shrubs have also been reported as important hosts of the bacterium *Erwinia amylovora* (Sherald 2007). Since ornamental plants from the species *Pyracantha* and *Cotonaster* are grown in many parks in city centers, the examination of characteristics of *Erwinia amylovora* strains isolated from these plants is very important. *Cotoneaster horizontalis* and *Pyracantha coccinea* have an important place in the flora of parks. Identification test done in a plant pathology laboratory indicates the existence of this bacterium in ornamental shrubs (Bastas et al. 2012).

Material and methods

Identification of *Erwinia amylovora* was made according to internationally recognized methods, such as: Biolog test, immunofluorescence, ELISA test and agglutination test.

Biolog TEST

The Biolog GP2 MicroPlate is designed for identification and characterization of a very wide range of aerobic gram-positive bacteria (Vojinović 2010). The Biolog GP2 MicroPlate performs 95 discrete tests simultaneously and gives a characteristic reaction pattern called a “metabolic fingerprint”. These fingerprint reaction patterns provide a vast amount of information conveniently contained in a single Biolog MicroPlate. The metabolic fingerprint patterns are compared and identified using the MicroLog™ database software (Brennan et al. 2002). The procedure is fast and simple, involving only 5 steps, and requiring only 2 to 3 minutes hands-on time per sample (Brennan et al. 2002).

1) A pure culture of a bacterium was grown on a Biolog Universal Growth agar plate (Biolog catalog No 70101 for a 500 g jar of dehydrated powder.) Bacillus spp. was grown on a Biolog Universal Growth agar plate with 25% Maltose.

2) The bacteria was swabbed from the surface of the agar plate, and suspended to a specified density in GN/GP Inoculating Fluid (Biolog catalog No 72101).
3) 150 µl of bacterial suspension was pipetted into each well of the GP2 MicroPlate (Biolog catalog No 1014).
4) The MicroPlate was incubated at 30°C or 35 °C (depending upon the nature of the organism) for 4-24 hours.
5) The MicroPlates was read either visually or with the Biolog MicroStation or OmniLog System and compared to the GP Database (Biolog catalog No 22404A) and a result is displayed.

Enrichment DASI-ELISA
The only commercial kit for Enrichment DASI-ELISA has been validated in the ring tests. It is based on the monoclonal antibodies and technique described by Gorris et al. 2005. As positive controls, use aliquots of a sample extract that previously gave a negative result on testing were used, mixed with 108 cells of *Erwinia amylovora* per mL. As negative controls, include a sample extract that has previously given a negative result for E. amylovora and a suspension of a non-*Erwinia amylovora* strain in PBS were used. The necessary amount of the enriched extracts and controls was boil in a water bath (or in a thermoblock) at 100° for 10 min before ELISA, making sure that the tubes are not opened. The remaining enriched samples for isolation and /or PCR were keep. The boiled samples was process to ELISA (once at room temperature) on the same day or they were freeze at −20 °C for subsequent analysis. This heat treatment is necessary for optimum sensitivity and specificity using the monoclonal antibodies obtained by Gorris et al. (1996a). The appropriate dilution of rabbit anti-E. amylovora polyclonal immunoglobulins in carbonate buffer, pH 9.6. was prepared 200 µL to each well of a Nunc Polysorp (or equivalent) ELISA plate was added. Incubate at 37 °C for 4 h or at 4 °C for 16 h.was perfomed. The wels were washed three times with washing buffer. 200 µL per well of the plant macerates previously enriched in the two media and boiled was added. Two wells per sample enriched on each medium, and two of the positive and negative controls were used. The negative controls of the extraction buffer and of the enrichment media were used (previously prepared as additional negative controls of the enrichment). Incubation for 16 h at 4 °C was the step. The wells were next washed three times with washing buffer (as above). The appropriate dilution of specific *Erwinia amylovora* monoclonal antibodies was prepared in PBS plus 0.5% bovine serum albumin (BSA) and added 200 µL to each well. Incubate was at 37 °C for 2 h. The wells were washed three times with PBS-Tween. The appropriate dilution of goat antimouse immunoglobulins conjugated with alkaline phosphatase in PBS was prepared. 200 µL to each well was added. Incubate was at 37 °C for 2 h. The wells were washwed three times as above. 1 mg mL−1168 Erwinia amylovora (Bulletin OEPP/EPPO, 2008) alkaline phosphatase substrate (p-nitrophenylphosphate) in substrate buffer was prepared. 200 µL to each well was added. Incubation was at room temperature and reading was at 405 nm after 30, 45, and 60 min. The ELISA test was negative if the average optical density (OD) reading from duplicate sample wells was < 2x OD of that in the negative sample extract control wells (provided the OD for the positive controls were above 1.0 after 60 min incubation and were greater than twice the OD obtained for negative sample extracts). The ELISA test was positive if the average OD readings from duplicate sample wells was > 2x OD in the negative sample extract wells provided that 2x average OD readings in all negative control wells were lower those in the positive control wells. Negative ELISA readings in positive control wells indicate that the test has not been performed correctly and/or the reagents were not well prepared. Positive ELISA readings in negative control wells indicate cross-contaminations or non-specific antibody binding has occurred. In either case, the test should be repeated or a second test based on a different biological principle should be performed.

Immunofluorescence (IF)
For this test it is necessary to use a validated source of antibodies to *Erwinia amylovora* (Ivanović et al. 2012). Three commercial antibodies have been validated in the ring test. It is recommended that

the titre and dilution of use are determined for each new batch of antibodies (Ivanović et al. 2012). The IF test should be performed on freshly prepared sample extracts. If the extracts stored at −80 °C under glycerol are used, remove glycerol it by adding 1 mL of PBS, centrifuge for 15 min at 7000 g, discard supernatant and resuspend in PBS (Jerffreger 2012). For each set of tests, prepare a positive control slide using a suspension of 106 cells mL^{-1} of a known pure culture of *Erwinia amylovora* (Jerffreger 2012). For large-scale survey work, it is recommended to include blind positive control slides (Ivanović et al. 2012). Use PBS and an aliquot of a sample extract, which was negative by several techniques, as negative controls. Use undiluted macerates and 1 : 10 and 1 : 100 dilutions in PBS to spot windows of the IF slides. Prepare one slide for each sample and its dilutions. Allow to air-dry and fix by flaming or by absolute or 95° ethanol according to the characteristics of the antibodies used. Store slides at −20 °C until required. Use the monoclonal or polyclonal antibodies at the appropriate dilutions in PBS. Spot 25 –30 µL per well. Incubate slides in a moist chamber for 30 min at room temperature. Use of two dilutions of the antibodies is advised when working with polyclonal antibodies, to detect cross reactions with other bacteria. Shake droplets off the slide and rinse slides carefully with PBS. Wash 10 min with the same buffer. Carefully remove excess moisture. Dilute the appropriate FITC conjugates in PBS: antimouse for the monoclonal antibodies (GAM-FITC) and antirabbit (GAR-FITC) or antigoat. Cover the windows of all slides with the corresponding diluted conjugate and incubate in a moist chamber for 30 min at room temperature. Repeat the washing step. Pipette 5 –10 µL 0.1 m phosphate-buffered glycerol mountant with antifading (0.5% phenylenediamine or other) on each window and apply a cover slip. View slides under oil immersion at 500 –1000× magnification by scanning windows across 2 diameters at right angles and around the perimeters. Calculate the number of cells per mL = l of the sample, according to EU (1998). The test is negative if green fluorescing cells with morphology typical of *Erwinia amylovora* are observed in positive controls but not in sample windows. The test is positive if green fluorescing cells with typical morphology are observed in positive control and sample windows, but not in negative control windows. As a population of 103 cells per mL is considered the limit of reliable detection by the IF test, for samples with > 103 cells per mL, the IF test is considered positive. For samples with < 103 cells per ml, the result of the IF test may be considered doubtful. In such a case, further testing or re-sampling should be performed. Samples with large numbers of incomplete or weakly fluorescing cells compared to the positive control need further testing, with different dilutions of antibody or pellet or a second source of antibodies.

Agglutination test

Suspected *Erwinia amylovora* colonies levan-positive, non fluorescent in King's B medium, can be tested for slide agglutination mixing them in a drop of PBS with a drop of *Erwinia amylovora* specific antiserum (not diluted or only at 5 or 10 fold dilution) on a slide (Gavrilović et al. 2007).

Biochemical and physiological characteristics of the isolates of Erwinia amylovora were determined using the following parameters:

Production of levan on a medium supplemented with sucrose (NAS)

Levan is a complex polysaccharide macromolecule which is produced by sucrose metabolism. Bacteria that produce levan on media supplemented with sucrose form very prominent and large colonies, which are shiny, smooth and slimy (levan type) (Gavrilović et al. 2008). A mesopeptone medium supplemented with 5% sucrose was used for the production of levan (Vojinović et al., 2008).

Gelatin hydrolysis

The nutrient medium of peptone, yeast extract and gelatin was dispensed (5 ml) into test tubes (16 x 160 mm) and sterilized in an autoclave at 121^{o}C for 15 minutes. Three test tubes with the medium were inoculated with each isolate. The results were read at 3, 7, 14 and 21 day. Before reading, the test tubes were placed in a refrigerator at 4^{o}C for 30 minutes. If there is no hardening of the medium after this period, the bacteria break down gelatin.

Esculin hydrolysis
The corresponding medium was angled after sterilization and after solidification it was inoculated with the bacteria from 24-hour-old culture. The results were read after 4 days of growth at 27°C. A positive test resulted in a color change of the medium to dark brown.

Glucose metabolism (O / F)
After sterilization and cooling to 50 ° C the filter-sterilized glucose solution was added to Hugh-Leifson medium, to make a final concentration of 1%. Four test tubes were inoculated with each analyzed isolate. In each group half of the test tubes were sealed with sterile paraffin oil to create anaerobic conditions. The results were read after three days of growth in a thermostat at 27°C. A positive test resulted in a color change of the medium to yellow, under aerobic and anaerobic conditions.

Catalase production
The concentration of 20% H_2O_2 solution was used as a test reagent to detect the presence of catalase. A small amount of a well-isolated, 24-hour-old colony was taken with a bacteriological loop and emulsified in a drop of H_2O_2 on a microscopic plate. A positive test resulted in the appearance of gas bubbles and formation of free oxygen.

Oxidase activity
Kovacs method was used (Vojinović et al. 2009). The strips of filter paper were soaked in 1% solution of tetra-methyl-*p*-phenylenediamine-dihydrochloride. A well-isolated colony from the angled medium was taken with a glass rod and streaked on the filter paper strips. Microorganisms are oxidase positive when the color changes to dark purple within 10 to 15 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 30 to 40 seconds.

Methyl red and VP test (acetoin production)
This test is used to detect acetoin that produce some bacteria from the family *Enterobacteriaceae*. The test was performed by adding a few drops of 5% alpha-naphthol solution and a few drops of 40% potassium hydroxide solution to the laboratory-prepared Voges-Proskauer medium (Torlak, Belgrade), and the results were read after the second and the fifth day. If the color turns red within 2 hours, the test is positive. The methyl red test was used as an indicator of the final pH value of the end-products derived from the breakdown of glucose. Methyl red turns red when the pH of the medium is 4.2 or less.

Breeding characteristics of Erwinia amylovora isolates were determined using the following parameters:

Growth at 34°C and 36°C and in a medium containing 5% and 7% NaCl
Growth at 34°C and 36°C was monitored on the medium of yeast extract and mineral salts. The medium was dispensed as 5 ml portions into 16x160 mm test tubes and sterilized by autoclaving at 115°C, the usual treatment being 20 minutes at a pressure of 1.2 atmospheres. Three test tubes with the medium were inoculated with each isolate. After inoculation, test tubes with the medium were incubated in a water bath that maintained the proper temperature for 14 days. If the medium becomes turbid, the bacteria will grow at the mentioned temperature. In order to study the growth of *Erwinia amylovora* in the presence of 5% and 7% NaCl, test tubes with the inoculated medium were maintained in a thermostat at 27° C for 14 days. A positive test resulted in turbidity of the culture medium. Non-inoculated media were used as controls.

Results and discussion

A total of 10 *Erwinia amylovora* strains were analyzed in this study. They were isolated from two different host plants in various geographic regions. Biolog test is a very reliable and simple method for the identification of *Erwinia amylovora* (Vojinović 2010). This test results showed that all strains analyzed were affiliated with the bacterium *Erwinia amylovora*, and similarity index ranged from 0,65 to 0,93. Different types of carbon dispensed into the microwells, in reaction with the bacterial suspension produce a color change in microwells to purple due to redox reactions. All analyzed strains metabolize 17 to 23 carbon compounds: n-acetyl-d-glucosamine, d-fructose, d-galactose, gentiobiose, α-d-glucose, m-inositol, d-mannitol, d-mannose, β-methyl-d-glucoside, d-psicose, d-sorbitol, sucrose, d-trehalose, succinic acid monomethyl ester, d-gluconic acid, succinic acid, bromosuccinic acid, l-aspartic acid, l-glutamic-acid, inosine, glycerol, α-d-glucose-1-phosphate, d-glucose-6-phosphate. The strains isolated from *Cotoneaster horizontalis* and *Pyracantha coccinea* react positively with the corresponding serum in ELISA test, they are positive in immunofluorescence tests and they cause agglutination reaction (Table 1).

Table 1. Methods for the identification of Erwinia amylovora

Strain	Elisa test	IF test	Agglutination test	Biolog test similarity index
Pc-1	+	+	+	0,72
Pc -2	+	+	+	0,93
Pc -3	+	+	+	0,91
Pc -4	+	+	+	0,67
Pc -5	+	+	+	0,87
Ch-1	+	+	+	0,65
Ch 2	+	+	+	0,75
Ch -3	+	+	+	0,72
Ch -4	+	+	+	0,73
Ch -5	+	+	+	0,65
Ap-55 control	+	+	+	0,91

These strains produce levan, they are catalase positive, they hydrolyze gelatin and produce acetoin. They showed a negative reaction for oxidase production, esculin hydrolysis and methyl red test. All analyzed strains metabolize glucose under aerobic and anaerobic conditions (O / F test), as shown in Table 2.

Table 2. Biochemical and physiological characteristics of the strains of Erwinia amylovora

Strain	Levan production	Gelatin hidrolysis	Esculin hidrolysis	Glucose metabolism		Catalase activity	Oxidase activity	VP test	Methyl red test
				O	F				
Pc-1	+	+	-	+	+	+	-	+	-
Pc -2	+	+	-	+	+	+	-	+	-
Pc -3	+	+	-	+	+	+	-	+	-
Pc -4	+	+	-	+	+	+	-	+	-
Pc -5	+	+	-	+	+	+	-	+	-
Ch-1	+	+	-	+	+	+	-	+	-
Ch 2	+	+	-	+	+	+	-	+	-
Ch -3	+	+	-	+	+	+	-	+	-
Ch -4	+	+	-	+	+	+	-	+	-
Ch -5	+	+	-	+	+	+	-	+	-
Ap-55 control	+	+	-	+	+	+	-	+	-

Strains used in this study produce acids from the following carbohydrates: fructose, glucose, ribose, sucrose, sorbitol, mannitol and glycerol. They showed a negative result for dulcitol and inositol (Table 3 and 4).

Table 3. Breaking down of monosaccharides and oligosaccharides from the analyzed strains of *Erwinia amylovora*

Strain	Fructose	Glucose	Ribose	Sucrose
Pc-1	+	+	+	+
Pc -2	+	+	+	+
Pc -3	+	+	+	+
Pc -4	+	+	+	+
Pc -5	+	+	+	+
Ch-1	+	+	+	+
Ch 2	+	+	+	+
Ch -3	+	+	+	+
Ch -4	+	+	+	+
Ch -5	+	+	+	+
Ap-55 control	+	+	+	+

Table 4. Metabolism of polyhydric alcohols and polysaccharides

Strain	Sorbitol	Manitol	Inositol	Glycerol	Dulcitol
Pc-1	+	+	-	+	-
Pc -2	+	+	-	+	-
Pc -3	+	+	-	+	-
Pc -4	+	+	-	+	-
Pc -5	+	+	-	+	-
Ch-1	+	+	-	+	-
Ch 2	+	+	-	+	-
Ch -3	+	+	-	+	-
Ch -4	+	+	-	+	-
Ch -5	+	+	-	+	-
Ap-55 control	+	+	-	+	-

All *Erwinia amylovora* isolates inoculated in a liquid medium grow at 34^{o}C, but not at 36^{o}C. They grow in a liquid medium with 5% NaCl, but not in a medium with 7% NaCl. A positive test resulted in turbidity of the culture medium in test tubes (Table 5).

Table 5. Growth at a 34 ^{o}C and 36 ^{o}C and in a medium containing 5% and7% NaCl

Strain	Growth at a temperature of		Containing NaCl	
	34^{o}C	36^{o}C	5%	7%
Pc-1	+	-	+	-
Pc -2	+	-	+	-
Pc -3	+	-	+	-
Pc -4	+	-	+	-
Pc -5	+	-	+	-
Ch-1	+	-	+	-
Ch 2	+	-	+	-
Ch -3	+	-	+	-
Ch -4	+	-	+	-
Ch -5	+	-	+	-
Ap-55 control	+	-	+	-

Characteristics of *Erwinia amylovora* strains isolated from *Cotoneaster horizontalis* and *Pyracantha coccinea* on the territory of Nis District fully coincide with the control strains and data from the literature (Brennan et al., 2002, Schaad et al., 2002, Beer, 2005, Triplett et al., 2006, Postinikova et al., 2008, Smits et al., 2010, Vojinović 2010, Balaž et.al 2012, Bastas 2012, Bastas et al. 2012, Ivanović et al.2012.).

Conclusions

Erwinia amylovora, the causative agent of fire blight, has been experimentally detected up to now in almost all major areas of the Republic of Serbia where pome fruits and ornamental shrubs are cultivated. In Nis district, it is detected on apple, pear, quince, medlar, rowan, *Cotoneaster horizontalis* and *Pyracantha coccinea*. Characteristics of the isolated strains fully coincide with the control strains and with the strains already detected. Identification of *Erwinia amylovora* strains was made according to internationally recognized methods, such as: Biolog test, immunofluorescence, ELISA test and agglutination test. Biochemical and physiological characteristics of the isolates of *Erwinia amylovora* were determined by levan production, oxidase and catalase activity, hydrolysis of gelatin and esculin, VP - test, MR - test, and glucose metabolism. Breeding characteristics were determined with the growth of *Erwinia amylovora* at 34^{o}C and 36^{o}C and in a medium containing 5% and 7% NaCl. Characteristics of *Erwinia amylovora* strains isolated from *Cotoneaster horizontalis* and *Pyracantha coccinea* on the territory of Nis District fully coincide with the control strains and data from the literature. Any possible differences could be determined by the use of new methods which are not available at present.

References

1. Balaž J, Radunovic D, Krstic M, 2012. Status of Erwinia amylovora in Montenegro. In: Proceedings of the International Symposium on Current Trends in Plant Protection, Belgrade, Serbia, 25-28th September, 2012 [ed. by Marisavljevic, D.]. Belgrade, Serbia: Institute for Plant Protection and Environment, 373-378.
2. Balaž J. Knežević T., Smiljanić A., Stojšin V. Chaenomeles japonica and Cotoneaster horizontalis, new hosts of *Erwinia amylovora* in Serbia, 10th international Workshop on Fire Blight Bologna, Italy 5-9 juli 2004. Book of abstraks p. 22.
3. Bastas KK, 2012. First report of Erwinia amylovora on firethorn (Pyracantha coccinea) and mountain ash (Sorbus sp.) in Turkey. Plant Disease, 96(12):1818. http://apsjournals.apsnet.org/loi/pdis
4. Bastas KK, Sahin F, 2012. First report of fire blight disease caused by Erwinia amylovora on rockspray (Cotoneaster horizontalis) in Turkey. Plant Disease, 96(11):1690. http://apsjournals.apsnet.org/loi/pdis
5. Beer SV. Molecular genetics of *Erwinia amylovora* involved in the development of fire blight. FEMS Microbiol Lett 2005, vol. 253: 185–192.
6. Brennan JM, Doohan FM, Egan D, Scanlan H, Hayes D. Characterization and differentiation of Irish *Erwinia amylovora* isolates. J Phytopathol 2002, vol. 150: 414–422.
7. Buletion 34 OEPP/EPPO. Buletion 34, 2008 p. 155-157. Organization Europeene et Mediterranne pour la Protection des Plantes 1, rue Le Notre, Paris, France
8. Gavrilović V., Obradović A., Milijašević S.,Arsenijević M. Vojinović M. Sorbus spp.-a new host of *Erwinia amylovora* in Serbia. 11th International Workshop on Fire Blight. Portland, Oregon, 12-17 August 2007. Program and Abstracts p. 69
9. Gavrilović V., Živković S., Ivanović Ž., Vojinović M. Sorbus domestica and Sorbus torminalis new host *Erwinia amylovora* in Serbia. "Plant protection" 2008, vol 58 (1-4) No.263-266, p. 69-79. Belgrade
10. Gavrilović V., Vojinović M., Gudžić S. The bacteriosis of the fruits in Serbia. Symposium: „Agriculture, lokal development and tourism“ 21-23. 12. 2009. Vrnjačka Banja.

11. Ivanović M., Obradović A., Gašić K., Gerald V. Minsavaqe, Ellen R. Dickstein, Jerffreg B. Jones „Exploring diversity of *Erwinia amylovora* population in Serbia by conventional and automated techniques and detection of new PFGE patterns. – European Journal of Plant Pathology. DOI: 10.1007/s10658-012-9950-3., 2012.
12. Postinikova E., Baldwin C., Whitehouse CA., Sechler A., Schaad NW., Sampath R., Harpin V., Li F., Melton R., Blyn L., Drader J., Hofstadler S., Schneider WL. Identification of bacterial plant pathogens using multilocus polymerase chain reaction/electrospray ionization-mass spectrometry. Phytopathology. 2008 Nov; vol. 98(11) p. 1156-64.
13. Smits THM., Rezzonico F., Kamber T., Blom J., Goesmann A., et al. Complete genome sequence of the fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* spp. Mol Plant-Microbe Interact 2010 vol 23
14. Smits THM., Rezzonico F., Duffy B. Evolutionary insights from *Erwinia amylovora* genomics. J Biotechnol. doi:10.1016/j.jbiotec. 2010.10.075.
15. Schaad N. W., and R. D., Frederick. Real-time PCR and its application for rapid plant disease diagnostics. Canadian Journal of Plant Pathology-Revue Canadienne de Phytopathologie 2002, vol 24(3) p. 250-258.
16. Schaad N. W., et al. Use of portable real-time PCR for same-day on-site field diagnosis of bacterial diseases. Phytopathology 2002, vol 92S(6s) p. 110-111.
17. Sherald J.L. Bacterial leaf scorch of landscape trees; what we know and what we do not know. Arboriculture and Urban Forestry 2007, vol 33(6) p. 376-385.
18. Triplett L., Zhao YF., Sundin GW. Genetic differences among blight-causing *Erwinia* species with differing host specificities identified by suppression subtractive hybridization. Appl Environ Microbiol 2006, vol 72 p. 7359–7364.
19. Vojinović M. Hosts and distribution of *Erwinia amylovora* (Burrill) Winslow et al. In district of Niš. Master of science thesis, Faculty of Agriculture in Kosovska Mitrovica 2006.
20. Vojinović M. Hosts and fundamental characteristics typical spp. of *Erwinia amylovora* in district of Nis. Plant doctor XXXVI, vol 5/2008, p. 329-337.
21. Vojinović M., Gavrilović V. Characterization of *Erwinia amylovora* strains isolated from hawthorn in region of Sjenica. "Plant protection" Vol 60 (3) No. 269, p. 177-186, 2009. Belgrade
22. Vojinović M. Comparative characteristics of *Erwinia amylovora* strains obtained from different hosts, areas and altitudes in Serbia. Phd thesis, Faculty of Agriculture in Kosovska Mitrovica 2010.

PATHOGENIC VARIABILITY OF *ASCOCHYTA LENTIS* IN BULGARIA

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Abstract
Ascochyta blight, caused by *Ascochyta lentis* Vassiljevsky, is an important foliar disease of lentil (*Lens culinaris* L.) with worldwide occurrence and is a serious threat to lentil production. The investigation involved 13 isolates of *Ascochyta lentis*. The pathogen was isolated from naturally infected lentil plants from various locations in Bulgaria, using an agar plate method. Inoculum was also produced by growing isolates on LDA. Ten plants (3 weeks old) of each of the cultivars were sprayed with a freshly prepared spore suspension (10^5 conidia mL-1). Disease symptoms were scored 14 days after inoculation. The virulence of the isolates was determined with the help of 11 lines and cultivars of lentil (ILL 358, ILL 5480, ILL 2429, ILL 5725, ILL 7537, Laird, Ilina, Bella, Nadejda, Naslada, Zornitsa). Based on the reaction of the used genotypes, the investigated isolates of *Asc. lentis* were grouped into seven pathotypes. The observations in the present study revealed a significant variation among the isolates of *Ascochyta lentis* for morphological traits as well as for pathogenicity.

Keywords: Pathotype, virulence, isolates, *Lens culinaris*.

Introduction

Ascochyta blight caused by *Ascochyta lentis* (Vassiljevsky) is an important disease on lentil (*Lens culinaris* L.) of wide distribution, causing serious damages (Nene *et al.,* 1988). The yield losses as a result from the disease vary from 40 to 70 % (Gossen and Morrall, 1983; Kaiser, 1992; Malik, 1983). The use of resistant varieties is considered to be the most cost-effective way for the control of the disease. Some resistant lines have been established (Singh *et al.,* 1982; Iqbal *et al*., 1990; Sugha *et al*.,1991; Erskine *et al*., 1996; Nasir and Bretag, 1996), but the presence of significant pathogenic diversity in the *Asc. lentis* population may lead to its overcoming (Ahmed *et al*.,1996). In their investigation, Kaiser *et al*. (1994) described differences in the cultural variability of *Asc. lentis* isolates from 24 countries. Ahmed and Morrall (1995) reported variation of *Asc. lentis* isolates from Canada, and Ahmed *et al.* (1996) found presence of sexually compatible types in isolates of *Asc. lentis*. Discrepancies were also observed in some lentil lines with regard to their reaction to ascochyta blight (Tay, 1989; Ahmed and Morrall, 1996; Nasir and Bretag, 1996). Any additional information on the variation of the virulence of *Asc. lentis.* can contribute to the development of cultivars with higher and more durable resistance. The aim of this investigation was to determine the pathogen diversity among the Bulgarian *Asc. lentis* isolates.

Material and methods

The pathogen *Asc. lentis.* was isolated from naturally infected lentil plants collected from different locations in Bulgaria. The isolation was carried out on PDA, and then the cultures were purified to monospores. All isolates were examined for pathogenicity. Single-spore lines of representative isolates were maintained on lentil seed extract agar (LSA) (Nasir and Bretag, 1997) at 4°C. Inoculum was also produced by growing isolates on LSA. Inoculated plates were incubated at 21°C and observed for colony color, diameter (mm), number of pycnidia. For quantification of pycnidia, 1 cm^2 was cut at a distance of 1 cm from the center of a well sporulating culture on LSA medium 12 days after incubation. The disc was observed under magnifying lens to count the number of pycnidia. The pathogenicity of the isolates was established by inoculating of 11 lens varieties (ILL 358, ILL 5480, ILL

2429, ILL 5725, ILL 7537, Laird, Ilina, Bella, Nadejda, Naslada, Zornitsa). The seeds of each variety were planted in plastic pots (ø 12 cm) containing soil-and-sand mixture at ratio 1:1. Ten plants of the genotype have been grown in the pots at 18-20° C in the greenhouse. Three weeks after emergence, the plants were sprayed with spore suspension (10^5 conidia mL-1). Plants sprayed with sterile distilled water were used as a check. After inoculation, the plants were placed in a moist chamber at 95-100 % relative humidity and temperature 20°C ± 2°C. After 72 h, they were transferred again to the greenhouse and were sprayed with water twice a day to maintain humidity. Disease symptoms were scored 14 days after inoculation using the following scale (Iqkbal et al. 2006): 1 – no spots; 3 – small spots on the leaves; 5 – many spots on the leaves with or without a halo around them and small spots on the stem; 7 – large spots on the leaves and defoliation, numerous spots on the stem and occurrence of fruit bodies in them; 9 – spots merging and covering the stem, perishing of the plants. The lines with score 1-3 were marked as resistant (R), while those with score above 3 were considered susceptible (S).

Results and discussion

The investigation showed significant differences in the linear growth of the isolates. The radial growth of the colonies of the investigated 13 isolates varied from 53.9 to 88.6 mm 14 days after incubation. The colonies of seven of the isolates were with faster growth rate, and the other six - with lower. Isolates AL 10, AL 5, AL 11, AL 9, AL 3, AL 1 and AL 13, with respective radial growth of the colonies 88.6, 87.3, 82.1, 81.5, 80.1, 77.7 and 75.9 mm were with fast growth rate. The radial growth of the rest of the isolates was less than 75.0 mm. Differences were also observed in the color of the colonies and the density of the pycnidium among the investigated isolates (Table 1). No correlation was found between the growth rate of the colonies and the virulence of the isolates, nor between the strength of sporulation and the virulence of the isolates. The investigation showed that there was variation in the morphological and cultural characteristics of the investigated isolates. Such variation among the *Asc. lentis* isolates has also been found by Kaiser *et al*. (1994) and Ahmed and Morrall (1995). In 1984, Grewal reported that the comparatively rapidly growing isolates with low sporulation were less virulent, while the isolates with slow growth and abundant sporulation were with higher virulence. Our investigation did not confirm these correlations.

Table 1. Cultural variability among the 13 Bulgarian isolates of *Ascochyta lentis*

Izolates	Origin	Radial growth mm	Colony color	pycnidial density /cm^2
AL 1	Selanovtsi	77.7	Brown	61
AL 2	Dobrich	68.9	Dark grey	59
AL 3	Ruse	80.1	Black	85
AL 4	Pordim	67.0	Dark grey	56
AL 5	Pordim	87.3	Grey	69
AL 6	Lipnitsa	73.3	Brown	43
AL 7	Spasovo	56.8	Dark brown	77
AL 8	Dobrich	59.7	Dark brown	82
AL 9	Vardim	81.5	Dark brown	74
AL 10	Brashlen	88.6	Black	68
AL 11	Selanovtsi	82.1	Dark brown	83
AL 12	Vardim	62.2	Black	87
AL 13	Sitovo	75.9	Dark brown	79

The disease reaction of the investigated lentil accessions 14 days after inoculation varied from 1 to 9. All tested isolates caused the symptoms typical of the disease on the susceptible accessions. The data from the investigation showed that none of the used genotypes possessed complex resistance to the involved isolates (Table 2). Cultivars Ilina and Bella demonstrated resistance to eight of the investigated isolates. Cultivar Nadezhda and line ILL 358 showed resistance to seven isolates. Line ILL

5480 and cultivars Zornitsa and Laird exhibited resistant reaction to six isolates. Line ILL 2429 showed resistance to five isolates, while line ILL 5725 was resistant to only one isolate. Line ILL 7537 was susceptible to all tested isolates. According Iqbal et al. (2006), line ILL 358 was resistant to all isolates. In our study, line ILL 358 has resistance to seven isolates. The discrepancy between our results and those of Iqbal et al. (2006) is probably due to the differences between virulence of the pathogen populations in Bulgaria and Pakistan.

Table 2. Variation in the virulence of *Asc. lentis* isolates collected from different locations in Bulgaria[a]

Genotypes	AL1	AL2	AL3	AL4	AL5	AL6	AL7	AL8	AL9	AL10	AL11	AL12	AL13
ILL 358	R	S	R	S	S	R	R	R	S	R	S	S	R
ILL 5480	S	R	R	R	S	S	R	R	R	S	S	S	S
ILL 2429	S	R	R	R	S	S	R	R	S	S	S	S	S
ILL 5725	S	S	R	S	S	S	S	S	S	S	S	S	S
ILL 7537	S	S	S	S	S	S	S	S	S	S	S	S	S
Laird	S	R	R	R	S	S	R	R	S	R	S	S	S
Ilina	R	R	R	R	S	R	R	R	S	S	S	S	R
Bella	R	R	R	R	S	R	R	R	S	S	S	S	R
Nadejda	S	R	R	R	S	S	R	R	S	R	S	S	S
Naslada	R	R	R	R	S	R	S	S	S	R	S	S	R
Zornitsa	R	S	R	S	S	R	R	R	S	S	S	S	R

[a] – Based on a 0- to- 9 scale, where 0 to 3 = resistant (R) and 3.1 to 9 = susceptible (S)

Among the 13 isolates included in the investigation, AL5, AL 9, AL 11 and AL 12 were with the highest virulence. Isolate AL 3 was with lowest virulence, followed by AL 7 and AL 8. Only some of the isolates demonstrated identical reaction to the genotypes involved in this investigation. Based on the reaction of the used genotypes, the investigated *Asc. lentis* isolates were grouped into seven pathotypes (*Pt*) (Table 3). Patotype *1* was with the lowest virulence, it was virulent to only one genotype. The virulence of *Pt* 7 and *Pt* 6 was the highest. Pathotype 7 was virulent to all investigated genotypes, while *Pt* 6 – to ten genotypes. The presence of isolates belonging to *Pt* 7 which overcame the resistance of all investigated genotypes, implies a necessity to search for new sources of resistance.

Table 3. Pathotype groupings of 13 *Ascochyta lentis* isolates based on disease reaction of 11 lentil genotypes

Pathotype	Nomb. of isolates	Differential line or cultivar										
		ILL 358	ILL 5480	ILL 2429	ILL 5725	ILL 7537	Laird	Ilina	Bella	Nadejda	Naslada	Zornitza
1	1	**R**	**R**	**R**	**R**	S	**R**	**R**	**R**	**R**	**R**	**R**
2	2	**R**	**R**	**R**	S	S	**R**	**R**	**R**	**R**	S	**R**
3	2	S	**R**	**R**	S	S	**R**	**R**	**R**	**R**	**R**	S
4	3	**R**	S	S	S	S	S	**R**	**R**	S	**R**	**R**
5	1	**R**	S	S	S	S	**R**	S	S	**R**	**R**	S
6	1	S	**R**	S	S	S	S	S	S	S	S	S
7	3	S	S	S	S	S	S	S	S	S	S	S

The reactions of the 11 lentil genotypes to individual isolates of *Asc. lentis* revealed differences in the virulence of the isolates. Although the isolates in this investigation differed by their virulence, they were not designated as races because there is no unified methodology for racial identification of *Asc. lentis*. The few publications, presenting researches on the resistance to *Asc. lentis,* have used

different differential lines and score scales, as well as different techniques for inoculation and incubation, which makes the results from these different investigations difficult to compare (Gossen et al., 1986; Kaiser and Hannan, 1986; Ahmed and Morrall, 1995).

Conclusions

There is variation in the morphological and cultural characteristics of *Ascochyta lentis* isolates. Seven pathotypes of *Asc. lentis* were identified in North-East Bulgaria, the most frequent of them being *Pt* 4 and *Pt* 7. None of the investigated genotypes possessed complex resistance to the 13 isolates of *Asc. lentisi* included in the investigation.

References

1. Ahmed, S.K. and R.A.A. Morrall, 1995. Population changes in *Ascochyta fabae* f.sp. *lentis* over 15 years of lentil cultivation in Saskatchewan. *Second European Conference on Grain Legumes,* Pp: 76-77. 9-13 July, 1995 Copenhagen-Denmark
2. Ahmed, S. and R.A.A. Morrall, 1996. Field reactions of lentil lines and cultivars to isolates of *Ascochyta fabae* f.sp. *lentis*. *Canadian J. Pl. Pathol.,* 18: 362–9
3. Ahmed, S., R.A.A. Morrall and J.W. Sheard, 1996. Virulence of *Ascochyta fabae* f.sp. *lentis* on lentils. *Canadian J. Pl. Pathol.*, 18: 354-361
4. Erskine, W., B. Bayaa and M.C. Saxena, 1996. Registration of ILL 5588 lentil germplasm resistant to vascular wilt and Ascochyta blight. *Crop Sci.,* 36: 1080
5. Gossen, B.D. and R.A.A. Morrall, 1983. Effect of Ascochyta blight on seed yield and quality of lentils. *Canadian J. Pl. Pathol.,* 5: 168-173.
6. Gossen, B.D., J.W. Sheppard, C.J. Beauchamp and R.A.A. Morrall, 1986. *Ascochyta lentis* renamed *Ascochyta fabae* f.sp. *lentis*. *Canadian J. Pl. Pathol.,* 8: 154–60
7. Grewal, J.S. 1984. Evidence of physiologic races in Ascochyta rabiei of chickpea. In: Proceedings of the Workshop on Ascochyta Blight and Wintering of Chickpeas. (Eds.): M.C. Saxena and K.B. Singh. ICARDA, Aleppo, Syria, 55-65.
8. Iqbal, S.M., A. Bakhsh and B. A. Malik, 1990. Identification of resistant sources to Ascochyta blight in lentil. *LENS Newsleter,* 17: 26-27.
9. Iqkbal, S.M., C.A. Rauf and S. Ali, 2006. Pathogenic variability among the isolates of *Ascochyta lentis*. *International journal of agriculture & biology,* 618-620, http://www.fspublishers.org
10. Kaiser, W.J. and R.M. Hannan, 1986. Incidence of seed borne *Ascochyta lentis* in lentil germplasm. *Phytopathology*, 76: 355–60
11. Kaiser, W.J., 1992. Fungi associated with the seeds of commercial lentils from the US Pacific Northwest. *Pl. Disease,* 76: 605-610.
12. Kaiser, W.J., R.M. Hannan and J.D. Rogers, 1994. Factors affecting growth and sporulation of *Ascochyta fabae* f.sp. *lentis. Pl. Disease,* 78: 374-379
13. Malik, B.A., 1983. Discussion remarks on lentil breeding II. *In:* Saxena, M.C. and S. Verma, (eds.) *Proceedings of International Workshop on Faba bean, Kabuli Chickpea and Lentils in 1980s,* P: 105 May 1983, Aleppo, Syria. International Center for Agricultural Research in Dry Areas.
14. Morrall, R.A.A. and J.W. Sheppard, 1981. Ascochyta blight of lentils in Western Canada: 1978-1980. *Canadian Pl. Disease Survey,* 61: 7–13
15. Nasir, M. and T.W. Bretag, 1996. Screening lentil for resistance to Australia isolates of Ascochyta blight. *LENS Newsletter,* 23: 7-9
16. Nasir, M. and Bretag, T.W, (1997) - Prevalence of *Ascochyta fabae* f sp. *lentis* on lentil seeds from Victoria, Australia. *Australasian Plant Pathology 26:* 117-120.
17. Nene, Y.L., S.B. Hanounik, S.H. Qureshi and B. Sen, 1988. Fungal and bacterial foliar diseases of peas, lentils, faba bean and chickpea. *In:* Summer, R.J. (ed.), *World Crops: Cool Season Food Legumes,* Pp: 577-89. Kluwer Academic Publishers, Dordrecht, The Netherlands.
18. Singh, G., K.Singh, A.S. Gill and J.S. Brar, 1982. Screening of lentil varieties/lines for blight resistance. *Indian Phytopathol.,* 35: 678-679.

19. Sugha, S.K., B.M. Singh and S.K. Sharma, 1991. Performance of lentil varieties/germplasm liens against blight. *LENS Newsletter* 18: 34-35.
20. Tay, J., 1989. Inheritance of resistance to Ascochyta blight of lentil. *M.Sc. Thesis,* Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Canada

BIOLOGICAL CONTROL OF BOTRYTIS BUNCH ROT OF GRAPES IN THE REPUBLIC OF MACEDONIA

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Abstract
Bunch rot disease of grapes, caused by the necrotrophic fungus *Botrytis cinerea* is a chronic and serious problem in most of the vineyards in Republic of Macedonia. Its control is mostly achieved by application of synthetic fungicides. However, chemical control of *B. cinerea* is often difficult and incomplete, especially in vineyards where resistant strains have developed. A promising alternative strategy that could replace or be combined with fungicides are biofungicides. The main goal of the experiment was to observe the possibility for biological control of Botrytis bunch rot disease by the use of three novel biofungicides (Serenade Aso, Polyversum and Timorex gold). Experiment was conducted during the 2016 in two vineyard regions of Republic of Macedonia (Negotino and Kavadarci), on two grape varieties, Chardonnay and Cardinal. The destructive potential of this grape disease was confirmed in the untreated variant of the Chardonnay variety, where the intensity of infection was higher than 48%. Biofungicides Serenade Aso (a.m. *Bacillus subtilis* QST 713) and Timorex gold (extract of *Malaleuca alternifolia*) had similar efficacy, with average of 89% reduction of disease incidence in the region of Negotino and 91,66% in the region of Kavadarci. Biofungicide Polyversum (a.m. *Pythium oligandrum* M1), applied as preventive sprays achieved more than 85% reduction of the disease in both regions. The results showed that all tested biofungicides have a prospective use for control of bunch rot disease in grapes.

Keywords: biofungicides, Botrytis bunch rot, biocontrol.

Introduction

Botrytis cinerea Pers.:Fr. (*Botryotinia fuckeliana* (de Bary) Whetzel) is a polyphagous, necrotrophic fungal pathogen, which causes economically important pre- and postharvest diseases in more than 235 plant species (Williamson et al., 2007; Aboelghar and Wahab, 2013; Elad et al., 2015). Recently, *B. cinerea* was ranked second into the world Top 10 fungal plant pathogens list based on its scientific and economic importance (Dean et al., 2012). In grapevine, *B. cinerea* attacks leaves, developing shoots, inflorescences and berries, but symptoms become most evident at the stage of ripening when berries are most sensitive (Couderchet, 2003). During the last 60 years, chemical control was the most important mean of bunch rot disease control, with use of synthetic fungicides (Rosslenbroich and Stuebler, 2000; Leroux, 2002). However, rapid development of resistant strains to frequently applied fungicides (Latorre et al., 2002; Sergeeva et al., 2002; Leroux, 2004) and increased public concern about the human and environmental health (Janisiewicz and Korsten, 2002; Spadoro and Gullino, 2005), has opened the door for other, alternatives means of control of this pathogen, including plant defence stimulants and microorganisms to suppress disease epidemics (Elmer and Reglinski, 2006). Biological control agents has risen as a potential alternative to synthetic fungicides and offers economically sustainable and environmentally friendly control of many economically important pathogens, including *B. cinerea* (Diguta et al, 2016). They suppress the growth of plant pathogens through competition for space and nutrients, production of inhibitory metabolites, such as antibiotics and cell-well degrading enzymes, parasitism and induction of defense-related responses in host plants (Howell, 2003; Harman et al, 2004; Magnin-Robert et al.,

2007; Trotel-Aziz et al., 2008; Sharma et al., 2009). Although many *in vitro* studies confirmed the potential use of antagonistic microorganisms in biological control of many economically important fungal pathogens, only a small number has exhibited field efficacy and even a smaller number has been developed into commercial products (Elmer and Reglinski, 2006). As a result, the main objective of this study was to assess the efficacy of three novel commercial biofungicides (Serenade Aso, Polyversum and Timorex gold) for control of *Botrytis cinerea* in grapevine.

Material and methods

The research was conducted during 2016 in two main vineyard regions in the Republic of Macedonia: Negotino (variety Chardonnay) and Kavadarci (variety Cardinal). The experiment consisted of four variants for each grape variety, in randomized block design with three replicates per treatment. Three of the variants were treated with biofungicides, while one was untreated and used as control (Table 1).

Table 1. Variants represented in Negotino (cv. Chardonnay) and Kavadarci (cv. Cardinal) region

Variant no.	Name of commercial biofungicide	Active ingredient	Content of a. i.	Producer	Dosage
1	SERENADE ASO	*Bacillus subtilis* QST 713 (spores)	5.13 x 10 CFU/g	BASF SE Germany	8 L/ha
2	POLYVERSUM	*Pythium oligandrum* M1 (oospores)	$1x10^6$/g	Biopreparaty spol., Czech Republic	0.25 kg/ha
3	TIMOREX GOLD	*Melaleuca alternifolia* (oil extract)	222.5 g/L	Biomor Israel Ltd	2 L/ha
4	CONTROL	Untreated			

During the vegetative period, based on the biology of the pathogen and phenological stage of the grapevine, three treatments were performed with the tested biofungicides (end of flowering - 80% caps fall, pre-bunch closure and veraison). Evaluation of the efficacy of all tested biofungicieds was performed 10 days after the last treatment. For that purpose, the Unterstenhöfer scale from 0 to 5 was used (Stojanovikj et al., 1971): Cluster: 0 = healthy cluster; 1 = up to 10% infected berries in the cluster; 2 = 10 – 25% infected berries in the cluster; 3 = 25 – 50% infected berries in the cluster; 4 = 50 – 75% infected berries in the cluster; 5 = 75 –100% infected berries in the cluster. 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). The statistical differences among biofungicide efficacy were assessed using Tukey's HSD test at probability level of P = 0.05.

Results and discussion

The obtained results regarding the intensity of infection and efficacy of the tested biofungicides are presented in Table 2 and Table 3. During the evaluation, Botrytis bunch rot infections were observed in all tested variants in the region of Negotino (Table 2). Very high intensity of infection (48.2%) was observed in the untreated (control) variant, which confirmed the destructive potential of the disease, especially in varieties with compact clusters, such as Chardonnay. This is in agreement with the hypothesis of Savage and Sall (1983) that the development of bunch rot is most pronounced in cultivars that develop compact fruit clusters and dense canopies (van Rooi and Holz, 2003). Moreover, between the untreated variant and all treated variants statistically significant difference was detected. Among the treated variants, the lowest intensity of infection was observed in the variant treated with the biofungicide Serenade ASO (5.1%). In the variant treated with the biofungicide Timorex Gold very similar intensity of infection was observed (5.3%), while the highest intensity of infection (6.8%) was observed in the variant treated with the biofungicide Polyversum. There was statistically significant difference between all variants treated with biofungicides (Serenade Aso and Polyversum and Timorex Gold) and the control (untreated variant). Regarding the

efficacy, all tested biofungicides achieved great reduction of bunch rot incidence. Biofungicide Serenade Aso performed highest efficacy (89.41%), but almost equal efficacy (89%) was achieved by biofungicide Timorex gold. The lowest efficacy performed biofungicide Polyversum with ca 85% reduction of bunch rot incidence, significantly lower compared to Serenade Aso and Timorex Gold.

Table 2. Intensity of infection and efficacy of tested biofungicides in control of *Botrytis cinerea* on cultivar Chardonnay grown in the region of Negotino

No	Variant	Intensity of infection (%)	Efficacy of biofungicides (%)
1	SERENADE ASO	5.1a	89.41a
2	POLYVERSUM	6.8c	85.89b
3	TIMOREX GOLD	5.3b	89a
4	Control	48.2d	/

*The values marked with the different letter are significantly different

In the region of Kavadarci (Table 3), bunch rot infections were also observed in all tested variants during the evaluation, but the intensity of infection was considerably lower compared to region of Negotino. The intensity of infection in the untreated (control) variant was moderate to low (4.8), but between the untreated variant and all treated variants statistically significant difference was detected. In the variants treated with the biofungicides Serenade Aso and Timorex Gold equal intensity of infection (0.4%) was observed, while in the variant treated with the biofungicide Polyversum significantly higher intensity of infection (0.7%) compared to the other two biofungicides was observed. Concerning the efficacy of the tested biofungicides, even higher reduction of bunch rot incidence was observed in this region. The highest reduction of the gray mold incidence was achieved by the biofungicides Serenade Aso and Timorex gold, which performed equal efficacy of 91.66%. Biofungicide Polyversum performed the lowest efficacy, with 85.41% reduction of bunch rot incidence, significantly lower than Serenade Aso and Timorex Gold.

Table 3. Intensity of infection and efficacy of tested biofungicides in control of *Botrytis cinerea* on cultivar Cardinal grown in the region of Kavadarci

No	Variant	Intensity of infection (%)	Efficacy of biofungicides (%)
1	SERENADE ASO	0.4a	91.66a
2	POLYVERSUM	0.7b	85.41b
3	TIMOREX GOLD	0.4a	91.66a
4	Control	4.8c	/

*The values marked with the different letter are significantly different

The obtained results showed that all tested biofungicides were effective in suppression of bunch rot disease in grapes. This is in agreement with statement of Elmer and Reglinski (2006), that biocontrol treatments, whether operating by antibiosis, competition, and/or elevation of host resistance, can be highly effective against *B. cinerea* in grapes under controlled conditions and on occasions in vineyards. Moreover, levels of disease suppression can be equal those achieved with synthetic botryticides, particularly under low-to-moderate disease pressure conditions, as was the case in our study, but also in other studies. Thus, Esterio *et al*. (2000) reported that four applications of product Serenade (*B. subtilis* strain QST-713), which were compared with a traditional spray programme used to treat table grapes (cv. Thompson Seedless) resulted in postharvest disease control equivalent to a traditional botryticide programme.

Conclusions

Bunch rot disease, caused by *Botrytis cinerea* has great economic importance and in some cases can lead to complete loss of the grape production. Reducing these losses to a level that is acceptable still poses a great challenge for grape producers in the Republic of Macedonia. The results obtained in this study showed that all tested biofungicides (Serenade Aso, Timorex gold and Polyversum)

performed great reduction of bunch rot incidence and have a prospective use for control of *Botrytis cinerea* in grapes. Moreover, combination of this biofungicides together with some preventive methods that control the development of the pathogen (less sensitive varieties, reduced fertilization, leaf removal for cluster ventilation and adequate protection against other pathogens such as powdery mildew and berry moth) can guarantee highly effective control of Botrytis bunch rot in grapes.

References

1. Abbott W. S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.
2. Dean, R., van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., Foster, G.D. (2012). The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13: 414–430.
3. Diguță, C.F., Matei, F., Cornea, C.P. (2016). Biocontrol perspectives of *Aspergillus carbonarius, Botrytis cinerea* and *Pencillium expansum* on grapes using epiphytic bacteria isolated from Romanian vineyards. Romanian Biotechnological Letters 21(1): 11126 11132.
4. Elad, Y., Vivier, M., Fillinger, S. (2015). *Botrytis*: the good, the bad and the ugly. In: Fillinger, S., Elad, Y., Vivier, M. (Eds.), Botrytis—the Fungus, the Pathogen and Its Management in Agricultural Systems. Springer, Heidelberg, Germany, pp. 1–15.
5. Elmer, P.A.G and Reglinski, T. (2006). Biosuppression of *Botrytis cinerea* in grapes. Plant Pathology 55*:* 155–177
6. Esterio M, Auger J, Droguett A, Flanagan S, Campos F. (2000). Efficacy of *Bacillus subtilis* (Ehrenberg), Cohn., QST-713 Strain (SerenadeTM), on *Botrytis cinerea* control in table grape (*Vitis viniferea* L. cv Thomson Seedless). In: Proceedings of the XII International Botrytis Symposium, Reims, France. Europol Agro, Abstract L27.
7. Harman, E.G., Howell, R.C., Viterbo, A., Chet, I., Lorito, M. (2004). *Trichoderma* species - opportunistic, avirulent plant symbionts. Nature Reviews Microbiol. 2: 43-56.
8. Howell, R.C. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history of and evolution of current concepts. *Plant Disease* 87(1): 4-10.
9. Janisiewicz WJ, Korsten L. (2002). Biological control of postharvest diseases of fruits. Annual Review of Phytopathology 40: 411–441.
10. Latorre BA, Spadaro I, Rioja ME. (2002). Occurrence of resistant strains of *Botrytis cinerea* to anilinopyrimidine fungicides in table grapes in Chile. Crop Protection 21: 957–961.
11. Leroux P. (2004). Chemical control of *Botrytis and* its resistance to chemical fungicides. In: Elad Y, Williamson B, Tudzynski P, Delen N, eds. *Botrytis: Biology, Pathology and Control*. Dordrecht, The Netherlands: Kluwer Academic, 195–222.
12. Leroux P., Fritz R., Debieu D., Albertini C., Lanen C., Bach J., Gredt M., Chapeland F. (2002). Mechanisms of resistance to fungicides in field strains of *Botrytis cinerea*. Pest Management Science, 58: 876-888.
13. Couderchet. M. (2003). Benefits and problems of fungicide control of *Botrytis cinerea* in vineyards of Champagne. Vitis 42 (4): 165–171
14. Magnin-Robert M, Trotel-Aziz P, Quantinet D, Biagianti S, Aziz A. (2007). Biological control of *Botrytis cinerea* by selected grapevine-associated bacteria and stimulation of chitinase and β-1,3 glucanase activities under field conditions. European Journal of Plant Pathology 118(1): 43-57.
15. Aboelghar, M. and Wahab, H.A. (2013). Spectral footprint of *Botrytis cinerea*, a novel way for fungal characterization. Advances in Bioscience and Biotechnology, 4: 374-382.
16. van Rooi, C. and Holz, G. (2003). Fungicide efficacy against *Botrytis cinerea* at different positions on grape shoots. S. Afr. J. Enol. Vitic. 24(1): 11-15.
17. Rosslenbroich HJ, Stuebler D. (2000). *Botrytis cinerea* – history of chemical control and novel fungicides for its management. Crop Protection 19: 557–561.

18. Sergeeva V, Nair NG, Verdanega JR, Shen C, Barchia I, Spooner Hart R. (2002). First report of anilinopyrimidineresistant phenotypes in *Botrytis cinerea* on grapevines in Australia. Australasian Plant Pathology 31: 299–300.
19. Sharma RR, Singh D, Singh R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological Control 50: 205–221.
20. Spadoro D, Gullino ML. (2005). Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Protection 24: 601–613.
21. Stojanovikj, D., Kostikj, B., Dimitrievikj, B., Ostoikj, N., Mitikj-Muzina, N., Bogavac, M., Tomasevikj, B., Gruikj, G. and Ruzikj A. (1971). Handbook of methods in determining presence, evaluation of presence intensity and calculation ofplant loss caused from plant disease and pests. Secretariat for Agriculture, Belgrade, Serbia.
22. Townsend, G. R and J. W. Heuberger. (1943). Methods of estimating losses caused by diseases in fungicide experiments. Plant Disease Reporter 27: 340-343.
23. Trotel-Aziz P, Couderchet M, Biagianti S, Aziz A. (2008). Characterization of new bacterial biocontrol agents A*cinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas* spp. mediating grapevine resistance against *Botrytis cinerea.* Environmental and Experimental Botany. 64(1): 21–32.
24. Williamson, B., B. Tudzynski, P. Tudzynski, and J. A. L. van Kan. (2007). *Botrytis cinerea*: the cause of grey mould disease. Molecular Plant Pathology 8: 561-580.

SOME POSSIBILITIES OF VEGETATIVE PROPAGATION OF STRAWBERRY CULTIVARS *IN VITRO*

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Abstract
This work presents the production technology of five strawberry cultivars by micropropagation. Starting materials were taken in May or June from the stolons that were in the air and meristems of 0.5mm were isolated in the laboratory. The isolated strawberry meristems were cultivated in a medium containing macro and micro elements and phytohormones in different concentrations. The highest percentage of initiated culture was recorded by the cultivar Senga Sengana (81.48%) and the lowest by the cultivar Marmolada (48.00%). Optimal hormone balance for initiating culture strawberries was 1.0 mg / l BAP (Benzylaminopurine), 0,1 mg / l IBA (indole-3 -butyric acid) and 0.1 mg / l GA_3 (gibberellic acid). After 50 days of cultivation in the culture, the formed shoots were transferred to medium for multiplication. The multiplication of isolated buds was achieved on the medium with phytohormones BAP and IBA. The highest multiplication index was recorded by the cultivar Senga Sengana (8.77) and the lowest by the cultivar Marmolada (5.42). When planlets reached a height of 10-12mm they were transferred to the rooting medium. The optimal concentration of phytohormones IBA for rooting of the strawberry cultivars was about 5 mg / l. Number of roots ranged from 5.09 for the cultivar Idea to 6.11 for the cultivar Senga Sengana. After two months the plants with developed roots were transplanted into peat briquettes. Adjustment by external conditions was conducted in greenhouses under a mist system. The reception was submitted well by the planted strawberries (90%). The results presented in this paper indicate that micropropagation can speed up the process of getting healthy and HIGH quality planting material of strawberries. The procedure for obtaining strawberries by micropropagation can be accelerated by proper selection of the combination and concentrations of phytohormones.

Keywords: micropopagation, multiplication, phytohormones, adjustment, greenhouse.

Introduction

The district of Jablanica is known for the production of berry fruits. The most represented berry fruits are raspberries, strawberries and blackberries. By building the processing capacities of the cold storages in Leskovac, Vucje, Bojnik and Lebane, the task for improving the production of berry fruit was set, with a special emphasis on continuous improvement of technological usability and quality of the fruits. Special emphasis is given on the biological and pomological properties of strawberry cultivars for a particular region, as well as the use of varietally pure and healthy planting material. In practice, there are several ways of producing strawberry planting material. Regardless of the way, planting material should be high quality, healthy and monovarietal. The condition of planting material in the Republic of Serbia is in an unenviable situation. Strawberry producers, for economic reasons, mostly use planting material from production plants. On that occasion, rosettes are often mixed with seedlings, and very often the real name of the cultivar or degree of infection diseases and pests is unknown, contributing mostly to the low yield and poor quality fruit. In order to improve this situation, it is necessary to introduce newer and more fertile cultivars, and for the production of strawberry runner plants, for obtaining healthy planting material, the micropropagation methods should be used. Micropropagation of plants implies the production of plants from the cells of the

meristematic tissue. This vegetative propagation is provided only under laboratory conditions. Micropropagation is perfomed on a special medium, which contains a larger number of nutrients, especially minerals, sucrose and growth hormones. Modern production of strawberry runner plants consists of the production of selected and virus free planting material. The strawberries can be freed from the virus by the method of micropropagation. This method is based on the fact that viruses are not widespread throughout the plant. The apical bud of stolon does not contain viruses, even when the whole cluster is infected. On the other hand, meristematic tissue can be easily isolated from the plant under sterile conditions, and the whole plant can easily regenerate on the nutrient medium.

Material and methods

The research used five strawberry cultivars: Senga Sengana, Cortina, Idea, Marmolada and Humel's constantly giving birth. Explants of apical buds were used as starting material for the induction of callus, organogenesis and regeneration of plants of studied strawberry cultivars. The starting materials were taken in May or June from stolons that were still in the air. Preparation of the starting material and Murashige and Skoog's medium (Murashige and Skoog, 1962) was carried out in the biology laboratory "Zdravlje" Leskovac, and isolation in the microbiological laboratory.The explants were kept under controlled conditions, at a temperature 20-25 ^{o}C, with light intensity of 2000 – 2500 lx and the length of brightness of 16 hours per day and 8 hours per night. Adjustment to environmental conditions was carried out in the greenhouses under the a ''mist'' system, while adaptation to external conditions was carried out on the plots of "Porecje" Vučje. Strawberries were kept there for three years.

Results and discussion

Stolons of the runner plants of the following strawberry cultivars: Senga Sengana, Cortina, Idea, Marmolada and Humel's constantly giving birth were used for the isolation of meristems. Plant materials were collected during May or June. Meristems size of 0.5-1mm were isolated in the laboratory from the stolons of strawberries. The isolated strawberry meristems were cultured on Murashige and Skoog's medium containing macro and micro elements, sugar and agar. Phytohormones BAP, IBA and GA3 at different concentrations were added to this substrate. From the applied concentrations, the optimal hormone balance for the culture of primary explants was: 1.0 mg / l BAP, 0.1 mg / l IBA and 0.1 mg / l GA3. The influence of these hormone concentrations on the initiation of cultures varieties is shown in Table 1.

Table 1. The beginning of the development of various sorts of strawberry cultivars

Cultivar	The number of meristems present in the culture	Uninfected cultures		Activated cultures		% of success
		Number	%	Number	%	
Senga Sengana	30	27	90.00	22	81.48	73.33
Cortina	30	25	83.33	12	72.00	60.00
Marmolada	30	25	83.33	18	48.00	40.00
Idea	30	26	86.67	17	65.38	56.67
Humel's constantly giving birth	30	19	63.33	11	57.90	36.67

The highest success in culture initiation was achieved with the Senga Sengana cultivar (81.48%), and the lowest with the Marmolada cultivar (48%). The highest percentage of success was recorded in the Senga Sengana cultivar (73.33%), and the lowest in the Humel's constantly giving birth (36.67%). Milosavljevic S. (1999) achieved the highest success in culture initiation with the Selena cultivar (70%), Nikolic at al (2004) achieved a high percentage of culture initiation with the Selena cultivar

(76.67%). After six weeks of cultivation in a culture, formed scions size 10-20mm were transferred to the medium for multiplication. The multiplication of isolated buds was achieved at Murashige and Skoog's medium with phytohormones BAP and IBA. In our research, the influence of different concentrations of BAP hormone on the reproduction and extinction of shoots in all tested cultivars was also monitored. The optimal hormone balance for continual multiplication of strawberies is 1mg / l BAP and 1 mg / l IBA. Without the presence of BAP hormones in the nutrient medium there was no multiplication, while the omission of hormone IBA reduced the multiplication. For the most favorable concentrations of phytohormones, multiplication in the tested cultivars is shown in Table 2.

Table 2. The multiplication of various strawberry cultivars

Cultivar	Number of plants in culture	The number of produced plants per row	Index of multiplication
Senga Sengana	22	193	8.77
Cortina	18	111	6.17
Marmolada	12	65	5.42
Idea	17	96	5.65
Humel's constantly giving birth	11	47	4.27

The highest multiplication index was recorded in the Senga Sengana cultivar (8.77) and the lowest in the Humel's constantly giving birth cultivar (4.27). In his experiments, Milosavljević S. (1999), reached the highest index of multiplication with the Careca cultivar (6.00), and the lowest with the Sena cultivar (4.1). The presence of BAP hormone in the nutrient medium effectively prevented the rooting of strawberries. The omission of the hormone IBA resulted in a reduction in multiplication, but not in the omission of reproduction. By eliminating BAP hormones, multiplication is absent. When obtained in the multiplication and elongation phase strawberry planlets are distinguished by the fact that they have no roots. When the plantlets reached a height of 10-20 mm, they were transferred to the rooting medium. The composition of this medium includes ½ MS macro and micro salts, sucrose, agar and inizitol, and hormones, IBA hormones at different concentrations. The optimal concentration of IBA hormone for the rooting of strawberry cultivars is 0.5 mg/l. At a higher concentration of IBA hormone (1mg/l) the larger mass of callus was formed at the basal part of the scion, but at a lower concentration of hormone (0,1mg/l IBA), the number of rooted scions was quite limited. The results of rooting of strawberry cultivars with a concentration of 0.5 mg / l IBA are given in Table 3.

Table 3. The rooting of various strawberry cultivars after 60 days

Cultivar	Number of plants in culture	Number of rooting plants	% of rooting	The average number of root/culture
Senga Sengana	30	26	86.67	6.11
Cortina	30	27	90.00	6.04
Marmolada	30	25	83.33	5.96
Idea	30	22	73.33	5.09
Humel's constantly giving birth	30	20	66.67	4.95

The first root initials could be spotted after 15 days, and after 4-5 weeks the veins reached a length of about 40 mm. The roots were slender and long, and on the cutting site, there was a little plant with little callus. The number of roots per plant ranged from 4.95 in the cultivar Humel's constantly giving birth, to 6.11 in the cultivar Senga Sengana. The highest percentage of rooting is in the Cortina cultivar, and the lowest in the Senga Sengana cultivar. Petrovic D. (1990) recorded the highest

success in culture initiation in the Selena cultivar (70%), and the lowest in the Sena cultivar (10%). Nikolic et al. (2004) achieved a high percentage of the initiation of the cultures with the cultivar Selena (76.67%). Petrović et.al (1990) achieved rooting of 80.00% in the Senga Sengana cultivar. After rooting the most convenient moment for transplanting the obtained plantlets is after two months of incubation of scions on agrarian 1/2 Murashige and Skoog's medium when the rootlets have reached the length of 30-50 mm. Then the roots of young plants were carefully rinsed with water to remove the agar, in order to avoid harmful settled microflora. Thus washed, young plants were placed into sterile plastic dishes with a sterile mixture of vermiculite, peat, and sand, in a ratio of 1:1:1. 1/2 Murashige and Skoog's mineral solution was added to this mixture. The courts were placed in plastic tubs with water and covered with jar. Thus planted plants were kept for a month under plastic sheets, and after that they were transplanted into pots with soil. The results of adaptation of strawberry cultivars are presented in the following table.

Table 4. The adaptation of various strawberry cultivars to the outdoor conditions

Cultivar	The number of planted plants	The number of plants that survived	% of survived plants
Senga sengana	40	31	77.50
Cortina	40	34	85.00
Marmolada	40	32	80.00
Idea	40	29	72.50
Humel's constantly giving birth	40	27	67.50

The highest number of adapted plants was recorded in the Cortina cultivar (85.00%), and the lowest in the Humel's constantly giving birth cultivar (67.50%).

Conclusions

Considering the conducted analyses, we can conclude that vegetative multiplication of five strawberry cultivars is possible in *in vitro* conditions. The optimal hormone concentration balance for strawberry cultivars is 1mg / l BAP, 0.1mg / l IBA and 0.1mg / l GA3. The highest success in the initiation of cultivars is in the Senga Sengana cultivar (84.48%), and the lowest in the cultivar Marmolada (48%). For the multiplication of strawberries, the hormone BAP plays the most important role. The best strawberry multiplication was achieved by a combination of phytohormones 1.0 mg / l BAP and 1 mg / l IBA. The highest multiplication index is with the Senga Sengana cultivar (8.77), and the lowest with the Humel's constantly giving birth cultivar (4.27). The optimum concentration of BAP phytohormone for rooting of strawberries is 0.5 mg / l. The highest percentage of rooting is found in the Cortina cultivar (90%), and the lowest in the Humel's constantly giving birth cultivar (66.75%). The highest number of roots was recorded in the Senga Sengana cultivar (6.11), and the lowest in the Humel's constantly giving birth cultivar (4.95). When adapting to external conditions, the largest number of plants have been adapted with the Cortina cultivar (85.00%), and the lowest with the Humel's constantly giving birth cultivar (67.50%).

References

1. Blagojević, R., Nikolić, Z. 2012. Jagodasto voće. DIS Publik, Beorad.
2. Milosavljević, S,. et al. 1999. Razmnožavanje novih sorti jagoda metodom kulture meristema *in vitro.* Zbornik naučnih radova, 5: 295-300.
3. Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and biossays with tobacco tissue cultures. Physiol.Plant.15, 473-497.
4. Nikolić, M., Milivojević, J. 2010. Jagodaste voćke. Grafika Jureš, Čačak.
5. Nikolić, Z. et al. 2004. Meristemskoto razmnožuvanje kako metod za dobivanje na zdrav saden materijal kaj neki sorti jagoda. Godišen zbornik za zaštita na rastenijata. Vol. XV, 153-162. Skopje

6. Nikolić, Z., 2006. Mikrorazmnožavanje i njegov uticaj na agrobiološke i tehnološke osobine kod nekih sorti jagoda. Doktorska disertacija. Lešak. 134.
7. Nikolić, Z. et al. 2013. Micropropagation of some strawberry sorts. Research people and actual tasks on multidisciplinary sciences. Vol. 1, 151-154. Lozenec, Bulgari
8. Petrović, D et al. 1990. Razmnožavanje cv. Senga sengana metodom kulture meristema *in vitro*. Nauka u praksi, 20, 11-18.

USING DRONES IN AGRICULTURAL AREAS

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Abstract

When the world population has approached eight billion, the worldwide focus shifted to supply food for it in a sustainable way. A new grip on precision agriculture has been led. This was identified as a farm management. This emerging concept is established to observe, measure, respond the crops and their inter and intra-field variability and versatility. To gain yield in a maximum level from the limited sources of a farm, this notion can be an efficient way for farmers to reach this aim. Meantime, the outcomes in terms of crop yields, plant health and other data have to be observed constantly while having a real-time feedback. They also require an analysis to be done objectively and equitably. Drones are considerably convenient to the farmers for helping to optimize the use of inputs such as fertilizers, seed and water. They are helpful for them to react quickly and on time to threats like weeds, pests and fungi. The farmers can also save time in treatment validation of the crop scouting, can improve the variable rate prescriptions in real time and can do an estimation about the yield to be given from a field in harvest seasons. Our environment and our food can be cleaned more and more from chemical load. The reduction of water use can be figured out more and better. Despite being a young technology, drone usage in agriculture is expected to increase significantly. Encouraging the farmers to use the drone with the aid of the government support and agricultural extension efforts, will help them to warm towards this technology and to become adherent to future technologies. In this paper, secondary data was used to show drone usage and benefits in agriculture and how it effects environment positively.

Keywords: Drone, environment, agriculture, sustainability, extension.

Introduction

In parallel with the developments in the economy and population growth in the world as a whole, the agriculture will come into prominence ascendantly. The estimations made by the scientists indicate that we will need more production of crop, in other words we will have to double it to meet the food supply, by the year 2050. To increase the productivity in agriculture is extremely significant for this demand (Snow, 2016). When the world population has approached eight billion, the worldwide focus shifted to supply food for it in a sustainable way. A new grip on precision agriculture has been led. This was identified as a farm management. This emerging concept is established to observe, measure, respond the crops and their inter and intra-field variability and versatility. To gain yield in a maximum level from the limited sources of a farm, this notion can be an efficient way for farmers to reach this aim. Meantime, the outcomes in terms of crop yields, plant health and other data have to be observed constantly while having a realtime feedback. They also require an analysis to be done objectively and equitably. The aerial images are easily taken by the aid of drones, which means they are very efficient tools to assemble these significant data (Snow, 2016). This paper will be an essay of analysing the drones that we have used as remote sensing devices in agriculture, up to the present. Additionally, we will be reviewing some approaches, such as competitive and traditional, which apply necessary technology; and we will be discussing the technology itself that provide many opportunities and challenges; we will find out our knowledge and finally we will talk about the drones in the future in agricultural era.

Definition of drone

The word 'drone' is the common name for an Unmanned Aerial Vehicle (UAV). Wikipedia describes drone as "An unmanned aerial vehicle (UAV), commonly known as a drone, is an aircraft without a human pilot aboar" and as "'*a powered, aerial vehicle that does not carry a human operator, uses aerodynamic forces to provide vehicle lift, can fly autonomously or be piloted remotely, can be expendable or recoverable, and can carry a lethal or nonlethal payload*.' By (Office of Secretary of Defense, 2005). The augmentation of the productivity and the regulation of the crop quality are due to the unmanned drones which are a particular innovation for the farmers. Today, they prefer to use drones more frequently and regularly to obtain best results from the informations taken. Before, more traditionally methods such as crop-monitoring from satellite photography or manned airplanes were remaining unsufficient while providing incomplete and/or delayed informations. The drones also are a member of the manned airplanes. But they seem more efficient than them because they can fly much closer to the crops and to the leaf level at the same time which make the drones more valuable in the eyes of the farmers who have more possibility to capture additional and expanded detailed information. Needing to combine photos taken by manned airplanes can be a problem since those ones have traditional flight paths. To overcome this issue, the drones are a beneficial choice by being able to fly over a fixed point for a period of time. The pesticides, herbicides, fertilizer and irrigation are detected on site-monitoring by the aid of the drones that can be equipped with infrared cameras, sensors, and other technologies. The drones can ensure them and fix them up within the collection of a variety of relevant data. And this will help the farmers to make a decision and to take precautions. The use of the infrared cameras mounted on drones is an example of this site-monitoring. It helps to mesure the productivity of the crops by visible and infrared radiation to designate whether the plants are in good conditions or not (Sunding et al. 2016).

The significance and the usage of drone

I. We can mention UAS as an agricultural tractor. But singlehandedly it is not very useful in the field work without some kind of implement. Due to this, we can mount on some kind of tool such as a platform mounted cameras which will lead it to become useful for agriculture together with the farmers. The different aspects of plant health are assessed from simple visible-light photographs or more scientific multi-spectral imagery. The farmers can put inputs about seeds, fertilizers or chemicals according to their quotations to the larger and sophisticated UAS. However, we must take into consideration that the data will be collected by the UAS but its interpretation will be done by the farmer. In an agricultural area, to use of the aerial photographs or videos of the problematic part or parts visually explored, and then to visit it/them to diagnose the cause of the problem directly seems easy. Nevertheless, the diagnosis of problems is more like a direct interpretation and one of the biggest challenges when any aerial technology is used. Collecting many different potentially useful data is a possibility, but there is a use of information that can be employed to make management decisions - and the question is how to receive these useful informations. To comment and to provide management tools that work together to develop methods in several different universities, companies and services can lead us, however, this is not a trivial task (Anonymous1, 2017). Drones can be very useful while starting the crop cycle. One of their advantages can be that having higher quality and precision of the images. A satellite who takes pictures only in limited times such as once a week or once a month could not be sufficient for planting. Here comes the importance of the drones. However the multidimensional maps for early soil analysis are produced precisely by the satellites. The farmers use them in their planning seed planting configurations. Once the field and soil analysis are done, they can efficiently use drones to plant their land. The pods with seeds and plant nutrients can be shot by the drones into the soil. Thus and so, the plants can provide all the necessary nutrients for sustaining life. This also leads to lower the costs of planting, therefore is very advantageous for the farmers. Accordingly, the ground can easily be scanned by the drones. They can modulate and adjust the distance of spraying from the ground and the amount of liquid correctly; thus, they can realize the spraying in real time even in coverage. It results to a

satisfying degree of effectiveness: The efficiency is increased and the amount of chemicals that intends to penetrate into the soil and then the groundwater is reduced. And this is one of the most important motive. Furthermore, the different parts of a field can be identified with the sensors of the drones if they are dry and if they need an irrigation. Drones can also be used to tailor the usage of the pesticides, herbicides, fertilizer of the farmers. They help them for other applications too depending to their need and at a specific point of their field.

Where can we use drone?

1. To scout crop in a simple way

The farmers are watching, observing and checking up their crops to be aware of the time to step in and help as we are checking up our own health. They regularly examine their crops for signs of stress and of potential stress. This is significant for crop protection from weeds, pests, and disease (Anonymous2, 2017).

2. Health Assessment and its valuation

Visible and near infrared light is used by some drones to scan crops. The plants reflect and near-infrared light and its amount is identified by on-board light processing devices such as thermal sensor. The water use of the plants is one of the occurrence that can be identified by a thermal sensor. Accessing more water is appeared to be cooler in an image. The multi-spectral images are developed by the use of these datas. These images illustrate the health of the plants. Crop health is also painted and tracked by these images. In occurrence of any sickness, the farmers can also monitor the administered remedies (Anonymous3, 2017).

3. The inspection of irrigation

Many fields are spreaded out across a region and lage growers need more control for them. When the crops such as corn are reached a certain height, the inspection of irrigation nozzles and sprinklers commited in the mid-season become a troublesome situation, because the inspectors have to wade through crops to find the troubled ones. This task is very time-consuming. The better way to avoid the waste of time is to have professional drones that have camera zoom functionality.The parts of the field that have become dry can be identified by the drones which are equipped with hyper-spectral or thermal sensors. This ensures the precision and time gaining on promptly making irrigation(Anonymous3, 2017). Like water which is used more efficiently as input, the environmental resources are also conserved by them. The sustainable agriculture put emphasis to conserve the water in the production. Today, sustainable agriculture comes to meet society's needs without compromising the welfare of future generations. In addition, wireless technologies allow farmers to use water more efficiently and increase their earnings. Excessive irrigation of the plants causes the nutrient to dissolve the groundwater and mix with it. They are particularly mixed with fertilizer and nitrogen nutrients which have a negative effect on both local and global water quality. The food stream can contaminate the water in the surface. A major problem arising from the flow of water is the creation of “dead zones”. The nutrient flow from agriculture and other human activities causes the overgrowth of algeas which consume oxygen, creating anoxic conditions that can kill all marine life in this area. Stimulating algae overgrowth is emerging areas of dead zones. The significant impact of these dead zones are detrimental to the ecosystems, the seafood and the tourism industries(Sunding et al. 2016).

4. Precision spraying

Sprayed drones are designed for precise variable rate application of liquid insecticides, fertilizers and herbicides. Unmanned spray helicopters have been used for many years. The use of these drones in agriculture is a more efficient route in terms of cost and cost in managing their products. But the agent does not hold excess liquid. Thanks to the use of ultrasonic echo and laser, the drones can adjust the altitude by changing the topography and the geography. They have the ability to scan and

modulate the distance from the site, and evenly dispense the right amount of the desired liquid in real time. This results in increased productivity; because the amount of water penetrating the ground is reduced the most. It has been proven that spraying using Drones is faster than other conventional methods (Anonymous3, 2017).

5. Maps of individual fields or segments

Individual fields or pieces require more knowledge and expertise than pressing the flying drone's record button to create maps. "Orthomosaic photography" requires knowledge of how to create it. An orthomosaic geometric scale is an aerial photograph that can be corrected : there is the same imperfection as distortion of the photo map. Not like uncorrected aerial photographs, an orthophotograph can be used to measure distances on the Earth as it has a representation (such as areas and field segments). The greatest advantage of an agricultural drone via satellite or a plane is shown by taking the photo of a specific area. The pictures taken are sufficiently clear to determine whether the difficulties are in which of the sections if there are any. Detailed informations that is collected by the drones is so useful by showing the only part to be fixed, you'll be able to treat the problem. This detailed information for each portion of the field allows you to specify which part of the field requires cure. So, you don't have to spend money for spraying the whole area, you can save your money by targeting a specific area. You can develop three-dimensional maps by your agricultural drones and your nearby field can allow you to have a better idea of the layout. You can be help by the maps that can help you to better identify your direction with tools such as water flow and sink holes. You can develop water and land management plans for your area and even register your areas for insurance purposes with the aid of this data. It can additionally be used to determine the nutritional and fertility levels of different parts of individual areas, at the same time, with the power of computers and people (Anonymous4, 2015).

6. Analysis

We can use drones to make also the soil and field analysis. 3-D maps created by the drones helps us to analyzing soil on soil property, moisture content, and soil erosion. Seed planting patterns need this study. Once the processing of planting finished, we can keep this information to use in the irrigation and the management of the nitrogen level in the soil both (Anonymous3, 2017).

7. Planting

Although not yet widespread, some manufacturers have found any systems which unable to shoot capsules that contain seeds into prepared soil. This deeply lowers the costs of planting.

Disadvantages

In addition to the advantages, various problems arise when using the UAV. The performance of current UAVs, in terms of transport load, range and/or accuracy, is still limited. VTOL-tools (able to set up vertically) can be well positioned, but have limited payload due to the power required for lift. With fixed-wing vehicles, you can carry more tools, but they can not be placed having a suitable position when flying. In existing models, there is an autonomy and they can mostly follow the road signs, but still unlikely to intervene if an operator is needed for task scheduling and some problems occur. Moreover, the UAV should resolve the ethical issues when implementing various commercial applications. Especially while UAV makes its observational tasks, the ownership, the security and the privacy questions arise about the breach. One of the disadvantages is that they should be kept under continuous observation in the sense that is seen as a privacy invasion because of their constantly surveillance. Drones can carry high-power zoom lenses, night vision and imaging properties. These clearly need to be addressed and must be sorted out. Like all new technology, it's probably desirable for efficiency and reliability reasons, however, there is a discussion on the replacement of human labour of the people in the field (Vroegindeweij et al. 2014).

Results and discussion

Drones are considerably convenient to the farmers for helping to optimize the use of inputs such as fertilizers, seed and water. The drone, especially in the field of precision farming, includes various aspects that can show greater benefits when applying it to the agriculture. One of its facility is the monitoring tasks. Its human observation from the satellites or based on the repeatability of the current system, has the potential to achieve much better performance in terms of accuracy and timing. Whenever the appropriate processing technology is provided, at the same time, the coordination of operations are performed by farmers, machines or robots in the field, as well as some methods to be developed allows for the manipulation of AUAV by themselves (Vroegindeweij et al. 2014). They are helpful for them to react quickly and on time to threats like weeds, pests and fungi. The farmers can also save time in treatment validation of the crop scouting, can improve the variable rate prescriptions in real time and can do an estimation about the yield to be given from a field in harvest seasons. Our environment and our food can be cleaned more and more from chemical load. The reduction of water use can be figured out more and better. Despite being a young technology, drone usage in agriculture is expected to increase significantly. Encouraging the farmers to use the drone with the aid of the government support and agricultural extension efforts, will help them to warm towards this technology and to become adherent to future technologies. While supplying the product increasing within the on-site and on-time interventions, the sustainability will be supported by increasing the efficiency of the resource utilization. The drone's use in agriculture is assumed to keep on growing. New wireless technologies as found in computing or smart phones, is also available for agriculture; it has made possible the use of drones for farmers to indicate the entities such as soil moisture levels, weather, and irrigation equipment to be able to access critical information in real time about tools. With this information, farmers can make a more effective decision for irrigation of crops and. The farmers increase their profits within this increased decision-making authority, they can save on water and water quality is improving correspondingly. Finally, as a matter of fact, the use of drones becomes extemely popular (Sunding et al. 2016).

References

1. Anonymous1, (2017). file:///C:/Users/TU%C4%9E%C3%87E%20U%C4%9EUR/Desktop/TheUseofUnmannedAircraftSystemsinAgricultureUASFactsheetFinal.pdf
2. Anonymous2, (2017). https://modernag.org/innovation/crop-scouting-technology/
3. Anonymous3, (2017). http://www.droneguru.net/the-pros-and-cons-of-drones-in-agriculture/
4. Anonymous4, (2015). https://www.sensat.co.uk/single-post/2015/07/25/The-Advantages-Of-Drone-Remote-Sensing-For-Agricultural-Crops
5. Snow, C. (2016). The Truth about Drones in Precision Agriculture They’re great scouting tools, but can they unseat the incumbents?, August 08, 2016.
6. Sunding, D., Rogers, M., Bazelon, C., (2016). The Farmer and the Data: How Wireless Technology is Transforming Water Use in Agriculture. Prepared for CTIA Wireless Foundation, April 22, 2016.
7. Vroegindeweij, B. A., van Wijk, S. W., & van Henten, E. (2014). Autonomous unmanned aerial vehicles for agricultural applications.

VIRUSES OF SOME GARLIC ECOTYPES IN CROATIA

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Abstract
Recent, there is increasing demand for autochthonous ecotypes of garlic (*Allium sativum* L.) in Croatia. Many local ecotypes of garlic are developed due to specific environmental conditions and producer's selection. However, the average yield of garlic is not in the European average range, since the classical vegetative propagation from cloves usually results with virus infections that cause significant yield reduction. Viruses are considered significant garlic's pathogens. The research was set up to determine infection rate in plant material of different garlic ecotypes grown from cloves in different regions. Plants were collected from commercial fields in Zadar County (coastal part of Croatia) and Vukovar-Srijem County (eastern Croatia) and were tested by enzyme-linked immunosorbent assay (ELISA) on the presence of three viruses: *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV) and *Garlic common latent virus* (GCLV). As a potential source of antigen leaf tissue was used and tests were conducted according to manufacturer's instructions (Bioreba AG, Switzerland). In plants from Zadar County dominant was OYDV, followed by GCLV and LYSV. All tested plants originating from Vukovar-Srijem County were infected with three tested viruses. Out of 316 analyzed plants only 4 (1.3%) showed to be free from viruses included in survey. Plants infected with OYDV showed symptoms of leaf yellowing and reduced growth, while those infected with LYSV expressed yellow stripes. Symptoms were most evident at the beginning of vegetation. Due to high infection rate, deteriorated sanitary status, and increased interest in use and production of local garlic ecotypes, work on sanitary selection will be important part of revitalization program.

Keywords: *Allium sativum* L., ELISA, *Onion yellow dwarf virus*, *Leek yellow stripe virus*, *Garlic common latent virus*.

Introduction

Garlic (*Allium sativum* L.) is one of the most important and widely widespread culinary plants used as a medicinal herb and spice (Abou El-Magd et al., 2013). According to FAO (2015) in period from 2000 to 2013 garlic is among top five food ingredients with annual production incensement of 7.7%, indicating its worldwide importance. In the Republic of Croatia production of garlic is insufficient for domestic consumption with no data about export, while most of the imported garlic comes from China (Dumičić et al., 2015). According to Toth et al. (2015) the scarcity of domestic production is a consequence of two major problems: poor quality of planting material and inadequate storage conditions. Garlic is produced in all agriculture regions, but mostly on small areas for local markets and personal consumption (Dumičić et al., 2015). By introducing virus-free planting material production could increase and represent a significant source of income (Radat, 2014). The average globally yield of garlic is about 10 t/ha (Lešić et al., 2016). Twice higher yields from world average have Egypt and USA, while in Croatia the average yield is 8.58 t/ha (Matotan, 2004). In Croatia most garlic cultivars are obtained by clonal selection of local ecotypes (Lešić et al., 2016). Transferring the ecotype of desirable properties in the new production area adaptive capacity of plant play significant role (Dumičić et al., 2015). The average bulb mass of domestic ecotypes is usually between 30 and 40 g, while the French non-virulent cultivars have an average mass of bulbs between 70 and 100 g (Lešić

et al. 2016). Viral diseases usually have influence on growth and yield of different crops (Hull, 2009). However, there is a little data about their impact on different crops in Croatia (Juretić, 2002). Species from the genus *Allium* are economically important cultures in the Mediterranean basin, and viruses are among the most important pathogens affecting their yield (Loebenstein and Lecoq, 2012). Since viral infections are usually systemic, they are problem in vegetative propagated crops. Vegetative propagation ensures the transmission of viruses to progeny and, when used on large scale without appropriate sanitary control, can lead to massive dissemination of viruses. Such material can serve as primary source of virus and led to secondary contaminations which, especially in case of viruses transmitted by insects, can lead to disease epidemic with significant consequences on crop growth and yield (Astier et al. 2006). Garlic can be infected with viruses from genera *Potyvirus*, *Carlavirus* and *Allexvirus*. These viruses are transmitted by vegetative propagation and different vectors (Chodorska et al. 2014). Some viruses, like *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV), cause mosaic symptoms and reduction of yield over 25% (Messianen et al. 1981; Vunsh et al. 1991). Other viruses are usually latent with no significant impact on yield (Van Dijk et al., 1991). However, simultaneous infection by *Carlavirus* or *Alexivirus* together with *Potyviruses* may have synergistic effect. It is estimated that viruses can reduce garlic yield during consecutive cultivation of infected plants up to 50% (Conci et al. 2003; Lot et al. 1998). In addition, using virus-free planting material resulted in 32-216% higher total bulb's mass in different garlic cultivars (Conci, 1997; Conci et al. 2003; Melo Filho et al. 2006; Walkey and Antill, 1989). The aim of this study was to determine occurrence of OYDV, LYSV, and *Garlic common latent virus* (GCLV) as the most common viruses of *Allium* species in Mediterranean region. Only OYDV has been previously reported in Croatia, but just on onion (Štefanac, 1977).

Material and methods

Survey was conducted on four garlic ecotypes (E1-E4) originating from Zadar County and one ecotype (E5) from Vukovar-Srijem County. Plants were divided in three categories: I) plants/ecotypes E1-E4 grown from cloves collected from commercial fields located in Zadar County; II) plants/ecotypes E1-E4 originating from Zadar County, but grown from cloves in Zagreb (experimental field of University of Zagreb Faculty of Agriculture); III) plants/ecotype E5 originating from Vukovar-Srijem County, but grown from cloves in Zagreb. Collecting of leaves, used as a potential source of antigen, was done in the stage 3-5 well developed leaves. Collected leaves, symptomatic or asymptomatic, were tested by enzyme-linked immunosorbent assay (ELISA) on the presence of three viruses: OYDV, LYSV and GCLV. ELISA was performed using commercial kits provided by Bioreba (Switzerland) according to manufacturer's instructions. Each sample was tested twice on each virus. Final results were measured on spectrophotometer EL800 (Biotek, USA) two hours after adding the substrate. Samples with spectrophotometric values at least two times greater than average value of negative controls were considered positive. After laboratory tests, more detailed analysis of the symptoms and their correlation with the results of laboratory tests was done.

Results and discussion

ELISA results showed high infection rates of all five garlic ecotypes, especially with OYDV. Garlic collected in Zadar County showed prevalence of OYDV (93%), while GCLV (9.3%) and especially LYSV (2.3%) had significantly lower occurrence. Only in 3 plants (out of 43 tested) from that region presence of viruses was not confirmed. Similar situation was confirmed in garlic originating from Zadar, but grown in Zagreb, with prevalence of OYDV (99.4%) and presence of GCLV (21.9%) and LYSV (5.8%) with only 1 plant (out of 155) free of tested viruses. The highest infection rate was confirmed in Zagreb on plants originating from Vukovar-Srijem County where all 118 plants included in survey showed mixed infection with three viruses. Significant difference in virus composition and infection rate was discovered in material originating from Zadar County, while planting material from Vukovar-Srijem County showed significantly higher rate of GCLV and LYSV when compared to

material from Zadar County. Detailed review of sanitary status determined in this survey is given in Table 1. Infected plants showed reduced growth that was most evident at the beginning of vegetation. Additionally, plants infected with OYDV expressed symptoms of leaf yellowing, while yellow stripes were present on plants infected with LYSV. Yellowing, reddening and necrosis of leaf tips were detected on plants simultaneously infected with three viruses (Figure 1.). According to literature three viruses determined in this survey have worldwide distribution and are commonly found in all garlic grown regions, especially without adequate sanitary selection (Loebenstein and Lecoq 2012). In Europe, viruses infecting garlic have been reported from France (Lot et al. 1998), Greece (Dovas et al. 2001), Czech Republic (Klukácková et al. 2004; Smékalová et al. 2010), Poland (Chodorska et al. 2014) and Spain (Lunello et al. 2005). The vast majority of information relates to viruses economically important for production region of mentioned countries. Klukáčková et al. (2007) showed that five tested garlic varieties in Czech Republic were infected in average 75.4% by OYDV, 31.2% by LYSV and 99.6% by GCLV. Total, 80.9% of examined garlic plants had visible symptoms of virus infection on leaves. Dovas et al. (2001) and Dovas and Vovlas (2003) confirmed presence of OYDV in almost all tested plants from Greece and Italy. Symptoms observed in this survey are in accordance with those described by other authors (Van Dijk 1993, 1994). Although negative impact of viral infections is well documented in other countries (Loebenstein and Lecoq 2012), effect on Croatian garlic ecotypes is unknown. According to obtained results sanitary selection will be necessary for revitalization of the production of Croatian autochthonous garlic ecotypes either by finding virus-free plants from production field or elimination of viruses by cryotherapy, thermotherapy and/or meristem-tip culture.

Table 1. Virus infection rates determined in different garlic ecotypes (E1-E5) from Croatia

Origin	Ecotype	Total number of analyzed samples	GCLV (%)	OYDV (%)	LYSV (%)	Virus free (VF) (%)
Zadar County	E1	2	2 (100)	2 (100)	1 (50)	0
	E2	14	1 (7.1)	12 (85.7)	0	2 (1.4)
	E3	9	0	9 (100)	0	0
	E4	18	1 (5.6)	17 (94.4)	0	1 (5.6)
	Total	43	4 (9.3)	40 (93)	1 (2.3)	3 (6.9)
Zagreb *	E1	20	8 (40)	20 (100)	5 (25)	0
	E2	31	4 (12.9)	31 (100)	0	0
	E3	15	5 (33.3)	15 (100)	0	0
	E4	89	17 (19.1)	88 (98.9)	4 (4.5)	1 (1.1)
	Total	155	34 (21.9)	154 (99.4)	9 (5.8)	1 (0.7)
Zagreb**	E5	118	118 (100)	118 (100)	118 (100)	0
TOTAL	5	316	156 (49.4)	312 (98.7)	128 (40.5)	4 (1.3)

Legend: GCLV - *Garlic common latent virus;* OYDV - *Onion yellow dwarf virus;* LYSV - *Leek yellow stripe virus;* Zagreb*- plants grown in Zagreb, but cloves originating from Zadar County; Zagreb** - plants grown in Zagreb, but cloves originating from Vukovar-Srijem County

Figure 1. Symptoms of yellowing, reddening and leaf tip necrosis on garlic infected with *Garlic common latent virus*, *Onion yellow dwarf virus* and *Leek yellow stripe virus*.

Conclusions

Survey gives additional knowledge about occurrence and distribution of three viruses of garlic indicating deteriorated sanitary status of Croatian garlic ecotypes originating from Zadar and Vukovar-Srijem County. In the future adequate clonal and sanitary selection should be undertaken in order to give producers choice of using virus-free planting material as a prerequisite for stable production. This could have positive impact on revitalization and enhancement of production based on Croatian autochthonous garlic ecotypes which represent original Croatian value and are important segment of Croatian heritage.

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References

1. Abou El-Magd, M.M., Zaki, M.F., Abd El-Al, F.S. and Abd El-Samad, E.H. (2013). Growth analysis and chemical constituents of garlic plants in relation to morphological growth stages. Journal of Applied Sciences Research 9(2): 1170-1180.
2. Astier, S., Albouy, J., Maury, Y., Robaglia, C. and Lecoq, H. (2006). Principles of Plant Virology: Genome, Pathogenicity, Virus Ecology. Enfield, New Hamshire, USA: Science Publishers.
3. Chodorska, M., Paduch-Cichal, E., Kalinowska, E. and Szyndel, M.S. (2014). Assessment of Allexiviruses infection in garlic plants in Poland. Acta Scientiarium Polonorum Hortorum Cultus 13 (2): 179-186.
4. Conci, V.C., Canavelli, A. and Lunello, P. (2003). Yield losses associated with virusinfected garlic plants during five successive years. Plant Disease 87: 1411–1415.
5. Conci, V.C. (1997). Virus y Fitoplasmas de ajo. In ‘‘50 Temas Sobre Produccion deAjo’’ (J. L. Burba, ed.) EEA-INTA La Consulta, Mendoza. 267–293.
6. Dovas, C. I. and Vovlas, C. (2003). Viruses infecting Allium spp. In Southern Italy. Journal of Plant Pathology 85: 135.
7. Dovas, C. I., Hatziloukas, E., Salomon, R., Barg, E., Shiboleth, Y. M. and Katis, N. (2001). Incidence of viruses infecting Allium spp. In Greece. European Journal of Plant Pathology 149: 1-7.

8. Dumičić G., Miloš B., Žanić K., Urlić B., Jukić Špika M. and Čagalj M. (2015). Jadranski češnjak. Institut za jadranske kulture i melioraciju krša, Split.
9. FAO (2015). FAO Statistical Poketbook (2015). Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/3/a-i4691e.pdf
10. Hull, R. (2009). Comparative plant virology. Second edition, Elsevier Academic Press, Burlington, 24.
11. Juretić, N. (2002). Osnove biljne virologije. Školska knjiga, Zagreb: 48.
12. Klukáčková, J., Navrátil, N. and Duchoslav, M. (2007). Natural infection of garlic (*Allium sativum* L.) by viruses in the Czech Republic. Journal of Plant Diseases and Protection 114 (3): 97–100.
13. Klukáčková, J., Navrátil, N., Veselá, M., Havránek, P. and Šafářová, D. (2004). Occurrence of garlic viruses in the Czech Republic. Acta Fytotechnica et Zootechnica 7: 126–128
14. Loebenstein, G. and Lecoq, H. (eds.) (2012). Advances in Virus Research, Vol. 84, Burlington: Academic Press: 163-208.
15. Lešić, R., Borošić, J., Butorac, I., Herak-Ćustić, M., Poljak, M. and Romić, D. (2016). Povrćarstvo III. dopunjeno izdanje. Zrinski d.d. Čakovec: 138-145.
16. Lot, H., Chevelon, V., Souche, S. and Dellecolle, B. (1998). Effects of Onion yellow dwarf virus and Leek yellow stripe virus on symptomatology and yield loss of three French garlic cultivars. Plant Disease 82: 1381–1385.
17. Lunello, P., Ducasse, D. and Conci, V. (2005). Improved PCR detection of Potyviruses in Allium species. European Journal of Plant Pathology 112: 371–378.
18. Matotan, Z. (2004). Suvremena proizvodnja povrća. Nakladni zavod globus, Zagreb. 153-158.
19. Melo Filho, P., Resende, R.O., Torres Cordeiro, C.M., Buso, J., Torres A.C. and Dusi, A.N. (2006). Viral reinfection affecting bulb production in garlic after seven years of cultivation under field condtions. European Journal of Plant Pathology 116: 95–101.
20. Messianen, C., Youcef-Benkada, M. and Beyries, A. (1981.) Rendement potentiel et tolerance aux virus chen l'ail. Agronomie 1: 759-762.
21. Radat, B. (2014). Poslovni plan za proizvodnju češnjaka. Završni rad. Sveučilište Josipa Jurja Strossmayera, Poljoprivredni fakultet u Osijeku.
22. Smékalová, K., Stavlíková, H. and Dusek, K. (2010). Distribution of viruses in the garlic germplasm collection of the Czech Republic. Journal of Plant Pathology 92(1): 273–274.
23. Štefanac, Z. (1977). Virus žute kržljavosti crvenog luka u Jugoslaviji. Acta Botanica Croatica 36: 39—45.
24. Toth, N., Fabek, S. and Vončina, D. (2015). Završno izvješće: Revitalizacija proizvodnje češnjaka u Zadarskoj županiji. Agronomski fakultet, Zagreb.
25. Van Dijk, P. (1994). Virus diseases of Allium species and prospects for their control. Acta Horticulture 358: 299–306.
26. Van Dijk, P. (1993). Survey and characterization of potyviruses and their strains of Allium species. Netherlads Journal of Plant Pathology 99(Suppl. 2): 1–48.
27. Van Dijk, P., Verbeek, M. and Bos, L. (1991). Mite-borne virus isolates from cultivated Allium species and their classification into two new rymoviruses in the family Potyviridae. Netherlands Journal of Plant Pathology 97: 381-399.
28. Vunsh, R., Rosner, A. and Stein, A. (1991). Detection of bean yellow mosaic virus in Gladioli corms by the polymerase chain reaction. Annals of Applied Biology 119: 289-294.
Walkey, D.G.A. and Antill D.N. (1989). Agronomic evaluation of virus-free and virus infected garlic (*Allium sativum* L.). Journal of Horticulutral Science 64: 53.

MONITORING OF POTATO CYST NEMATODES (*GLOBODERA* SPP.) IN SOUTHWEST BULGARIA

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Abstract
The potato cyst nematodes, *Globodera pallida* and *Globodera rostochiensis*, can cause significant economic damage to potato production and their conduct is a difficult process. They are quarantine pests for Europe, which are regulated by different directives specific to different regions. The main objective of the study was to provide data of the determination of the spread of potato cyst nematodes in Southwest Bulgaria in order to limit their further dissemination outside the infected areas. In the period 2015-2016, the monitoring of potato cyst nematodes was carried out in six major potato-producing regions in Bulgaria (Samokov, Pernik, Kyustendil, Pazardzhik, Blagoevgrad and Smolyan). The monitoring has covered seed production areas and potato areas intended for human consumption and/or for processing. The results confirm the presence of both species in the observed potato-growing areas. The distribution and density of nematodes of the genus *Globodera* varies between regions. *G. rostochiensis* was the most common species in all areas. The species was found by itself in a higher level than in joint populations of *G. pallida*.

Key words: *Globodera pallida*, *G. rostochiensis*, distribution.

Introduction

Potato (*Solanum tuberosum*) is one of the most important food sources in the world (FAO 2008, Lutaladio and Castaldi 2009). The potato production in Bulgaria is concentrated in four main regions: Pazardjik, Plovdiv, Smolyan and Samokov. Significant economic damage to potato production worldwide can cause the plant parasitic nematodes. The estimation of potato yield showed that the losses can reach up to 9% per year as a result of attack by the potato cyst nematodes of the genus *Globodera* (Olsson, 2009). Many different control strategies have been developed to reduce the damage effect caused on potato crops, as a chemical control (Norshie et al. 2016), crop rotation (Evans and Haydock 2000, Eberlein et al. 2016) and use of resistant cultivars of potatoes (Sparkes, 2016). Potato cyst nematodes *Globodera pallida* (white potato cyst nematode) and *Globodera rostochiensis* (yellow potato cyst nematode) are quarantine pests and are regulated by various plant protection directives specific to the different regions. In Europe the spread and control of potato cyst nematodes is regulated by COUNCIL DIRECTIVE 2007/33/EC of 11 June 2007. The main ways of preventing and limiting the spread of potato cyst nematodes are the areas intended for seed propagation and the plants or plant parts intended for planting to be free of these nematodes. The current situation in the EU requires extensive studies on the spread and density of potato cyst nematodes and to develop new strategies to control them. The main objective of the study was to provide data of the determination of the spread of potato cyst nematodes in Southwest Bulgaria, in order to limit their further dissemination outside the infected areas.

Material and methods

The results of this work were obtained on the basis of field observations and laboratory analysis from March 2015 to October 2016. Six observation points were selected (Pernik, Kyustendil, Samokov, Pazardzhik, Blagoevgrad and Smolyan), on which potatoes were grown. The studies on the

presence and spread of potato cyst nematodes *Globodera pallida* (Stone, 1973), *Globodera rostochiensis* (Wollenweber, 1923) (Heteroderidae) included potato-growing areas intended for planting and for consumption and processing.

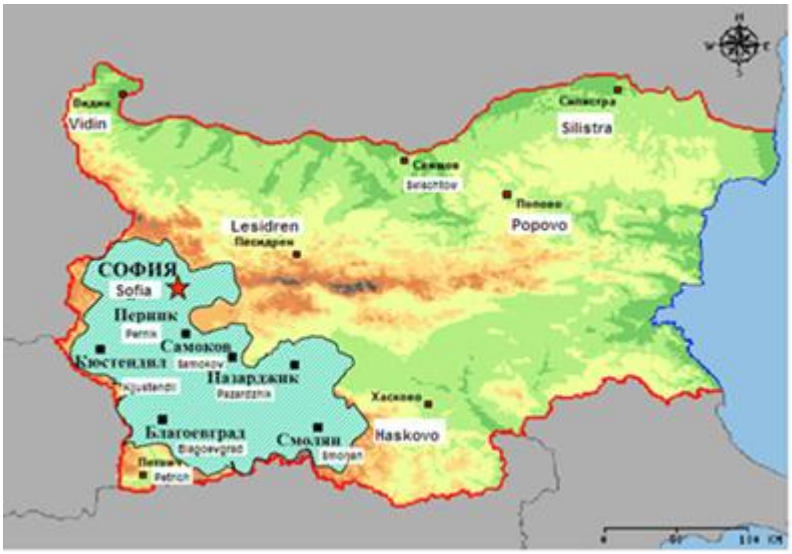

Figure 1. Regions in Southwestern Bulgaria, subject to monitoring of potato cyst nematodes

The soil samples were taken according to the procedure under Ordinance 17 of 3 June 2010 on the control of potato cyst nematodes (Ministry of Agriculture, Food and Forestry of Republic Bulgaria) during harvesting and before planting the potatoes. After mixing of the samples taken, average samples of 100 cm3 were compiled using a measuring cylinder. The resulting quantitative and qualitative data refer to this volume. The samples were placed in plastic bags, sealed and stored in a refrigerator at 4-5 °C to carry out laboratory analysis. Various extraction methods were used for the isolation and quantification of the cysts according to established methodology. The methods for the extraction of the life stages of the potato cyst nematodes (second-stage juveniles J2 and male) from the soil are according to the Cobb and Baermann technic. The vitality of eggs and juvenile stages was determined by macroscopic and microscopic analysis and by an egg-hatch test. The cysts, live juvenile stages and males in soil samples and live juvenile stages and cysts eggs were quantified. The average number of live nematodes in the sample (100 cm3) at the starting suspension of 100 ml was determined by Peters (2013). Species characterization and identification were based on morphology of various life stages ad cysts (Subbotin et al. 2010, EPPO 2013). In addition, the samples were examined for the presence of cyst nematodes by flotation methods.

Results and discussion

Monitoring of areas for the production of potatoes for consumption and processing

In 2016, the surveys were carried out in potato fields amounts to 18.400 ha, which is 15% of the total harvested areas in 2015 (122.484 ha). In 2015-2016, the production of 597 registered and 71 unregistered producers of potatoes was controlled.The plant-health control of potatoes has been carried out in the potato-growing areas of 30.1528 ha, but only of potato cyst nematodes in 9.8 ha areas; 660 soil samples were analysed. The identified plant-parasitic nematodes belong to the order Tylenchida and order Dorylaimida. The cyst nematodes were identified as: *Globodera rostochiensis* (Wollenweber 1923) *Globodera pallida* (Stone 1973) and *Heterodera* spp. All *Globodera* cysts, isolated from the six regions of Southwestern Bulgaria were morphologically identified to species level as *G. rostochiensis* and *G. palida*, located in the following areas:

- *G. pallida* - in 50 soil samples (5 Smolyan, 9 Sofia-District, 34 Samokov, 2 Pazardzhik);
- *G. pallida* /dead stages/ - in 1 soil samples (1 Pazardzhik);
- *G. rostochiensis* - in 63 soil samples (42 Sofia-District, 20 Samokov, 1 Pazardzhik);

- *G. pallida* + *Globodera rostochiensis* - in 1 soil sample (Sofia-district).

Table 1. Distribution of established species *G. pallida* and *G. rostochiensis* in the observed areas

	G. pallida	*G. rostochiensis*	*G. pallida + G. rostochiensis*	Total number of infected samples
Blagoevgrad				
Pazardzhik	2+1 /dead stages/	1		3
Smolyan	5	20		25
Sofia district	9	42	1	52
Samokov	34			34
Pernik	0			
Kyustendil	0			
Total:	50	63	1	113

As a result of the control, in the year 2015-2016, a total of 1.6 ha (124 areas) were mapped:

Monitoring of potato seed production areas

In the soil samples from the surveyed areas before planting (1.9 ha) and in those, taken during the vegetation period from the seed production areas, the nematodes of genus *Globodera* were not found. The obtained results overlap with the studies conducted by Samaliev and Stoyanov (2007) and Samaliev (2011) and confirm the prevalence of the two species of potato cyst nematodes in Southwestern Bulgaria. Samaliev (2011) reported the identification of pathotypes 1, 3 and 4 of the *G. rostochiensis* (Ro1, Ro3, Ro4) and pathotypes 2 and 3 of *G. pallida* (Pa2, Pa3) and found differences in their pathogenicity. The pathotype Ro1 dominated in *G. rostochiensis* -populations, followed by and then Ro3 and Ro4, whereas Pa3predominated in *G. pallida populations*.

Quantitative analysis of live stages of Globodera in soil samples

An assessment of the ecological and physiological status of *G. rostochiensis* and *G. palida* were made on the basis of the cyst viability analysis (Figure 2). Because of the labor-intensive process, data on the presence of male individuals in soil samples are not presented. In Fig. 2 are the values of the mean number of individual life-stages of nematodes.

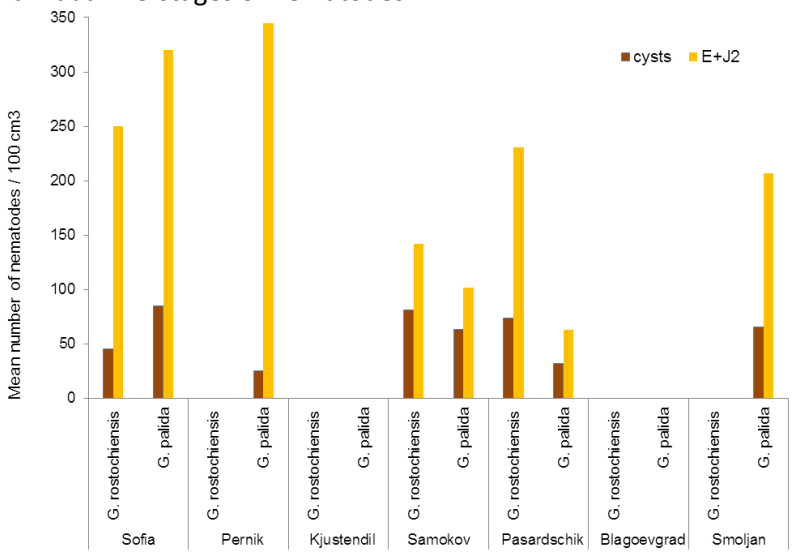

Figure 2. Average number of live eggs and juvenile stages in the cysts of *G. rostochiensis* and *G. pallida* in the observed areas

The mean number of live eggs and juvenile stages J1 in the cysts, as an indication of the extent of distribution, was lower than the incidence of cyst density in the soil. For research period and for the areas where the monitoring was carried out, it could be concluded that the potential risk of spread of infection is decreased. The results obtained by Samaliev (2011) over the period 2006-2008 overlap with our research, but the population density of *Globodera* spp. was lower. For example, for the region of Samokov, the author reports 7-1980 eggs / 100 g of soil, in our studies the number of live eggs and juvenile stages in the cysts of *G. rostochiensis* was 142 E + J2 / 100 cm3, and for *G. palida* - 102 E + J2 / 100 cm3). Considering that the seasonal dynamics of nematodes of Globodera is determined by numerous factors (Ebrahimi et al. 2014). The reasons for the observed differences may be due to the microclimatic characteristics of the area and the meteorological changes in the studied periods. In order to answer this question, the dynamics of the species should be tracked over a longer period of time in close relation to soil and climatic conditions. However, the research was carried out under real production conditions and makes it possible to detect changes in the species composition and density of potato cyst nematodes.

Conclusions

The study revealed the following conclusions:

- Two species of potato cyst nematodes on *G. rostochensis* and *G. pallida* were found in the six potato-growing regions in Bulgaria (Samokov, Pernik, Pazardzhik, Blagoevgrad and Smolyan), with the highest number of cysts were isolated from the Samokov region.
- Rates of spread and density of *Globodera* spesies ranged between regions. *G. rostochiensis* was the most common species in all areas. This species occurred alone in a higher degree than in joint populations of G. pallida.
- The mean number of live eggs and juvenile stages in the cysts as an indicator of the spread of potato cyst nematodes was lower than the frequency of isolation cyst in the soil.

References

1. Council Directive 2007/33/EC of 11 June 2007 on the control of potato cyst nematodes (and repealing Directive 64/465/ EEC).
2. Eberlein, C., Heuer, H., Vidal, S. and Westphal, A. (2016). Population dynamics of globodera pallida under potato monoculture. Nematropica, 46(2): 114-120.
3. Ebrahimi, N., Viaene, N., Demeulemeester, K., and Moens, M. (2014). Observations on the life cycle of potato cyst nematodes, Globodera rostochiensis and G. pallida, on early potato cultivars. Nematology, 16(8): 937-952.
4. EPPO (2013) Diagnostic Protocol PM 7/40(3) for Globodera rostochiensis and G. pallida. Bulletin OEPP/EPPO Bulletin 43: 119–138.
5. Evans, K. and Haydock, P. P. J. (2000). Potato cyst nematode management-present and future. Aspects of Applied Biology, (59): 91-97.
6. FAO, 2008. <http://www.potato2008.org.en/aboutiyp/index.html> (2008-11-06)
7. Lutaladio, N.and Castaldi, L. (2009). Potato: the hidden treasure. Journal of Food Composition and Analysis, *22*(6): 491-493.
8. Norshie, P. M., Grove, I. G. and Back, M. A. (2016). Field evaluation of the nematicide fluensulfone for control of the potato cyst nematode Globodera pallida. Pest management science, 72(10): 2001-2007.
9. Olsson, C. (2009). Characterisation of mechanisms involved in hatching of the potato cyst nematode.
10. Peters, A. (2013). Application and commercialization of nematodes. Applied microbiology and biotechnology, 97(14): 6181-6188.
11. Sparkes, J. (2016). Potential trap crops for the control of Potato Cyst Nematode (PCN).
12. Subbotin, S.A., Mundo-Ocampo, M. and Baldwin, J.G. (2010). Systematics of Cyst Nematodes (Nematoda: Heteroderinae). Leiden, Netherlands: Brill.

CMV, AMV, PVY: DYNAMICS OF VIRUS ANTIGEN ACCUMULATION IN SINGLE AND MIXED INFECTIONS

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Abstract

Pepper plants cultivated in open fields are highly susceptible to plant viruses. That is why, apart from single, viruses appear and in mixed infections. The aim of this study was to examine the eventual interactions in the mixed infections between the three most common viruses on pepper plants in R. Macedonia, such as *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY). Virus antigen accumulation was measured with the use of DAS-ELISA method. The dynamics of the antigen accumulation was measured three times during the vegetation in a three-year trial. Single infections were observed to be more spread than mixed infections during the tested period. Mixed infections appeared in 2 – 12% from the inspected plants. Most of the mixed infections included CMV, being the most spread virus in the tested period. During this trial, a significant interaction between the virus antigen accumulations of the tested viruses in the mixed infections could not be observed, leaving space for further and more profound examinations.

Keywords: DAS-ELISA, viruses, pepper production.

Introduction

Pepper (*Capsicum annuum* L.) is one of the most important cultivated crops in the Republic of Macedonia (Jankulovski, 1997). Considering pepper production, R. Macedonia is amongst the top ten countries in Europe (FAO, 2015). Pepper cultivated in open fields is more susceptible to virus infections, than pepper cultivated in green houses (Bogatzevska et al., 2007). Since most of the pepper plants in R. Macedonia are cultivated in open fields (Tudzarov, 2011), plant viruses represent a major problem and limiting factor in pepper production (Jovanchev et al., 1996; Rusevski and Bandzo, 1998). The most common pepper viruses are: *Cucumber mosaic virus* - CMV, *Alfalfa mosaic virus* - AMV, *Tobacco mosaic virus* - TMV, *Tomato spotted wilt virus* - TSWV, *X* and *Y viruses of potato* - PVX and PVY etc. **(**Jovanchev et al., 1996; Choi et al., 2005; Ormeño et al., 2006; Kim et al., 2010; Milošević, 2013). These pathogens can cause damage in pepper production up to 100% (Šutić, 1995; Jovanchev et al., 1996). Previous findings on occurrence and distribution of viruses on pepper cultivated in open fields showed that in R. Macedonia the most widespread virus infections were by CMV, followed by AMV and PVY (Rusevski et al., 2009; 2010; 2011; 2013). The damage which these viruses cause to the plants is even more enhanced during mixed infections. In mixed infections, CMV expresses more severe symptoms and causes more extreme growth inhibition (Procházková, 1970; Murphy and Bowen, 2006; Kim et al., 2010), as well as increasing of the virus titer in the infected tissues (Wang et al., 2002; Murphy and Bowen, 2006). PVY is usually found in mixed infections, causing more severe damage (Šutić, 1995). Symptom manifestation of virus infections depends on various factors, amongst which are mixed infections caused by two or more viruses (Kim et al., 2010) and may vary from typical, to masked and atypical symptoms (Nair et al., 2009). Because of that, visual detection has only a preliminary role, while for a final diagnosis and virus determination, especially in mixed infections, laboratory tests such as DAS-ELISA are performed. That is why, during determination of virus occurrence of the most widespread pepper viruses on the territory of R. Macedonia, special attention was given to the mixed infections in the plants and their possible interactions.

Material and methods

Collection of plant samples

The study was conducted during 2012, 2013 and 2014 on pepper plants cultivated in open fields in R. Macedonia. Eight important pepper production regions were included in the survey: areas around Skopje (1 locality), Kumanovo (1 locality), Sveti Nikole (2 localities in 2012 and 1 locality in 2013 and 2014), Kochani (2 localities), Strumica (2 localities), Radovish (2 localities), Prilep (1 locality) and Bitola (2 localities). In each locality, field inspection was conducted three times during the vegetation: end of June, after planting of seedling material in the field; middle of August, during flowering and end of September, while harvesting. Sample collection was performed from seven randomly chosen plants. In order to perform serological testing, young pepper leaves were collected from the upper parts of the plants.

Serological analysis

The presence of the inspected viruses and the dynamics of the antigen accumulation were determined on collected leaf samples tested by Double Antibody Sandwich – Enzyme Linked Immunosorbent Assay (DAS-ELISA), as described by Clark and Adams (1977) and modified as proposed by Bioreba AG (Wernli, 1999), using commercial polyclonal antisera. Plant tissue samples were homogenized in extraction buffer (1:10 w/v). Commercial positive and negative controls produced from the same manufacturer were included on each plate. The tested samples were considered to be positive if the average optical density (OD) value after incubation of one hour at room temperature in the dark was higher at least twice than the average OD of the negative control, measured with an ELISA microplate reader MULTISCAN ASCENT at absorbance of 405 nm (Boonham et al., 2003; Vučurović et al., 2012).

Results and discussion

Virus occurrence in single and in mixed infections

During the whole three-year trial, CMV was observed to be the most prevalent virus of pepper plants cultivated in open fields in R. Macedonia (51% in 2012, 34% in 2013 and 61% in 2014) (Table 1). In 2012, AMV was detected as second (15%), while in 2013 and 2014 it was PVY (7% and 8%, respectively). Plant viruses regularly occur on pepper plants throughout the vegetation in R. Macedonia (Jovanchev et al., 1996; Rusevski et al., 2011; 2013) and other countries (Choi et al., 2005; Kim et al., 2010; Milošević, 2013), causing economic losses and representing a major threat for pepper production. The frequency of CMV on pepper was confirmed and in other studies (Choi et al., 2005; Ormeño et al., 2006; Kim et al., 2010). During the tested period, viruses appeared more in single, than in mixed infections. Mixed infections were primarily observed in 2012 (12%). Their frequency declined during the examined years, so in 2013 it was 5% and in 2014 mixed infections were determined only in 2% of the tested samples. Further investigation to why this frequency declining occurred during the years, should be performed. Mixed infections with CMV, AMV and PVY were also detected in other studies (Avilla et al., 1997; Kim et al., 2010; Milošević, 2013).

Table 1. Incidence of *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY) in single and mixed infections on pepper plants in R. Macedonia during 2012-2014

Year	Number of tested samples	Single infections			Mixed infections			
		CMV	AMV	PVY	CMV+AMV	CMV+PVY	AMV+PVY	CMV+AMV + PVY
2012	91	37 (41%)	4 (4%)	1 (1%)	7 (8%)	1 (1%)	2 (2%)	1 (1%)
2013	84	24 (29%)	1 (1%)	2 (2%)	0	3 (4%)	0	1 (1%)
2014	84	49 (59%)	2 (2%)	5 (6%)	0	2 (2%)	0	0

The occurrence of mixed infections was considered to be a common event (Murphy and Bowen, 2006). In our study, CMV, being the most widespread pepper virus, was detected in almost all of the

mixed infections. Because of its distribution, CMV was observed as part of many mixed infections with other viruses by various authors (Fraile et al., 1997; Kim et al., 2010; Chen et al., 2011; Vučurović et al., 2010; Rusevski et al., 2013). In the studies of Avilla et al. (1997) and Milošević (2013), the most common mixed infection was observed between CMV and PVY, which corresponded to our findings from 2013 and 2014. In 2012 and 2013, a triple mixed infection was observed in the area around Kumanovo. Many other authors have observed mixed infections with more than two viruses (Kim et al., 2010; Vučurović et al., 2010; Rusevski et al., 2013).

Table 2. Incidence of *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY) in single and mixed infections on pepper plants per regions in R. Macedonia during 2012-2014

Tested regions	Year	Single infections			Mixed infections			
		CMV	AMV	PVY	CMV + AMV	CMV + PVY	AMV + PVY	CMV + AMV + PVY
Kochani[2]	2012	0	1 (7%)	1 (7%)	0	0	2 (14%)	0
	2013	5 (36%)	0	0	0	1 (7%)	0	0
	2014	12 (86%)	0	0	0	0	0	0
Prilep[1]	2012	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0
	2014	2 (29%)	0	1 (14%)	0	0	0	0
Bitola[2]	2012	7 (50%)	2 (14%)	0	1 (7%)	0	0	0
	2013	0	1 (7%)	0	0	1 (7%)	0	0
	2014	7 (50%)	2 (14%)	0	0	0	0	0
Skopje[1]	2012	5 (71%)	0	0	2 (29%)	0	0	0
	2013	3 (43%)	0	0	0	0	0	0
	2014	5 (71%)	0	1 (14%)	0	0	0	0
Kumanovo[1]	2012	4 (57%)	0	0	0	1 (14%)	0	1 (14%)
	2013	2 (29%)	0	2 (29%)	0	0	0	1 (14%)
	2014	3 (43%)	0	1 (14%)	0	0	0	0
Strumica[2]	2012	9 (65%)	1 (7%)	0	2 (14%)	0	0	0
	2013	5 (36%)	0	0	0	1 (7%)	0	0
	2014	4 (29%)	0	2 (14%)	0	1 (7%)	0	0
Radovish[2]	2012	0	0	0	0	0	0	0
	2013	5 (36%)	0	0	0	0	0	0
	2014	10 (72%)	0	0	0	1 (7%)	0	0
Sveti Nikole	2012[2]	12 (86%)	0	0	2 (14%)	0	0	0
	2013[1]	4 (57%)	0	0	0	0	0	0
	2014[1]	6 (86%)	0	0	0	0	0	0

[1] 1 location per area was tested, 7 marked plants

[2] 2 locations per area were tested, 7 marked plants

Dynamics of virus antigen accumulation in mixed infections

During this study, three times during the vegetation virus antigen accumulation in the infected pepper plants was measured using the DAS-ELISA test. One of the aims of this study was to investigate if and how the viruses included in the mixed infections influence each other's dynamics and virus accumulation. The only mixed infection which did not include CMV was between AMV and PVY in the area around Kochani in 2012 on two infected pepper plants. Dynamics of the virus antigen accumulation of these two viruses is shown in Table 3.

In the mixed infections with AMV+PVY detected on the marked pepper plants included in this study, any higher OD values or significant virus accumulation fluctuations were not observed. Dynamics of virus antigen accumulation of the other types of mixed infections is given in Tables 4, 5 and 6.

Table 3. Dynamics of virus antigen accumulation of AMV and PVY (according to OD absorbance) measured in marked pepper plants with AMV+PVY mixed infection during the tested period 2012-2014

Tested regions	Year	Plant	Tested viruses and number of repetitions					
			AMV			PVY		
			I	II	III	I	II	III
Kochani	2012	1	-	-	0.261	-	-	0.368
		2	-	-	0.226	-	0.267	0.379

I, II, III – different testing periods (I – beginning of vegetation, II – middle of vegetation, III – end of vegetation)
[-] the sample is virus free from the inspected virus (OD absorbance bellow 0.100 is measured)

Table 4. Dynamics of virus antigen accumulation of AMV and CMV (according OD absorbance) measured in marked pepper plants with AMV+CMV mixed infection during the tested period 2012-2014

Tested regions	Year	Plant	Tested viruses and number of repetitions					
			AMV			CMV		
			I	II	III	I	II	III
Bitola	2012	1	-	-	0.273	-	0.777	0.555
Skopje	2012	1	-	-	0.249	-	0.684	1.457
		2	-	-	0.260	-	0.756	0.583
Strumica	2012	1	-	-	0.311	-	0.612	0.554
		2	-	0.192	0.362	-	-	0.727
Sveti Nikole	2012	1	-	-	0.241	-	0.695	0.255
		2	-	-	0.204	-	0.758	0.420

I, II, III – different testing periods (I – beginning of vegetation, II – middle of vegetation, III – end of vegetation)
[-] the sample is virus free from the inspected virus (OD absorbance bellow 0.100 is measured)

In most of the cases of mixed infections with CMV+AMV, after the inoculation with AMV on already infected plants with CMV, declining of CMV virus accumulation was observed. Most evident examples were on the infected plants in the area around Sveti Nikole. In contrary, in the area around Skopje after the AMV infection, the OD absorbance measured during the second control grew up during the third. Based only on the number of these tested plants, a conclusion can't be drawn, but a pattern may be sensed, which draws further investigations in the interactions between AMV and CMV in mixed infections.

Table 5. Dynamics of virus antigen accumulation of PVY and CMV (according OD absorbance) measured in marked pepper plants with CMV+PVY mixed infection during the tested period 2012-2014

Tested regions	Year	Plant	Tested viruses and number of repetitions					
			CMV			PVY		
			I	II	III	I	II	III
Kochani	2013	1	-	0.471	1.896	0.377	0.310	4.322
Bitola	2013	1	-	0.319	0.204	0.237	0.244	0.260
Kumanovo	2012	1	-	-	0.714	-	-	3.420
Strumica	2013	1	0.255	3.008	1.337	0.318	0.251	0.258
	2014	1	-	-	0.772	-	-	0.217
Radovish	2014	1	-	-	0.796	-	-	0.209

I, II, III – different testing periods (I – beginning of vegetation, II – middle of vegetation, III – end of vegetation)
[-] the sample is virus free from the inspected virus (OD absorbance bellow 0.100 is measured)

During the three-year trial, unlike the other types of mixed infections, the mixed infections with CMV+PVY were detected during the whole tested period (Table 5). It was determined that in some

cases CMV virus accumulation declined in the CMV+PVY infections (example from the area around Strumica). In other cases, the virus antigen accumulation of both viruses increased (in the area around Kochani. During these mixed infections, very high OD values for both of the tested viruses were measured, giving the assumption of synergistic interaction between these viruses. Choi et al. (2002) have established that in mixed infections between CMV and some *Potyvirus* on zucchini plants, an increase of the CMV virus antigen accumulation occurred.

Table 6. Dynamics of virus antigen accumulation of AMV, PVY and CMV (according to OD absorbance) measured in marked pepper plants with AMV+PVY+CMV mixed infection during the tested period 2012-2014

Tested regions	Year	Plant	Tested viruses and number of repetitions								
			AMV			CMV			PVY		
			I	II	III	I	II	III	I	II	III
Kumanovo	2012	1	-	-	0.280	-	0.561	0.405	-	-	4.016
	2013	1	2.575	-	0.703	-	0.263	0.733	1.699	4.866	3.999

I, II, III – different testing periods (I – beginning of vegetation, II – middle of vegetation, III – end of vegetation)
[-] the sample is virus free from the inspected virus (OD absorbance bellow 0.100 is measured)

Triple mixed infections with all of the tested viruses was detected in 2012 and 2013 in the area around Kumanovo on two occasions (Table 6). In 2012, a decrease in the CMV virus antigen accumulation was observed, after the pepper plant was infected with AMV and PVY. Unlike the previous year, in 2013 an increase in the virus antigen accumulation of CMV was observed. Also, an increase of AMV and decrease of PVY accumulation in the triple mixed infection was detected. Overall, after observing the fluctuations in the virus antigen accumulations of the inspected viruses (AMV, CMV and PVY) in the mixed infections on the marked pepper plants, several correlations could be pointed out: antagonistic effect in AMV+CMV and synergistic interaction in CMV+PVY. Because of the small number of tested samples, no significant correlation could be determined. Other authors also came to different conclusions regarding this matter. Kim et al. (2010), which were inspecting mixed infections with CMV on pepper, could not find any significant correlation between the interactions of the different viruses present in mixed infections. Unlike these results, Choi et al. (2002) have established that in mixed infections between CMV and some *Potyvirus* on zucchini plants, an increase of the CMV virus antigen accumulation occurred.

Conclusions

During the tested period on the pepper plants cultivated in the open fields in R. Macedonia, it was determined that single infections were more dominant and widespread, than mixed infections of AMV, CMV and PVY. The occurrence of the mixed infections declined from 12% in 2012, 5% in 2013, to only 2% in 2014. After analyzing the dynamics of the virus antigen accumulation of the inspected viruses in the mixed infections, a door has opened for further investigations in this field, leaving space for speculation of possible interactions between tested viruses. The dynamics of replication of the virus particles and their distribution in the systemically infected host plants needs to be understood and studied further, in order to better understand the interactive influence of various external and internal factors that impact virus fluctuations in mixed infections.

References

1. Avilla, C., Collar, J. L., Duque, M. and Fereres, A. (1997). Yield of bell pepper (*Capsicum annuum*) inoculated with CMV and/or PVY at different time intervals, zeitschrift fur pflanzenkrankheiten und pflanzenschutz. Journal of plant diseases and protection, 104: 1-8.

2. Bogatzevska, N., Stoimenova, E. and Mitrev, S. (2007). Bacterial and Virus diseases spread in Bulgaria and Macedonia on field and greenhouse pepper. Plant protection, 18: 17-21.

3. Boonham, N., Walsh, K., Smith, P., Madagan, K., Graham, I. and Barker, I. (2003). Detection of potato viruses using microarray technology: towards a generic method for plant viral disease diagnosis. Journal of Virological Methods, 108: 181-187.
4. Chen, S., Gu, H., Wang, X., Chen, J. and Zhu, W. (2011). Multiplex RT-PCR detection of Cucumber mosaic virus subgroups and Tobamoviruses infecting Tomato using 18S rRNA as an internal control. Acta Biochim Biophys Sin, 43: 465–471.
5. Choi, G.S., Kim, J.H., Lee, H.D., Kim, J.S. and Ryu, K.H. (2005). Occurrence and distribution of viruses infecting pepper in Korea. Plant Pathol. J, 21(3): 258-261.
6. Choi, S. K., Yoon, J. Y., Ryu, K. H., Choi, J. K., Palukaitis, P. and Park, W. M. (2002). Systemic movement of a movementdeficient strain of Cucumber mosaic virus in zucchini squash is facilitated byg a cucurbit-infecting potyvirus. Journal of General Virology, 83: 3173–3178.
7. Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of the plant viruses. J. Gen. Virology, 34 (3): 457-483.
8. FAO. (2015). http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor
9. Fraile, A., Alonso-Prados, J. L., Aranda, M. A., Bernal, J. J., Malpica, J. M. and Garcia-Arenal, F. (1997). Genetic exchange by recombination or reassortment is infrequent in natural populations of a tripartite RNA plant virus. J Virol, 71: 934–940.
10. Jankulovski, D. (1997). Pepper and tomato. NIP "BAS – TRADE", Skopje: 1-126.
11. Jovanchev, P., Pejchinovski, F., Jankulovski, D., Rusevski, R., Bandzo, S. and Popsimonova, G. (1996). Health status of pepper plants in the Republic Macedonia in 1995. Annual Book of Proceedings of Plant Protection, 7: 159-169.
12. Kim, M.S., Kim, M.J., Hong, J.S., Choi, J.K. and Ryu, K.H. (2010). Patterns in disease progress and the influence of single and multiple viral infections on pepper (*Capsicum annuum* L.) growth. Eur J Plant Pathol, 127: 53–61.
13. Milošević, D. (2013). Diversity and characterization of pepper viruses in Serbia. Ph.D. Thesis. Faculty of Agriculture, University in Belgrade, Belgrade, Serbia.
14. Murphy, J. F. and Bowen, K. L. (2006). Synergistic disease in pepper caused by the mixed infection of *Cucumber mosaic virus* and *Pepper mottle virus*. Phytopathology, 96: 240-247.
15. Nair, R. M., Habili, N. and Randles, J. W. (2009). Infection of *Cullen australasicum* (syn. *Psoralea australasica*) with *Alfalfa mosaic virus.* Australasian Plant Disease Notes, 4: 46–48.
16. Ormeño, J., Sepúlveda, P., Rojas, R. and Araya, J.E. (2006). *Datura* Genus Weeds as an Epidemiological Factor of *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), and *Potato virus Y* (PVY) on Solanaceus Crops. AgriculturaTécnica (Chile), 66 (4): 333-341.
17. Procházková, Z. (1970). Interaction of Cucumber Mosaic Virus and Potato Virus Y with Tobacco Mosaic Virus. Biol. Plant., 12: 297-304.
18. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Gjamovski, V. (2009). Virus status of pepper plants in the area around Kochani in 2008. Plant protection, Skopje, 20: 84-88.
19. Rusevski, R., Bandzo, K., Kuzmanovska, B., Sotirovski, K. and Risteski, M. (2013). Occurrence, distribution and dynamics of virus antigen accumulation in pepper cultivation on open fields in Republic of Macedonia during 2008-2009. African Journal of Agricultural Research, 8 (28): 3836-3841.
20. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Stanoevska, M. (2010). Virus status of pepper plants cultivated on open fields in R. Macedonia in 2009. Plant protection, Skopje, 21: 44-50.
21. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Stanoevska, M. (2011). Virus status of pepper plants cultivated on open fields. In: XVI International meeting on Biotechnology – Book of Proceedings, Čačak, Serbia, 2011: 429-434.
22. Rusevski, R. and Bandzo, S. (1998). Influence of some microclimate characteristics (temperature and humidity) on the aphid population and virus infections on pepper in green houses. Annual Book of Proceedings of Plant Protection, Skopje, 9: 141-159.
23. Tudzarov, T. (2011). Novel plant species and technologies in the vegetable production. Alfa 94, Skopje, R. Macedonia.

24. Vučurović, A., Bulajić, A., Stanković, I., Ristić, D., Berenji, J., Jović, J. and Krstić, B. (2010). Occurrence, molecular detection and identification of Cucumber mosaic virus infecting pumpkin and squash in Serbia. In: 10th Meeting on plant protection, Zlatibor, 2010: 58-59.
25. Vučurović, A., Bulajić, A., Stanković, I., Ristić, D., Berenji, J., Jović, J. and Krstić, B. (2012). Non-persistently aphid-borne viruses infecting pumpkin and squash in Serbia and partial characterization of Zucchini yellow mosaic virus isolates. Eur J Plant Pathol, 133: 935–947.
26. Wang, Y., Gaba, V., Yang, J., Palukaitis, P. and Gal-On, A. (2002). Characterization of synergy between *Cucumber mosaic virus* and potyviruses in cucurbit hosts. Phytopathology, 92: 51-58.
27. Wernli, B. (1999). Bioreba AG
28. Šutić, D. (1995). Plant viruses. Institute for plant protection and environment, Belgrade: 1-394.

USE OF RT-PCR TECHNIQUES IN DETECTION OF VIRUSES OF PEPPER IN REPUBLIC OF MACEDONIA

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Abstract
Plant viruses can be a limiting factor and cause economically significant damage in the agricultural production. That is why, early and precise detection is very important in prevention of significant yield losses. In this study, early detection of plant viruses was performed on pepper, as one of the economically most important crops in Republic of Macedonia. Pepper plants were tested for *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY), most common RNA viruses in pepper production. Virus antigen accumulation was measured with the use of DAS-ELISA method on 259 plant samples. CMV was detected as most prevalent virus on pepper (51% in 2012, 34% in 2013 and 61% in 2014). In 2012, AMV was detected as second, while in 2013 and 2014 was PVY. Detection was performed using Reverse Transcription – Polymerase Chain Reaction (RT-PCR). The RNA was extracted using TRIzol method from the collected leaf samples. RT-PCR was performed for each of the viruses separately due to the differences in the annealing temperatures of the used primers. In order to prevent RNA degradation of the plant viruses, each step of manipulation with the plant material was carefully optimized. The gained amplicons after RT-PCR, associated with different viruses were: for CMV the amplicon was 773 bp, for AMV was 669 bp, while for PVY 902 bp, respectively. The RT-PCR method exhibited high sensitivity, detecting the viruses in the early stages of infection, before observation of any visible symptoms on the pepper plants and measuring negative OD absorbance by DAS-ELISA.

Key words: molecular detection, CMV, AMV, PVY.

Introduction

Pepper (*Capsicum annuum* L.) is one of the most important cultivated crops in the Republic of Macedonia (Jankulovski, 1997) with production amongst the top ten countries in Europe (FAO, 2015). Plant viruses represent a major problem and a limiting factor in pepper production (Jovanchev et al., 1996; Rusevski and Bandzo, 1998). The most common pepper viruses are: *Cucumber mosaic virus* - CMV, *Alfalfa mosaic virus* - AMV, *Tobacco mosaic virus* - TMV, *Tomato spotted wilt virus* - TSWV, Potato virus *X* and *Y* - PVX and PVY **(**Jovanchev et al., 1996; Milošević, 2013). These pathogens can cause damage in pepper production up to 100% (Šutić, 1995; Jovanchev et al., 1996). Symptom manifestation of plant virus infection depends on various factors, amongst which are weather conditions, overall condition of the plant itself, susceptibility of the pepper variety, virus virulence (Šutić, 1995), as well as time of virus infection and presence of mixed infections caused by two or more viruses (Kim et al., 2010). Because of these factors, symptoms may vary from typical, to masked or atypical symptoms (Nair et al., 2009). Because of that, visual detection of virus infections has only a preliminary role. Although some viruses, such as AMV, cause specific symptoms, still virus determination based solely on visual observation is not sufficient. Symptoms may point to general virus infection, while for a final diagnosis and virus determination, laboratory tests should be performed. For fast detection of the inspected viruses, usually serological methods, which detect the virus on a protein level, such as Double Antibody Sandwich – Enzyme Linked Immunosorbent Assay (DAS-ELISA), are performed.

They are relatively fast, robust and simple with satisfactory level of sensibility (Alonso-Prados et al., 2003; Chatzivassiliou et al., 2004). Comparing the serological with the molecular methods, the latter are distinguished with a greater sensitivity (Raj et al., 2002; Niimi et al., 2003). Because of that, in order to gain more accurate results, molecular methods such as Polymerase Chain Reaction (PCR) and Reverse Transcription – Polymerase Chain Reaction (RT-PCR) for RNA viruses are performed (Nie and Singh, 2000; Lee and Ryu, 2009).
This study was succeeding previous findings of virus occurrence on pepper in R. Macedonia (Rusevski et al., 2009; 2010; 2011; 2013), promoting molecular methods as a means of detection of plant viruses in the agricultural production.

Material and methods

Sample collection

A total of 259 pepper plant samples (91 sample in 2012, 84 samples in 2013 and 84 samples in 2014) were collected after visual inspection at 13 different localities from eight pepper production regions in R. Macedonia: Skopje, Kumanovo, Sveti Nikole, Kochani, Strumica, Radovish, Prilep and Bitola. Samples were collected from symptomatic plants showing general virus symptoms, such as: bright yellow to white mosaic, chlorosis and deformation on leaves, plant tissue necrosis and stunted growth. In order to perform isolation and testing of the inspected viruses (AMV, PVY and CMV), pepper leaves were collected from the upper parts of the plant. The samples from the field were brought to the laboratory by placing them in liquid nitrogen, in order to prevent RNA degradation (de Wijs and Suda-Bachmann, 1979) and later on were stored at -80ºC until tested (Chen et al., 2011; Wang et al., 2012).

Serological analysis

The presence of the inspected viruses was determined on the collected leaf samples tested by DAS-ELISA, as described by Clark and Adams (1977) and modified and proposed by Bioreba AG (Wernli, 1999), using commercial polyclonal antisera. Plant tissue samples were homogenized in extraction buffer (1:10 w/v). The tested samples were considered to be positive if the average optical density (OD) value after one hour incubation at room temperature in the dark was at least twice higher than the average OD of the negative control. Measures were done on ELISA microplate reader MULTISCAN ASCENT at the wavelength of 405 nm.

Molecular tests

Total RNA was extracted from 30 selected samples, using TRIzol® Reagent (Ambion, Life Technologies) according to manufacturer's instructions (Xu and Nie, 2006; Chen et al., 2011). To prevent RNA degradation, homogenization of the plant material was performed in liquid nitrogen (Bertolini et al., 2003). RT was performed in a total volume of 20 µl reaction mixture consisting of 3µl of total RNA, 2µl 10xPCR Buffer Gold, 4µl $MgCl_2$ (25mM), 8µl dNTP's (2.5mM), 1µl (50pM/µl) of reverse (antisense) primer R, 1µl RNase Inhibitor and 1µl of MuLV Reverse Transcriptase (Applied Biosystems, USA). Detailed review of primers used, their sequence and amplicon sizes is given in Table 1. The RT was performed according to van Dongen et al. (1999).

Table 1. Primers used in RT-PCR for amplification of the capsid protein gene of CMV, AMV and PVY

Primer	Sequence (5' to 3')	Amplicon size (bp)	Reference
F2 CMV	ATGGACAAATCTGRATCWMCC	773	Deyong et al. (2005)
R2 CMV	CTGGATGGACAACCCGTTC		
F2 AMV	ATCATGAGTTCTTCACAAAAGAA	669	Xu and Nie (2006)
R2 AMV	TCAATGACGATCAAGATCGTC		
F2 PVY	CTAAGAAGCTTCACTGAAATG	902	Llave et al. (1999)
R2 PVY	ATATCGGATCCGGAGAGACAC		

The primer pair for CMV covered the capsid protein (CP) gene and part of the 3' NTR (3' non translated region). The set of primers for AMV included CP gene, while primer pair for PVY covered CP gene, part of the 3' end of the gene for NIb (Nuclear Inclusion protein b) and the beginning of the 3' NTR. The primers were chosen from conservative RNA regions of the inspected viruses. PCR was done in 25µl mixture volume, which contained 5µl cDNA, 2.5µl 10xPCR Buffer II, 2.5µl $MgCl_2$ (25mM), 2µl dNTP's (2.5mM), 0.5µl (100pM/µl) of forward (sense) primer F (Table 1), 0.5µl (100pM/µl) of reverse (antisense) primer R and 0.25µl of Taq DNA Polymerase (Sigma-Aldrich, USA). The PCR protocol for amplification of the CP gene for CMV was customized to cycling conditions specific for the CMV primer pair: initial melting at 95°C for 15 min, 35 cycles of 95°C for 45 s (melting), 59°C for 45 s (annealing) and 72°C for 90 s (extension) and final extension at 72°C for 10 min. The PCR for AMV was performed according to Xu and Nie (2006), and for PVY as described by Llave et al. (1999). RT-PCR was performed on thermocycler Techne, TC – 512 (Fisher Scientific, USA). Two types of negative controls were prepared, where cDNA was omitted and where cDNA from uninfected (ELISA-negative) leaf samples was used. The extracted RNA from pepper (its 18S rRNA used as internal control of RNA extraction quality) and the amplified products were analyzed on 1.5% agarose gel electrophoresis, in 1xTBE buffer and visualized under a UV transilluminator (Popovski et al., 2013).

Results and discussion

Virus occurrence determined by ELISA

After performing DAS-ELISA on the collected leaf samples, CMV was detected as most prevalent virus on pepper plants in R. Macedonia (51% in 2012, 34% in 2013 and 61% in 2014) (Table 2). In 2012, AMV was detected as second (15%), while in 2013 and 2014 it was PVY (7% and 8%, respectively). Plant viruses regularly occur on pepper plants throughout the vegetation in R. Macedonia (Jovanchev et al., 1996; Rusevski et al., 2011; 2013) and other countries (Choi et al., 2005; Kim et al., 2010; Milošević, 2013), causing economic losses and representing a major threat for pepper production. The frequency of CMV on pepper was observed by other authors (Choi et al., 2005; Ormeño et al., 2006; Kim et al., 2010).

Table 2. Incidence of *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY) on pepper plants in R. Macedonia in the period of 2012-2014

Year	Number of tested samples	Detected viruses		
		CMV	AMV	PVY
2012	91	46 (51 %)	14 (15 %)	5 (5 %)
2013	84	28 (34 %)	2 (2 %)	6 (7 %)
2014	84	51 (61 %)	2 (2 %)	7 (8 %)

Molecular detection of AMV

After optimization of the RT-PCR protocol, because of the small virus occurrence of AMV during the tested period, a specific AMV isolate KUA7-2013 was chosen. The primer set AMV-F2/R2 was used for amplification of the entire region of the CP gene, generating amplicon of 669 bp (Figure 1). Due to change of conductivity, which can be a result of the amount of salts in the restriction buffer, or the salt constitution of the marker dyes, the size standard and the obtained fragment ran differently. Too much salt made the sample run slower, so fragment looked larger. The problem can be solved by staining the gel after electrophoresis, which we intend to do in the future, so next time this kind of discrepancies should be avoided. In the negative control, no amplification product was observed.

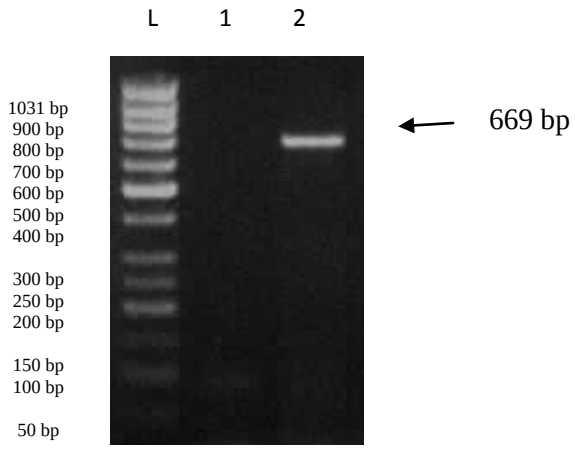

Figure 1. 1.5% agarose gel electrophoresis (AGE) analysis of the amplicon of the CP gene of *Alfalfa mosaic virus* (AMV) obtained by RT-PCR. L – GeneRuler™ 50bp DNA Ladder (Fermentas Life Sciences GmbH, Lithuania); lane 1 – negative control; lane 2 – isolate KUA7-2013.

The isolate KUA7-2013 was obtained from the area around Kumanovo in 2013. This particular isolate was chosen because of its unusual serological behavior. During the first test (performed after planting of the seedling material on field), a very high OD absorbance was measured (2.575), but during the second test (during flowering) a negative serological result was obtained (0.104). After performing a successful RNA isolation and RT-PCR, an amplicon of 669 bp was distinctly observed (Figure 1), showing that it is not a case of false positive serological result. This kind of fluctuations of the OD absorbance in AMV and some other plant viruses were observed also by other authors (Jaspars and Bos, 1980; Rusevski et al., 2013). With the help of RT-PCR, the problem was resolved, displaying the importance of the sensitivity of used methods.

Molecular detection of CMV

For the RT-PCR detection of CMV, ten leaf samples were chosen, having different concentrations of virus accumulation represented by OD values measured with DAS-ELISA and showing various symptom visibilities.

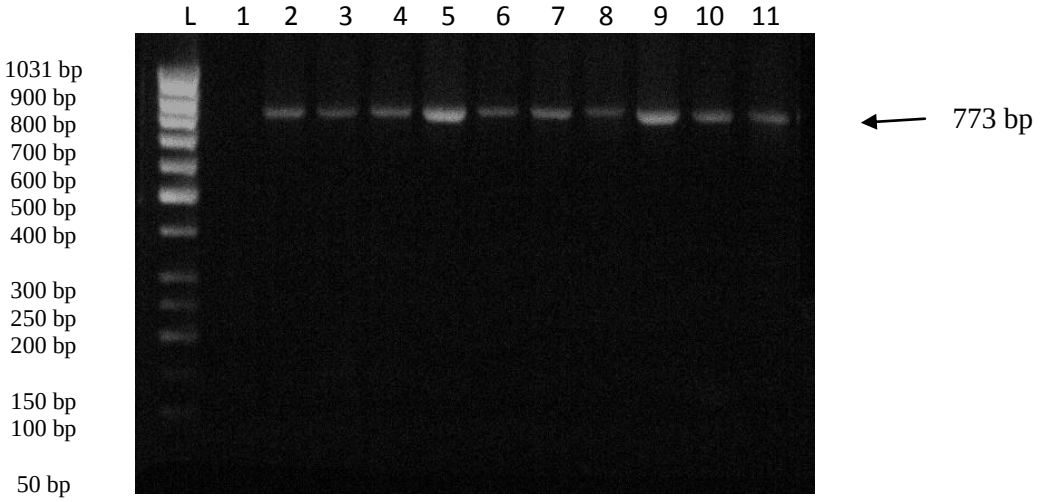

Figure 2. 1.5% agarose gel electrophoresis (AGE) analysis of the amplicon of the CP gene of *Cucumber mosaic virus* (CMV) obtained by RT-PCR. L – GeneRuler™ 50bp DNA Ladder (Fermentas Life Sciences GmbH, Lithuania); lane 1 – negative control; lane 2 – isolate SVNA3-2013; lane 3 – isolate SVNA6-2013; lane 4 – isolate SVNA7-2013; lane 5 – isolate SKA5-2013; lane 6 – isolate SKA3-2012; lane 7 – isolate KUA4-2014; lane 8 – isolate KUA7-2013; lane 9 – isolate STRA4-2013; lane 10 – isolate RAA6-2013 lane 11 – isolate SVNA3-2014.

The presence of CMV was positively identified in all of the tested samples, obtaining amplicons of the complete CP gene with the size of 773 bp. As it was in the case of the electrophoresis of AMV mentioned above, due to change of conductivity, the size standard and the obtained fragments ran differently. Too much salt made the samples run slower, so fragments looked larger. In the negative control, no amplification product was observed (Figure 2). RT-PCR was widely used for CMV detection (Vučurović et al., 2010; Fidan and Güllü, 2014) and amplicons were detected even in plant samples where measured OD absorbance (virus concentration) was very low (Yu et al., 2005; Chen et al., 2011).

Molecular detection of PVY

The presence of PVY was positively identified by the use of RT-PCR, obtaining amplicons of the complete CP gene with the expected size of 902 bp. In the negative control, no amplification product was observed (Figure 3).

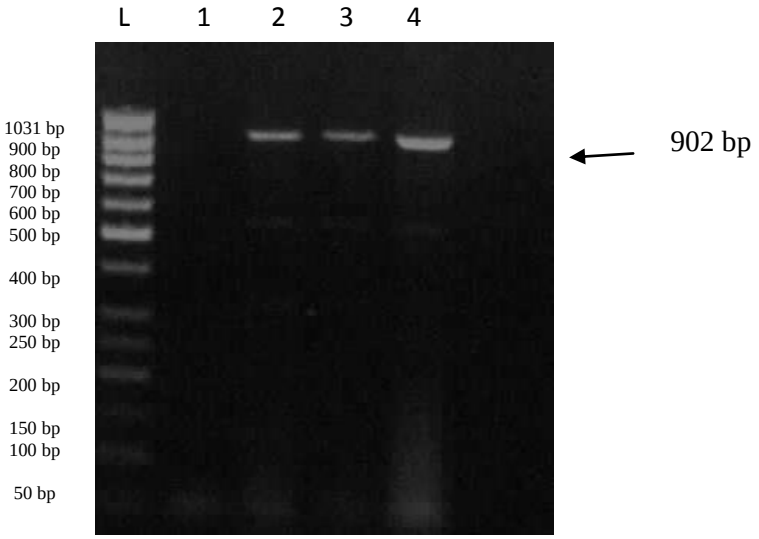

Figure 3. 1.5% agarose gel electrophoresis (AGE) analysis of the amplicon of the CP gene of *Potato virus Y* (PVY) obtained by RT-PCR. L – GeneRuler^{TM} 50bp DNA Ladder (Fermentas Life Sciences GmbH, Lithuania); lane 1 – negative control; lane 2 – isolate KUA6-2013; lane 3 – isolate KUA7-2013; lane 4 – isolate KOCB1-2013.

The PVY-isolates detected by RT-PCR were chosen as representatives of different types of infection. The isolate KUA6-2013 was part of a single infection, the isolate KUA7-2013 was part of a triple mixed infection (AMV+CMV+PVY) and the isolate KOCB1-2013 was part of a double mixed infection including CMV. After performing RT-PCR, bands of all amplicons with size of 902bp were clearly visible (Figure 3), regardless the type of infection. This proves again the sensitivity and the selectiveness of the method (Nie and Singh, 2000; Wang et al., 2012).

Conclusions

With the RT-PCR analysis performed during this trial on isolates of the inspected pepper plant viruses (AMV, CMV and PVY), the sensitivity and exclusivity of the method was confirmed. The type of infection (single or mixed), or the concentration of the virus accumulation in the plants represented by different OD values have not affected the accuracy of RT-PCR. Because of the obvious benefits from the use of the molecular methods, we hope that RT-PCR will take its rightful place in plant virus detection and controlling of the virus occurrence in the agricultural production in R. Macedonia.

References

1. Alonso-Prados, J. L., Luis-Arteaga, M., Alvarez, J. M., Moriones, E., Batlle, A., Laviña, A., García-Arenal, F. and Fraile, A. (2003). Epidemics of aphid-transmitted viruses in melon crops in Spain. European Journal of Plant Pathology, 109: 129–138.
2. Bertolini, E., Olmos, A., López, M. M. and Cambra, M. (2003). Multiplex nested reverse transcription-polymerase chain reaction in a single tube for sensitive and simultaneous detection of four RNA viruses and *Pseudomonas savastanoi* pv. *savastanoi* in olive trees. Phytopathology, 93: 286-292.
3. Chatzivassiliou, E. K., Efthimiou, K., Drossos, E., Papadopoulou, A., Poimenidis, G. and Katis, N. I. (2004). A survey of tobacco viruses in tobacco crops and native flora in Greece. European Journal of Plant Pathology, 110: 1011–1023.
4. Chen, S., Gu, H., Wang, X., Chen, J. and Zhu, W. (2011). Multiplex RT-PCR detection of Cucumber mosaic virus subgroups and Tobamoviruses infecting Tomato using 18S rRNA as an internal control. Acta Biochim Biophys Sin, 43: 465–471.
5. Choi, G.S., Kim, J.H., Lee, H.D., Kim, J.S., Ryu, K.H. (2005). Occurrence and distribution of viruses infecting pepper in Korea. Plant Pathol. J, 21(3): 258-261.
6. Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of the plant viruses. J. Gen. Virology, 34 (3): 457-483.
7. Deyong, Z., Willingmann, P., Heinze, C., Adam, G., Pfunder, M., Frey, B., Frey, J. E. (2005). Differentiation of Cucumber mosaic virus isolates by hybridization to oligonucleotides in a microarray format. Journal of Virological Methods, 123: 101-108.
8. de Wijs, J. J., Suda-Bachmann, F. (1979). The long-term preservation of potato virus Y and watermelon mosaic virus in liquid nitrogen in comparison to other preservation methods. Neth. J. Pl. Path., 85: 23-29.
9. FAO. (2015). http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor
10. Fidan, H. and Güllü, M. (2014). Newly report of *Cucumer mosaic virus* (CMV) in caper bush, *Capparis spinosa* (L.) (Capparacaea) in Turkey. Scientific conference "Challenges in modern agricultural production", Institute of Agriculture – Skopje, Skopje, R. Macedonia
11. Jankulovski, D. (1997). Pepper and tomato. NIP "BAS – TRADE", Skopje, R. Macedonia.
12. Jaspars, E. M. J. and Bos, L. (1980). Alfalfa mosaic virus CMI/AAB description of plant viruses, No. 229. http://www.dpvweb.net/dpv/showdpv.php?dpvno=229.
13. Jovanchev, P., Pejchinovski, F., Jankulovski, D., Rusevski, R., Bandzo, S. and Popsimonova, G. (1996). Health status of pepper plants in the Republic Macedonia in 1995. Annual Book of Proceedings of Plant Protection, 7: 159-169.
14. Kim, M.S., Kim, M.J., Hong, J.S., Choi, J.K. and Ryu, K.H. (2010). Patterns in disease progress and the influence of single and multiple viral infections on pepper (*Capsicum annuum* L.) growth. Eur J Plant Pathol, 127: 53–61.
15. Lee, B. Y. and Ryu, K. H. (2009). Incidence of virus diseases and RT-PCR detection of Daphne-infecting viruses in Korea. Eur J Plant Pathol, 124: 127–132.
16. Llave, C., Martínez, B., Díaz-Ruíz, J. R. and López-Abella, D. (1999). Serological analysis and coat protein sequence determination of potato virus Y (PVY) pepper pathotypes and differentiation from other PVY strains. European Journal of Plant Pathology, 105: 847-857.
17. Milošević, D. (2013). Diversity and characterization of pepper viruses in Serbia. Ph.D. Thesis. Faculty of Agriculture, University in Belgrade, Belgrade, Serbia.
18. Nair, R. M., Habili, N. and Randles, J. W. (2009). Infection of *Cullen australasicum* (syn. *Psoralea australasica*) with *Alfalfa mosaic virus.* Australasian Plant Disease Notes, 4: 46–48.
19. Nie, X. and Singh, R. P. (2000). Detection of multiple potato viruses using an oligo(dT) as a common cDNA primer in multiplex RT-PCR. Journal of Virological Methods, 86: 179–185.
20. Niimi, Y., Han, D.-S., Mori, S. and Kobayashi, H. (2003). Detection of cucumber mosaic virus, lily symptomless virus and lily mottle virus in *Lilium* species by RT-PCR technique. Scientia Horticulturae, 97 (1): 57–63.

21. Ormeño, J., Sepúlveda, P., Rojas, R. and Araya, J.E. (2006). *Datura* Genus Weeds as an Epidemiological Factor of *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), and *Potato virus Y* (PVY) on Solanaceus Crops. AgriculturaTécnica (Chile), 66 (4): 333-341.
22. Popovski, Z.T., Dimitrievska, B., Miskoska – Milevska, E. and Bandzo, K. (2013). PCR based methods – Manuel. University "Ss. Cyril and Methodius" – Skopje, Faculty of agricultural sciences and food, Skopje, R. Macedonia.
23. Raj, S. K., Srivastava, A., Chandra, G. and Singh, B. P. (2002). Characterization of cucumber mosaic virus isolate infecting *Gladiolus* cultivars and comparative evaluation of serological and molecular methods for sensitive diagnosis. Current Science, 83 (9): 1132-1137.
24. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Gjamovski, V. (2009). Virus status of pepper plants in the area around Kochani in 2008. Plant protection, Skopje, 20: 84-88.
25. Rusevski, R., Bandzo, K., Kuzmanovska, B., Sotirovski, K. and Risteski, M. (2013). Occurrence, distribution and dynamics of virus antigen accumulation in pepper cultivation on open fields in Republic of Macedonia during 2008-2009. African Journal of Agricultural Research, 8 (28): 3836-3841.
26. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Stanoevska, M. (2010). Virus status of pepper plants cultivated on open fields in R. Macedonia in 2009. Plant protection, Skopje, 21: 44-50.
27. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Stanoevska, M. (2011). Virus status of pepper plants cultivated on open fields. In: XVI International meeting on Biotechnology – Book of Proceedings, Čačak, Serbia, 2011: 429-434.
28. Rusevski, R. and Bandzo, S. (1998). Influence of some microclimate characteristics (temperature and humidity) on the aphid population and virus infections on pepper in green houses. Annual Book of Proceedings of Plant Protection, Skopje, 9: 141-159.
29. van Dongen, J. J., Macintyre, E. A., Gabert, J. A., Delabesse, E., Rossi, V., Saglio, G. Gottardi, E., Rambaldi, A., Dotti, G., Griesinger, F., Parreira, A., Gameiro, P., Diáz, M. G., Malec, M., Langerak, A. W., San Miguel, J. F. and Biondi, A. (1999). Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia, 12: 1901–1928
30. Vučurović, A., Bulajić, A., Stanković, I., Ristić, D., Berenji, J., Jović, J. and Krstić, B. (2010). Occurrence, molecular detection and identification of Cucumber mosaic virus infecting pumpkin and squash in Serbia. In: 10th Meeting on plant protection, Zlatibor, 2010: 58-59.
31. Wang, P., Guo, Y., Cheng, J.-H., Dong, Q.-F., Ding, X.-F., Guo, J.-F. and Jiang, Y.-H. (2012). Application of multiplex reverse transcription-ligase detection reaction-polymerase chain reaction (MRLP) mediated universal DNA microarray for detecting potato viruses in field samples. Eur J Plant Pathol, 132: 217–227.
32. Wernli, B. (1999). Bioreba AG.
33. Xu, H. and Nie, J. (2006). Identification, characterization, and molecular detection of *Alfalfa mosaic virus* in potato. Phytopathology, 96 (11): 1237-1242.
34. Yu, C., Wu, J. and Zhou, X. (2005). Detection and subgrouping of *Cucumber mosaic virus* isolates by TAS–ELISA and immunocapture RT–PCR. Journal of Virological Methods, 123: 155–161.
35. Šutić, D. (1995). Plant viruses. Institute for plant protection and environment, Belgrade: 1-394.

DIFFERENCES IN VIRULENCE OF *RHYNCHOSPORIUM COMMUNE* ISOLATES FROM CENTRAL ANATOLIA ON BARLEY CULTIVARS

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Abstract
Rhynchosporium commune, the causal agent of leaf disease and also known as leaf blotch or scald, affects plant growth and accordingly yield in barley worldwide. The aim of the study was to determine difference in virulence of isolates of *R*. *commune*(Rc) from main barley growing areas of Turkey. In the study, a total of 37 isolates were obtained from Central Anatolia (Ankara, Konya, Eskişehir, Kırşehir and Çorum provinces) in 2013 and 2014. To reveal difference in virulence of the isolates, susceptible cultivar Aydanhanımandcvs. Çetin 2000 and Tokak 157/37 were used as host plants. The study, set up a factorial design in randomized plots with three replications, was conducted under controlled conditions in Ankara. Cultivars at the seedling stage were inoculated by spraying inoculum on them. Using 0 to 4 scale, disease ratings were done. In the cluster analysis, the isolates were separated into four different groups. Mean disease severity (MDS) of the isolates on the cultivars was 1.92, while they were 2.59, 2.05 and 1.14 in cv. Tokak, Aydanhanım and Çetin 2000, respectively. Disease severity (DS) varied from 0.01 to 3,5 among the cultivars. With 2.83 and 2.66 DS rates, isolate 37 and 31 were the most virulent, respectively. However, the least virulent isolates were 10(1.17) and 11(1,0), 12(1,0), 13(1,0), 25(1,0). Differences determined among isolates and cultivars and isolate x cultivar interactions were significant statistically ($P<0.01$, R2 0,94). The study showed that virulences of the isolates of Rcwere significantly different on the hosts tested.

Keywords: *Rhynchosporium commune*, isolate, virulence, barley, seedling stage.

Introductıon

With a nearly 2,7 million ha cultivated area and 7.1 million tonnes production, barley (*Hordeum vulgare* L.) is ranked as the second cerealafter wheat in Turkey (Anonymous, 2015). It is mainly used in animal feeding and malt industry and usually grown in Central Anatolia under dry conditions in Turkey (Kün, 1988). Leaf blotch disease (LBD) of barley, caused by the fungus *R*. *commune* (Zaffarano, Mcdonald&Linde), is one of the most important diesease of barley in Turkey as well as the World (Zaffarano et al. 2011).LBD is generally leads to reduce yield by decreasing tillering and grain weight. Depending on the onset of the disease on the host, yield losses can reach up to 30-40% (Aktaş, 2001). However, yield losses ranging from 10 to 70% due to LBD was reported by Mathre (1982). Ensuing infection of *R*. *Commune* (Rc) on the second and third leaves of susceptible cultivars, leaf lesions covering leaf blade appear and then general chlorosis on the leaves occurs. On the other hand, on the leaves of resistant cultivars no lesions form or small, brownish-grey spots on the border and tips of the leaves emerge (Xue et al. 1991). Damage and intensity of LBD have tended to increase all around the world since 1980s. Growing barley in vast areas as a monoculture, using reduced tillage system, remaining infected crop residues in the soil, shifting climatic conditions and highly evolving genetic structure of the fungus, all of these factors could be responsible for those damage from LBD (Ellen, 2002). Thus, as the damage of LBD increases, fungicide usage against the disease goes up in the World. However, the fungicides used to control the disease causes soaring cost for production and adverse effects on the environment (Polley and King1993). In this regard, the best way to manage with the disease is to develop resistant barley cultivars and use them in barley

cultivation. There are many pathotypes of the fungus (Salamati and Tronsmo 1997, Lebedeva et al. 2006, Meles et al. 2008, Araz and Hekimhan 2017). Hence, determining reactions of existing barley cultivars against virulent pathotypes and monitoring genetic changes of the fungus are very important to manage with the disease (Avrova and Knogge 2012). The objective of the study was to determine differences in virulence of Rc isolates obtained from barley growing areas of Central Anatolia on widely grown cultivars.

Material and methods

Barley Cultivars

2-row Tokak 157/37 and Aydanhanım, 6-row Çetin-2000.

Isolates

A total of 37 isolates were obtained from diseased leaves during surveys conducted in barley growing areas of Central Anatolia (Konya, Ankara, Eskişehir, Kırşehir and Çorum Provinces) in 2013 and 2014.

Isolation

Infected leaf tissues were cut as pieces with a size of 5 mm^2 and exposed to 70% ethyl alcohol for 10 seconds and then 5% Sodium Hypochlorite (NaoCl) for 90 seconds. These pieces were soaked into sterile distilled water for 30 seconds and later transferred to blotter medium at 20 °C for 24 hour to ensure sporulation. Afterwards, the leaf pieces were dipped into sterile distilled water and rinsed and then a drop from this solution was taken and spreaded onto 1% water agar medium and incubated at 20 °C for 24 hour (Fowler and Owen 1971, Döken, 1979). Conidia germinating on the medium were taken single by single through a sterile needle under a microscope and transferred to PDA (Potato Dextrose Agar). Petri dishes were put in an incubator by adjusting 17 °C for 15 days and in this way colony of the fungus appeared (Figure 1). By subculturing these colonies, pure isolates of the fungus was attained. Single spore isolates obtained were transferred into glass tubes (Microbank) and stored at -18 °C (Lange and Boyd 1968, Xue et al. 1991).

Figure 1. Colonies of *R. commune* on PDA

Greenhouse tests and evaluations of host responses

Stored isolates were transferred on PDA and incubated at 17 to 20 ° for 14 to 20 days. Later, 5 to 10 ml sterile water per petri was poured on colonies developing on PDA and the colonies were taken using a sterile fine brush and filtered via a sterile cheese cloth into a sterile beaker. Conidia concentration of the inoculum was adjusted as $5x10^5$ spores/ml through a haemocytometer (Xue et al. 1991). Since barley is a long-day plant, in the greenhouse experiments, durarion of lighting was adjusted as 16 hour light and 8 hour darkness (Vardar, 1983). During lightning and dark periods, temperatures of the ambient were arranged as 18±2°C and 16±2°C, respectively. (Xue et al. 1991). 10 seeds of each cultivars were sown per pot and plants were watered twice a week. At the seedling stage of plants(11^{st} growth stage of Zadoks) (Zadoks et al. 1974), plants were inoculated by spraying adjusted inoculum. However, sterile water was sprayed on cv. Aydanhanım (susceptible check cultivar). Experiments were set up a factorial design in randomized plots with three replications. Ensuing inoculation, plants were covered with polyethylene bags and kept at 18±2 °C under 90 to 100% relative humidity and 48 hour dark period conditions (Mayfield and Clare 1991). Nearly 18 to

20 days later inoculation, disease ratings were done according to 0 to 4 scale in Table1 (El-Ahmed, 1981). In the scale; values of 0,1 and 2 were evaluated as resistant, as for 3 and 4 were rated as susceptible.

Table 1. Scale, 0 to 4, used for disease evaluations (El-Ahmed, 1981)

0	No disease symptom on plant
1	Small, brownish-grey spots on tip and/or borders of leaves
2	Small, brownish-grey lesions scattered on leaves
3	Large lesions on an area, over 50% of leaves
4	Large, coalesced lesions and general chlorosis and leaf death

Results and discussion

All cultivars tested showed resistant response to the isolates according to mean scale values. Differences in the isolates and cultivars and isolate x cultivar interactions were significant statistically ($P<0.01$, R^2 0,94). Mean disease severity (MDS) of the cultivars was 1.93. However, MDS of Tokak 157/37, Aydanhanım and Çetin-2000 were 2.59, 2.05 and 1.14, respectively (Table 2). Disease severity (DS) of the cultivars ranged from 0.01 to 3.5. Of the isolates, with 2.83 and 2.66 MDS values, isolate 37 and 31 were the most virulent ones, respectively. Whereas, with 1.17 MDS, the weakest virulent was isolate 10. Isolate 17 and 37obtained from Ankara and isolate 31 from Çorum constituted the highest DS on cv. Tokak 157/37. Isolate 37 also created the highest DS on cv. Çetin 2000. Besides, isolate 8 from Kırşehir and isolates 29 and 33 from Konya constituted the highest DS values on cv. Aydanhanım (Table 2). In the cluster analysis, isolates were separated into 4 different groups according to their virulence status. The isolates 10, 11, 12, 13, 25 and 31, 37 were grouped individually as the least and the most virulent, respectively (Figure 2). In the study, isolates obtained from Ankara, Konya and Eskişehir Provinces constituted both high and low DS on the hosts tested, indicating virulence difference in them. As it is known that responses of the cultivars to different isolates from different locations may vary. For example, cv. Çetin-2000, known as resistant to Rc, showed 2.50 DS against isolate 37, which near the value of 3.0 DS displayed by susceptible cv. Aydanhanım. In the study, the most virulent isolate was 37, obtained from Ankara. However, in a study conducted by Araz and Maden (2006), it was reported that the most virulent pathotypes of Rc in Central Anatolia were found on the samples from Eskişehir, Kayseri and Yozgat Provinces. In this regard, our finding revealed that isolates in one location could shift their genetic structure and accordingly, with time, new virulent isolates may appear. Likewise, although sexual stage of Rc has not been know, it has been stated that Rc has a high degree of genetic variation and evolving potential (Jackson and Webster 1976, Zhang et al. 1992,Forgan et al. 2007, Zhan et al. 2012). In the present study, DS values changed according to isolate and host. For instance, the isolates, e.g. 33, 34 and 35, constituted high DS on cv. Aydanhanım whereas, they displayed low DS on cv. Çetin-2000. In addition, the isolates, e.g. 2 and 3, showed higher DS on cv. Tokak 157/37 than cv. Aydanhanım. As for, Azamparsa et al. (2015) stated that 3 Rc isolates from Gaziantep, Eskişehir and Manisa Provinces created high DS on Tokak 157/37 under greenhouse conditions. However, Düşünceli et al. (2008) emphasised that Of 36 barley cultivars, cv. Çetin-2000, 6-row barley variety, was the most resistant one against Rc both under greenhouse conditions and in the field. This finding corroborated the ones of Barradas (1984). In that study, author reported that 6-row barley cultivars have more resistance sources. Additionally, virulence of isolates can change depending on the pathotype isolated from different location and hosts. Barradas (1984) stated that genetic and morphological structure of the hosts, cultural practices, climate could play an important role in Rc development on barley. Likewise, Bockelman (1984) tested 9 different isolates of Rc on 20 barley cultivars and reported that virulence of the isolates were different one another. In fungi, alterations in virulence may appear through gene flow, recombination, mutation and sexual production. As a result of these phenomenon, with time, cultivars, known as resistant, could become susceptible to those new emerging virulent fungi.

Thus, new emerging pathotypes of Rc could be more virulent than existing ones (Jorgensen and Smedegaard-Petersen 1995, Zencirci and Hayes 1990, Düşünceli et al. 2008).

Table 2. Origin of Rc isolates and disease severity(DS)of the cultivars

No	Origin of isolates	DS of the Cultivars			Mean DS
		Tokak 157/37	Çetin-2000	Aydan hanım	
1	Konya, Altınekin, Koçyaka	2,00 ef	1,00 h	3,00 b	2,00 dh
2	Ankara, Temelli	2,33 ce	1,00 h	2,00 ef	1,78 hj
3	Ayaş, Bayram	2,33 ce	1,00 h	2,00 ef	1,78 hj
4	Ankara, Ş.koçhisar	2,67 bc	2,00 ef	2,50 cd	2,39 b
5	Konya, Sarayönü, Baş höyük	2,50 cd	2,00 ef	2,50 cd	2,33 bc
6	Çubuk, Saraycık	1,50 g	1,83 fg	1,50 g	1,61 j
7	Eskişehir Agr.Res.Inst.BVD 44	2,50 cd	2,00 ef	2,67 bc	2,39 b
8	Kırşehir, Çiçekdağı, Safalı	2,00 ef	1,00 h	3,50 a	2,17 be
9	Ankara, Gölbaşı, İkizce	2,00 ef	1,00 h	3,00 b	2,00 dh
10	Ankara, Çubuk, Yazır	1,00 h	0,01 ı	2,50 cd	1,17 k
11	Konya, Yunak, Böğrüdelik	1,00 h	0,01 ı	2,00 ef	1,00 k
12	Eskişehir, Mahmudiye, Şerefiye	1,00 h	0,01 ı	2,00 ef	1,00 k
13	Eskişehir, Çifteler, Zaferhamit	1,00 h	0,01 ı	2,00 ef	1,00 k
14	Ankara, Sincan	2,00 ef	2,00 ef	2,50 cd	2,17 be
15	Konya, Kulu, Zincirlikuyu	2,67 bc	1,00 h	3,00 b	2,22 bd
16	Konya, Çumra, Karapınar yolu	2,33 ce	1,00 h	2,50 cd	1,94 eh
17	Ş. Koçhisar, Demirciobası	3,00 b	1,00 h	3,00 b	2,33 bc
18	Sivrihisar, Yeniköy	2,67 bc	1,00 h	2,50 cd	2,06 dg
19	Ankara, Akyurt, Kalaba	2,00 ef	1,00 h	3,00 b	2,00 dh
20	Konya, Çeltik, Göktepe	2,50 cd	1,00 h	3,00 b	2,17 be
21	Ş. Koçhisar, Evren	1,00 h	1,00 h	3,00 b	1,67 ıj
22	Ankara, Akyurt, Bügdüz	2,00 ef	2,00 ef	3,00 b	2,33 bc
23	Bala, Merkez	2,00 ef	1,00 h	2,50 cd	1,83 gj
24	Konya, Cihanbeyli, Acıkuyu	2,67 bc	1,00 h	2,00 ef	1,89 fı
25	Ankara, Kızılcahamam,Akdoğan	2,00 ef	1,00 h	0,01 ı	1,00 k
26	Konya, Cihanbeyli, K. Beşkavak	2,17 df	1,00 h	3,00 b	2,06 dg
27	Ankara, Başayaş	2,00 ef	1,00 h	2,50 cd	1,83 gj
28	Ankara, Polatlı	2,00 ef	1,83 fg	2,50 cd	2,11 cf
29	Konya, Kulu, Yaraşlı	2,00 ef	1,00 h	3,50 a	2,17 be
30	Konya, Kaşören, Çeltik	1,00 h	1,00 h	3,00 b	1,67 ıj
31	Çorum, Sungurlu, Tuğcu	3,00 b	2,00 ef	3,00 b	2,67 a
32	Ankara, Haymana,Yeşilyurt	2,00 ef	1,00 h	2,50 cd	1,83 gj
33	Konya, Kulu, Bahadırlı mah.	2,00 ef	1,00 h	3,50 a	2,17 be
34	Konya, Cihanbeyli, Karatepe	2,00 ef	1,00 h	3,00 b	2,00 dh
35	Ankara, Bala, İsmetpaşa	2,00 ef	1,00 h	3,00 b	2,00 dh
36	Eskişehir Agr.Res.Inst. Parcel 57	2,00 ef	1,00 h	2,00 ef	1,67 ıj
37	Ankara, Susuz	3,00 b	2,50 cd	3,00 b	2,83 a
Mean		2,59 a	1,14 c	2,05 b	1,93

%CV: 13,65, $LSD_{0,05}$Variety:0,07, Isolate:0,24, Variety x Isolate: 0,42

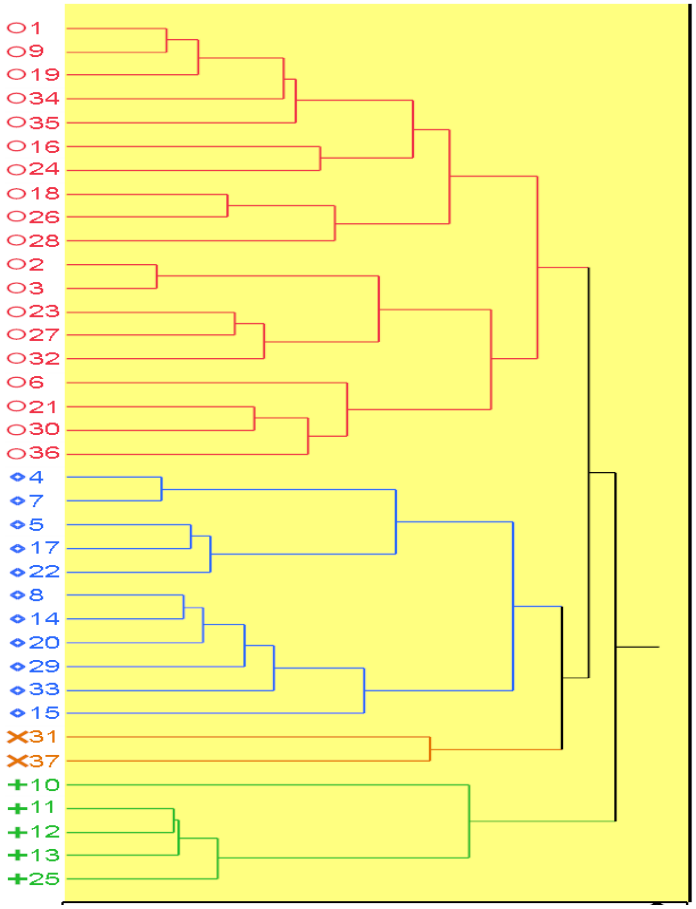

Figure 2. Dendogram of the virulence status of the isolates

Conclusions

Monitoring changes in virulence of Rc on the hosts is very crucial to manage with Rc. In this regard, in the present study, Rc isolates were obtained from main barley producing areas of Central Anatolia (Konya, Ankara, Eskişehir, Kırşehir and Çorum Provinces) in Turkey and difference in virulence of the isolates were determined. This study suggested that considerable changes in virulences of Rc isolates of Central Anatolia exist.

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References

1. Aktaş, H.(2001). Önemli Hububat Hastalıkları ve Survey Yöntemleri. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü Bitki Sağlığı Araştırmaları Daire Başkanlığı, Bağdat cad. No: 208, PK78, 06171 Yenimahalle-Ankara (in Turkish).
2. Anonymous (2015). www.tuik.gov.tr
3. Araz, A. and Hekimhan, H. (2017). Determination of pathotypes of barley scald disease [*Rhynchosporium commune* (Zaffarano,McDonald&Linde)] in some provinces in central Anatolia. II. International Iğdır Symposium, 9-11 October 2017, Iğdır, Turkey.
4. Araz, A. and Maden, S. (2006). Pathogenic variation among isolates of Rhynchosporium secalis from cultivated barley growing in Central Anatolia, Turkey. Plant Pathology Journal, 5(2): 244-247.

5. Avrova, A. and Knogge, W. (2012). *Rhynchosporium commune*: a persistent threat to barley cultivation. Molecular Plant Pathology, 13(9): 986-997.
6. Azamparsa, M. R., Karakaya,A., Mert, Z., Aydın, G., Peşkircioğlu, H., Seçer, E., Özmen, D., Tutluer, İ. and Sağel, Z.(2015). İki arpa çeşidinin ve Gamma ışınlanması ile elde edilmiş ileri kademe arpa hatlarının *R. commune*'ye fide dönemi tepkileri. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 24 (1), 75-78 (in Turkish).
7. Barradas, M.T. (1984). Leaf Scald - A Major Barley Disease in Portugal. Barley diseases and Associated Breeding Methodology Workshop, p90-94, Rabat, Morocco.
8. Bockelman, H.E. (1984). Virulence Patterns of *Rhynchosporıum secalis* from Several Barley-Growing Regıons. Barley diseases and Associated Breeding Methodology Workshop, p88-89, Rabat, Morocco.
9. Döken, M.T. (1979). Erzurum'da arpadan izole edilen *Rhynchosporium secalis* (Oudem) J.J.Davis'in morfolojisi, biyolojisi, zarar durumu ve savaş yöntemleri üzerinde çalışmalar (Unpublished, in Turkish).
10. Düşünceli, F., Çetin, L., Albustan, S., Mert, Z., Akan, K. and Karakaya, A. (2008). Determination of the Reactions of Some Barley Cultivars and Genotypes to Scald Under Greenhouse and Field Conditions. Tarım Bilimleri Dergisi, 2008, 14 (1) 46-50, Ank. Üniv. Ziraat Fakültesi.
11. Ellen, O. (2002). Plant Protection in spring cereal production with reduced tillage III. Cereal Diseases. Crop Protection, 21: 195-201.
12. El-Ahmed, A.M.(1981). Seedling reaction of the 7th IBON to *R. secalis* in the greenhouse and source of resistance. Barley diseases and Associated Breeding Methodology Workshop, p95-105, Rabat, Morocco.
13. Forgan, A. H., Knogge, W. and Anderson, P. A. (2007). Asexual genetic exchange in the barley pathogen *Rhynchosporium secalis*. Phytopathology,97:650-654.
14. Fowler, A. M. and Owen, H. (1971). Studies on leaf blotch of barley (*R. secalis*). Trans. Br. Mycol. Soc., 56: 137-152.
15. Kün, E., (1988). Serin İklim Tahılları, Ankara Üniversitesi Ziraat Fakültesi Yayınları: 1032, Ders Kitabı:299, Ankara
16. Jackson, L.F. and Webster, R.K. (1976). The dynamics of a controlled population of *Rhynchosporium secalis*, changes in race composition and frequencies. Phytopathology, 66: 726–8.
17. Jorgensen, H.J. L. and Smedegaard-Petersen, V.(1995). Pathogenic variation of *Rhynchosporium secalis* in Denmark and sources of resistance in barley. Plant Disease, 79: 297-301
18. Lange, B.J. and Boyd, W.J.R. (1968). Preservation of fungal spores by drying on porcelain beads. Phytopathology, 58: 1711.
19. Lebedeva, L.V., Tvaruzek, L. and Konovalova, G.S. (2006). Virulence analysis of Russian and Czech barley scald populations causes by *Rhinchosporium secalis*. Mikologiya I, Fitopatologiya, 40(4): 337-343.
20. Mathre, D.E., (ed.) (1982). Compendium of Barley Diseases. APS Press., 78 pp.
21. Myfield, A., and Clare, B.G., (1991). Effects of *Rhynchosporium secalis* at specific growth stages on barley grown in a controlled environment. Plant Pathology, Volume 40, Issue 4, December 1991 , Pages 561–567
22. Meles, A. K., Yahyaoui, A., Abu-Blan, H. and Ahmed, M. (2008). Variation in virulence in ethiopian and Jordanian barley scald (*Rhinchosporium secalis*) field populations. Proceedings of the 10th International Barley Genetics Symposium, 5-10 April 2008, 391-392, Alexandria, Egypt.
23. Polley, R.W. and King, J.E. E. (1993). Surveys of diseases of spring barley in England and Wales, 1976-1980. Annals of Applied Biology, 123: 271-285.
24. Salamati, S. and Tronsmo, A.M. (1997). Pathogenicity of *Rhinchosporium secalis* isolates from Norway on 30 cultivars of barley. Plant Pathology, 46, 416-424.
25. Vardar, Y.(1983). Bitki Fizyolojisi Dersleri (Bitkilerin Büyüme ve Gelişme Olayları) . Ege Üniv. Yayınları No: 69-221 s(in Turkish).

26. Xue, B., Hall, R. and Falk, D. (1991). Pathogenic variation in *Rhynchosporium secalis* from southern Ontario. Plant Disease, 75: 934-938.
27. Zadoks, J.C., Chang, T.T. and Konzak, C. F. (1974). A Decimal Code For The Growth Stages Of Cereals. Weed Research, 14: 415-421.
28. Zhang, Q., Webster, R.K, Crandall, B.A., Jackson, L.F.and Saghai Maroof, M.A. (1992). Race composition and pathogenicity associations of *Rhynchosporium secalis* in California. Phytopathology, 82: 798–803.
29. Zhan, J., Yang, L., Zhu, W., Shang, L. and Newton, A. C. (2012). Pathogen populations evolve to greater race complexity in agricultural systems – evidence from analysis of *Rhynchosporium secalis* virulence data. PLoS ONE, 7(6): e38611.
30. Zaffarano, P. L., Mcdonald, B. A. and Linde, C. C. (2011). Two new species of *Rhynchosporium*. Mycologia, 103 (1): 195-202.
31. Zencirci, N. and Hayes,P. M. (1990). Effect of scald (*Rhynchosporium secalis*) on yield components of twelve winter barley genotypes. Journal of Turkish Phytopathology, 82: 798 –803.

ASSESSMENT OF NEW APPLE HYBRIDS

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Abstract

As a result of breeding activity at the Institute of Agriculture in Kyustendil are received a large number of apple hybrids. In the article are presented data for 5 promising hybrids. The study was carried out during the period 2012-2016. The trees are grafted on rootstock MM 106 and planted in the spring of 2007 at distances of 4.5 x 2.5 m. Ten years after planting the largest trunk cross-sectional area (TCSA) had the trees of hybrid № 2/30 ('Prima' x 'Sekai Ichi') and the smallest of hybrid № 2/28 ('Prima' x 'Florina'). The highest average yield per tree was obtained from hybrid № 1/3 ('Mollie's Delicious' – open pollinated) - 25.8 kg, followed by № 1/5 ('Malus robusta' x 'Liberty') - 24.9 kg. The fruit of № 1/5 were the largest - 230 g. The fruit of hybrid № 1/26 ('Melrose' x 'Kent') had the highest fruit flesh firmness - 9.05 kg/cm^2, while those of hybrid № 2/28 and № 2/30 had the lowest firmness - 7.28 and 7.36 kg/cm^2, respectively. The highest percentage of soluble solids (17,8%) and total sugar (8,7%) were contained in the fruit of hybrid № 1/3.

Keywords: TCSA, yield, fruit size, fruit firmness, chemical composition.

Introduction

The cultivar as an essential element in the production of high quality fruits has been and will be subject to continuous improvement and renewal. The main method for breeding new cultivars is the controlled hybridization, resulting in what were created a lot of apple cultivars. The most important criteria for new cultivars are: better fruit quality, higher tree productivity, early and regular fruit-bearing, weaker growth of the trees, late flowering, resistance to biotic and abiotic stressors, a long storage period of fruit and others (Laurens, 1999, White, 2000, Lukic et al, 2005, Blagov et al., 2009, Dzhuvinov et al., 2014, Lukic and Maric, 2015). A purposeful selection work in the apple at the Institute of Agriculture - Kyustendil was started in 1945 and in different stages were selected and studied too many hybrids (Blagov, 2005, 2011). In 2010 were recognized as new the cultivars Besapara (Florina x Macfree), Marlena (Florina x Macfree), Gorana (Prima x Cooper 4), Martinika (Prima x Secai Ichi) and Elegia (Prima x Cooper 4) and in 2015 the cultivar Siyana (Florina x Macfree). The most part of hybrids are under investigation yet. The purpose of this study was to determine the growth characteristics, productivity and fruit quality of five pre-selected apple hybrids.

Material and methods

The study was carried out in the period 2012 – 2016 with five apple hybrids: № 1/3 ('Mollie's Delicious' – open pollinated), № 1/5 ('Malus robusta' x 'Liberty'), № 1/26 ('Melrose' x 'Kent'), № 2/28 ('Prima' x 'Florina') and № 2/30 ('Prima' x 'Sekai Ichi') selected at the Institute of Agriculture - Kyustendil. The hybrids were grafted on MM 106 rootstock and the trees planted in the spring of 2007 at distances of 4.50 x 2.50 m. Each hybrid was represented with five trees and each individual tree was treated as a repetition. Trees were trained as free-growing crowns. The soil is Chromic Luvisols, slightly sandy-loamy with a neutral reaction. The soil surface at the plantation was maintained in clean cultivation and irrigation was done by sprinkling. The trees were fertilised annually with 18 kg/da nitrogen in the form of ammonium nitrate. A routine manegement programme was aplied. The yearly recorded parameters included: trunk cross-sectional area (TCSA, in cm^2, 30 cm above the grafting zone), crown volume (m^3), average and cumulative yield (kg/tree), yield efficiency (kg/cm^2 of TCSA), fruit weight (g), fruit size (mm) and chemical composition of the

fruit - soluble solids (%, determined by refractometer), total sugars (%, by Luff-Schoorl method) and titratable acids (%, titrimetrically with 0.1 N NaOH). Results 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 discussion

During the 6th-10th year after planting, hybrid № 2/28 had the weakest growth rate of the trunk cross-sectional area (Figure 1). Although at the first year of the study the trees of hybrid № 1/26 had thinner trunks after the seventh year their increase was the most intensive. At the end of the studied period according to this indicator were identified three groups of trees with different growth. With weak growth were the trees of hybrid № 2/28. Moderate growth showed the trees of hybrids № 1/5, № 2/30 and № 1/3 and strong – hybrid № 1/26. Differences between the hybrids were statistically proven ($p \leq 0.05$).

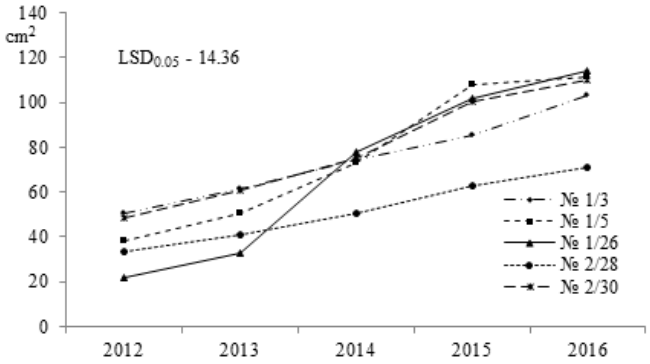

Figure 1. Dynamics of growth of the trunk cross-sectional area of apple hybrids

The trees of hybrid № 1/3 had the largest crown volume (7.2 m^3), while those of № 2/28 - the lowest (4.0 m^3), i.e. their volume was reduced by 55.6% (Figure 2). In the other hybrids the trees were with almost identical size - from 5.3 to 5.7 m^3.

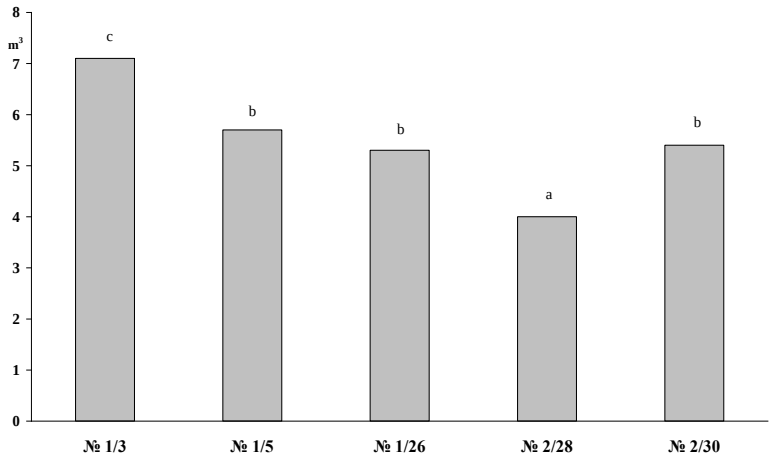

Figure 2. Trees crown volume of apple hybrids at the end of 10th growing year

Average yields per tree varied significantly over the years. The highest yields were registered in 2012 and 2016. In 2013 except hybrid № 1/3, and a certain extent of № 1/26, yields were significantly reduced as a result of damage from late spring frost. The highest 5-year cumulative yield per tree was obtained from hybrids № 1/3 (128.8 kg) and № 1/5 (124.3 kg). Good fertility had also the trees of hybrids № 1/26 (116.0 kg) and № 2/28 (100.9 kg). The lowest yield was obtained from hybrid № 2/30 - 96.6 kg. The differences between different genotypes were statistically proven (Figure 3).

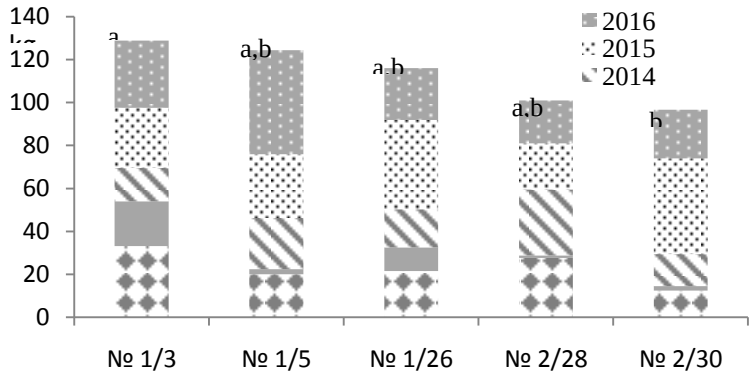

Figure 3. Average and cumulative yield of apple hybrids, kg/tree

The highest yield efficiency was calculated for hybrids № 2/28 (1.4 kg/cm^2 of TCSA) and № 1/3 (1.3 kg/cm^2) and the lowest for № 2/30 - 0.8 kg/cm^2. Hybrid № 1/5 had the highest fruit weight, average for the period, followed by № 1/26 and № 2/30, and the smallest were the fruit of № 1/3 (Table 1). The fruit mass was negatively affected by the yield, but to an insignificant extent degree, evidence of which is the low negative correlation coefficient and the percentage of determination (Figure. 4).

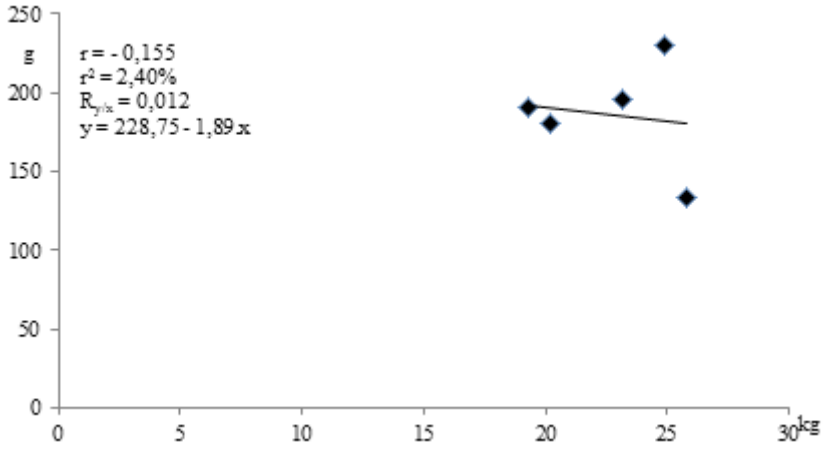

Figure 4. Relationship between the average yield per tree and fruit weight

The dimensions of the fruit largely corresponded to their weight. The studied hybrids had fruit with diameter over 65 mm, and cover the requirements for class 'Extra' quality according to Bulgarian standard. The measured fruit flesh firmness by hand penetrometer varied from 7.28 to 9.05 kg/cm^2 (Table 1).

Table 1. Biometrics data for fruit, average for 2012-2016

Hybrid	Fruit weight, g	Fruit height, mm	Fruit diameter, mm	Fruit flesh firmness, kg/cm^2
№ 1/3	133.6	58.6	67.3	8.68
№ 1/5	230.0	61.6	82.5	8.11
№ 1/26	195.1	68.5	74.8	9.05
№ 2/28	180.7	60.8	70.7	7.28
№ 2/30	190.0	66.7	78.9	7.36

Fruit of hybrid № 1/3 had the highest content of soluble solids and total sugars and at the same time the least titratable acids from where was calculated the highest sugar/acid index (Table. 2). The most

optimal ratio between sugars and acids was in fruit of hybrid № 1/5, which gives them a harmonious taste. The fruit of hybrid № 1/26 and № 2/28 had the highest acid content which gives them a sour taste.

Table 2. Chemical composition of the fruit, average for 2012-2016

Hybrid	Soluble solids, %	Total sugars, %	Titratable acids, %	Sugar/Acids index
№ 1/3	17.8	8.7	0.2	43.5
№ 1/5	15.9	8.1	0.4	20.2
№ 1/26	15.9	8.2	0.9	9.1
№ 2/28	14.8	8.1	0.5	16.2
№ 2/30	14.1	7.6	0.2	38.0

Conclusions

As a result of the study, hybrid № 1/5 proved to be the most promising among the tested apple hybrids. Although this hybrid indicated lower productivity in comparison with the other hybrids, at this stage its fruit were the largest, equal in shape and size. The ratio between sugars and acids was the most balanced, which gives them excellent flavor. The tree had moderate growth. The same hybrid can be used as a donor of these valuable qualities in future breeding programs in the apple.

Acknowledgements

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References

1. Blagov, A. (2005). Pomological characteristic of selected late ripening apple Hybrids. Scientific works of the National Center for Agrarian Sciences, Sofia, Vol, (3): 33-35.
2. Blagov, A., N. Christov, D. Sotirov and A. Stoyanova. (2009). Comparison of some new apple and sweet cherry cultivars under the environmental conditions of Kyustendil, Bulgaria. Acta Hortic, (825): 89-96.
3. Blagov, A. (2011). Some results of agrobiological investigation of apple cultivars and Hybrids in Kyustendil region. Plant Science, (48): 14-19.
4. Dzhuvinov, V., Penev, R., Slavov, I., Kutinkova, H., Gercheva, P., and Nacheva, L. (2014). The major results from the evaluation of genetic resourses and breeding of apple in the Fruit Growing Institute – Plovdiv. Plant Science, (51): 3-8.
5. Laurens, F. (1999). Review of the current apple breeding programmes in the world: objectives for scion cultivar improvement. Proc. of the Eucarpia Symp. on Fruit Breeding and Genetic Eds. K. R. Tobutt F. H. Alston. Acta Hortic, (484): 163-170.
6. Lukic, M., Tesovic, Z., Maric, S. and Sreckovic, M. (2005). Rezultati proucavanja novih sorti jabuke u uslovima Cacka. Vocarstvo, 39 (151): 233-239.
7. Lukic, M., S. Maric. (2015). Physiological and pomological properties of intense-colouring sports of 'Gala' and 'Elstar' apple cultivars. Journal of mountain agriculture on the Balkans. Vol. 18 (2): 344-360.
8. White, A. (2000). Breeding apple varieties for the world market. Hort. Research, (33): 116-118.

FIELD PERFORMANCE AND FRUIT QUALITY OF PRIMOCANE FRUITING RASPBERRY CULTIVARS GROWN IN SERBIA

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Abstract

The main focus of our study was to investigate differences in ripening time, vegetative and generative potential (number of canes per meter of hedgerow, cane height and diameter, number of fruiting laterals per primocane, yield per primocane and per meter of hedgerow) as well as fruit quality traits (fruit weight, index of fruit shape, number of drupelets per fruit, soluble solids content, total acids, total and inverted sugars, and sucrose content) in four newly introduced primocane fruiting raspberry cultivars (‘Erika’, ‘Paris’, ‘Versailles’, ‘Satine’) that are internationally competitive to both growers and marketers in the fresh markets. Studies were conducted at a commercial raspberry plantation located near Kraljevo (Serbia), in the period of 2015-2016. Results from this study showed that the average earliest harvest time was observed in ‘Versailles’ (11th August), whereas ‘Paris’ was the latest cultivar (10th September). Significantly higher cane height was registered in cvs. ‘Erika’ and ‘Paris’ (213 and 204 cm, respectively) in comparison to the two other tested cultivars. ‘Erika’ expressed the lowest values of all generative characteristics, while ‘Paris’ yielded much higher per primocane (840 g), followed by ‘Versailles’ (805 g). The latter one is also characterized by the highest average fruit weight (4.93 g) and the lowest level of soluble solids, total and inverted sugars (10.9%, 8.18% and 6.94%, respectively), whereas ‘Satine’ had significantly higher total acids content of the fruit (2.18%) compared to the rest cultivars. In general, cvs. ‘Versailles’ and ‘Paris’ demonstrated the best productivity and outer or inner fruit quality.

Keywords: *Rubus idaeus* L.*,* vegetative potential, productivity, physical and chemical fruit traits.

Introduction

Primocane-fruiting raspberries have many advantages over traditional summer-fruiting floricane raspberries. Most notably, they provide an opportunity to extend the season from late summer into fall (Pritts, 2008; Gwozdecki, 2004). Whereas the summer raspberry harvest lasts 5 weeks, fall fruiting can add an additional 3 months or longer to the season. These cultivars are usually grown in the form of a hedgerow system with annual removal of all canes after fall fruiting which decreases pruning costs and prevents winter injury (Milivojević et al., 2011a). Because of these advantages, primocane-fruiting raspberries were rapidly and widely planted in Republic of Serbia over the last several years (Milivojević et al., 2017; Milivojević et al., 2011b). Currently, primocane cultivars 'Polka' and 'Polana' are the most common in commercial raspberry plantings. These cultivars cover an area of about 500 ha in Serbia, mostly spreading in flat regions of the province of Vojvodina (Milivojević et al., 2017). However, some newer cultivars have large and flavorful fruit, earlier or later cropping, and exhibit higher yields. Certain new introduced cultivars also perform extremely well characteristics in specific locations. Hence the present study was undertaken over a 2-year period to describe vegetative potential, productivity, and physico-chemical fruit traits of four newly introduced primocane fruiting raspberry cultivars grown in South-Western Serbia.

Material and methods

Experimental work was performed in commercial raspberry plantation located near Kraljevo (Serbia), in the period of 2015-2016. The orchard was planted in the spring of 2014 in the form of a hedgerow system at spacing of 3.0 m × 0.5 m. Canes were simply mowed down early each spring and

the crop was only born on the primocanes during the summer and fall. Four newly introduced primocane fruiting raspberry cultivars ('Erika', 'Paris', 'Versailles' and 'Satine') were evaluated for their ripening time, vegetative and generative potential (number of canes per meter of hedgerow, cane height and diameter, number of fruiting laterals per primocane, yield per primocane and per meter of hedgerow) as well as fruit quality traits (fruit weight, index of fruit shape, number of drupelets per fruit, soluble solids content - SSC, titratable acidity - TA, total and inverted sugars, and sucrose content). Investigation of vegetative and generative characteristics was carried out on samples of 30 canes in 3 replications. Each replicate consisted of 10 canes selected for their uniformity. The cane height (cm) and diameter (mm) were measured after its growth was stopped using the PVC tape and caliper, respectively. Generative characteristics were investigated by counting the number of fruiting laterals per cane and weighing the harvested fruit to determine yield per cane (g). Yield per meter of hedgerow (kg) was calculated as a product of cane number per meter of hedgerow and yield obtained per cane for each harvest date. The date of the first harvest was considered as being the beginning of ripening when approximately 8 to 10% of fruits were technologically mature, whereas the end of ripening was recorded as being the date of the last harvest. Fruit samples were collected in triplicate at the commercial maturity stage of each cultivar to investigate the physical fruit properties (fruit weight, index of fruit shape and number of drupelets per fruit). Fruit weight was determined by weighing 30 fruit (± 0.1) per replication (90 per cultivar) and expressed in grams. For drupelets counting, the same fruit samples were used within each replication. An index of fruit shape was calculated as the ratio of the maximum height and width. Each sample consisted of 30 fruits pooled to obtain a composite sample and analyzed for soluble solids content using a digital refractometer (Pocket PAL-1, Atago, Japan). Titratable acidity was measured using a digital buret and 0.1 M NaOH, to titrate samples to an endpoint of 8.1, and acidity based as percent of malic acid equivalent. Determination of total and inverted sugars was done following the Luff – Schoorl method (Egan et al., 1981), whereas sucrose was obtained as the difference between the content of total and inverted sugars multiplied by 0.95. Statistical analyses were performed using software Statistica 8.0 for Windows (StatSoft Inc., Tulsa, OK, USA). Data from a 2-year investigation were calculated by ANOVA and significant differences among the means were determined by LSD test at a level of $P \leq 0.05$.

Results and discussion

The average earliest harvest time was observed in 'Versailles' (11th of August), whereas 'Paris' was the latest cultivar (10th of September). 'Versailles' expressed three days longer harvest duration compared to data registered in 'Erika', i.e. for four days compared to 'Satine' (Table 1). The shortest harvest duration was recorded in late cultivar 'Paris' (58 days).

Table 1. Ripening season of newly introduced primocane fruiting raspberry cultivars during 2015-2016

Cultivar	Beginning	End	Duration (days)
Erika	August 17	November 7	82
Paris	September 10	November 7	58
Versailles	August 11	November 4	85
Satine	August 15	November 4	81

Primocane growth was significantly affected by cultivar in our study, whereby primocanes of 'Versailles' and 'Satine' were shorter at the end of the season than those of 'Erika' and 'Paris' (Table 2). Cultivars with shorter canes also produced significantly lower number of canes per meter of hedgerow, while no significant differences in cane diameter among the tested cultivars were observed. Carew et al. (2000) reported that short canes produced larger berries and a higher yield. Interestingly, no effect in number of the fruiting nodes was found in their studies suggesting that the short canes were able to produce the same and even more number of fruiting laterals as the tall canes. In our study, 'Erika' had the highest primocane height with the lowest number of fruiting

laterals (213 cm and 7.47/cane, respectively), while 'Versailles' with significantly shorter canes produced much higher fruiting laterals (152 cm and 10.78/cane, respectively). Sønsteby and Heide (2009) reported that rate of growth in primocane-fruiting red raspberry was increased with temperature, but also cultivar and primocane management treatment may have a significant effect.

Table 2. Vegetative potential of newly introduced primocane fruiting raspberry cultivars

Cultivar	Number of canes per meter of hedgerow	Cane height (cm)	Cane diameter (mm)
Erika	6.17±0.20b	213±13.6a	0.99±0.04
Paris	6.10±0.21b	204±10.2a	1.12±0.04
Versailles	7.57±0.07a	152±13.7b	1.06±0.03
Satine	6.97±0.27a	159±12.1b	1.11±0.03
P	***	**	ns

Data are the means of 3 replications ± standard errors. Different letters in column denote significant differences (LSD test, $P \leq 0.05$). Statistically significant differences at ** $P \leq 0.01$, *** $P \leq 0.001$; ns=not significant.

Yield is a complex trait and also depends on cultural practices, environmental conditions and resistance to pest and diseases (Graham et al., 2007). This comparative study indicates that a wide variability in yield components occurs among the tested primocane fruiting raspberry cultivars (Table 3). The highest number of fruiting laterals per cane and consequently the highest yield were registered in 'Paris' (12.07/cane and 840 g/cane, respectively), whereby 'Erika' was the least productive cultivar (561 g/cane and 3.46 kg per meter of hedgerow). The yield per primocane obtained in our experiment was generally greater than that previously reported by Milivojević et al. (2011a), who found 2- to 4-fold lower values for eight primocane fruiting raspberry cultivars grown in Belgrade region (Serbia).

Table 3. Generative characteristics of newly introduced primocane fruiting raspberry cultivars

Cultivar	Number of fruiting laterals per primocane	Yield per primocane (g)	Yield per meter of hedgerow (kg)
Erika	7.47±0.73b	561±13.0c	3.46±0.12c
Paris	12.07±0.60a	840±22.1a	5.13±0.27b
Versailles	10.78±0.15a	805±32.1a	6.09±0.22a
Satine	11.57±0.52a	681±22.8b	4.73±0.14b
P	***	***	***

Data are the means of 3 replications ± standard errors. Different letters in column denote significant differences (LSD test, $P \leq 0.05$). Statistically significant differences at *** $P \leq 0.001$.

Large differences were also found among cultivars in terms of fruit quality characteristics (Table 4). Average fruit weight varied from 3.69 g ('Erika') to 4.93 g ('Versailles'). Since fruit size has been discussed as one of the main components of yield, large size is preferred for the fresh market and improves hand-harvest efficiency.

Table 4. Physical fruit properties of newly introduced primocane fruiting raspberry cultivars

Cultivar	Fruit weight (g)	Index of fruit shape	Number of drupelets per fruit
Erika	3.69±0.03c	1.10±0-04a	90.0±3.76
Paris	4.49±0.13b	0.91±0.01b	97.3±3.26
Versailles	4.93±0.14a	1.05±0.02a	99.8±2.52
Satine	3.32±0.08d	0.85±0.02b	88.6±1.33
P	***	***	ns

Data are the means of 3 replications ± standard errors. Different letters in column denote significant differences (LSD test, $P \leq 0.05$). Statistically significant differences at *** $P \leq 0.001$; ns=not significant.

Raspberry cv. 'Versailles' grown in a soilless system under a polyethylene 'umbrellas' in Switzerland gave higher average fruit weight of 6.16 g, but yield per plant was slightly lower than those obtained in our study (Andrianjaka-Camps et al., 2016). Great variability in the fruit weight of five tested primocane fruiting cultivars affected by the harvest date was also found by Milivojević et al. (2011b), i.e. significantly higher values were recorded in fruits of summer harvest than in autumn harvest. In our study, 'Erika' expressed the highest index of fruit shape (1.10), followed by 'Versailles' (1.05) corresponding to long conical fruit shape. 'Paris' and 'Satine' with lower values of fruit shape index than 1.00 mostly had broad conical and round forms. Without regard to differences in the fruit size, no significant differences were observed in number of drupelets per fruit among the tested cultivars. The obtained results of chemical fruit composition (Table 5) indicated that 'Versailles' yielded the lowest amounts of soluble solids, total and inverted sugars (10.9%, 8.18% and 6.94%, respectively). Probably, larger crop load of this cultivar had a negative effect on the sugar content. Beside crop load, weather conditions also may play a crucial role in the amount of primary metabolites in raspberry fruit (Milivojević et al., 2013). Conversely, the highest contents of soluble solids, total and inverted sugars, as well as sucrose were recorded in the late ripened cultivar 'Paris' (13.7%, 10.37%, 9.05% and 1.25%, respectively).

Table 5. Chemical fruit properties of newly introduced primocane fruiting raspberry cultivars

Cultivar	Soluble solids content (%)	Total acids (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)
Erika	12.4±0.52b	1.92±0.06b	9.50±0.53ab	8.41±0.47ab	1.04±0.05c
Paris	13.7±0.30a	1.88±0.04b	10.37±0.31a	9.05±0.30a	1.25±0.01a
Versailles	10.9±0.18c	1.72±0.08b	8.18±0.22c	6.94±0.21c	1.18±0.01ab
Satine	13.0±0.30ab	2.18±0.07a	9.09±0.06bc	7.90±0.05bc	1.13±0.01bc
P	**	**	**	**	**

Data are the means of 3 replications ± standard errors. Different letters in column denote significant differences (LSD test, $P \leq 0.05$). Statistically significant differences at ** $P \leq 0.01$.

Results from this study are comparable with the published data by Milivojević et al. (2011a) who evaluate the other eight primocane fruiting raspberry cultivars grown in Serbia. Since fruit taste depends not only on the total sugars, but also on organic acids content, their composition may reflect changes in fruit quality. This trait is generally desirable for both fresh and processed fruit, but without a proper balance of acids, a fruit may be perceived as bland and lacking fullness of flavor for fresh consumption. According to Milivojević et al. (2013), raspberries showed citric acid quantities three times higher than malic acid, which could present valuable information for the consumers. Among the tested cultivars in our study, the lowest sized fruit of cultivar 'Satine' had significantly higher total acids content (2.18%) compared to the rest cultivars.

Conclusions

These findings could meet the preference of demanding consumers for fresh raspberries out-of-season. Moreover, farmers who are focused on nearby and niche markets could have interest in these new cultivars to promote their further spreading in commercial raspberry production. All cultivars evaluated herein were acceptable based on their agronomic properties, but only the two cultivars, 'Versailles' and 'Paris', were clearly superior according to their productivity and outer or inner fruit quality. Further research on the impact of cultural practices is needed in order to provide high yields of raspberry fruit with undiminished amounts of phytochemicals, especially with an increasing trend of fresh raspberry consumption.

Acknowledgements

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References

1. Andrianjaka-Camps, Z.N., Wittemann, M.S., Ançay, A., Carlen, C. (2016): New cultivars for quality production of primocane fruiting raspberries enriched in healthy compounds. Acta Horticulturae, 1133, 345-352.
2. Carew, G.J., Gillespie, T., White, J., Wainwright, H., Brennan, R., Battey, N.H. (2000): Techniques for manipulation of the annual growth cycle in raspberry. Journal of Horticultural Sciences & Biotechnology 75(5): 504-509.
3. Egan, H., Kirk, R., Sawyer, R. (eds) (1981): *The Luff Schoorl method. Sugars and preserves*. 152-153. In Pearson's chemical analysis of foods. 8th edn. Harlow. UK: Longman Scientific and Technical.
4. Graham, J., Hein, I., Powell, W. (2007): CHAPTER 9: Raspberry. In: Genome Mapping and Molecular Breeding in Plants, Volume 4, Fruits and Nuts, C. Kole (Ed.), Springer-Verlag Berlin Heidelberg, pp 207-216
5. Milivojević, J., Nikolić, M., Radivojević, D. (2017): Modern raspberry and highbush blueberry production in Serbia – achievements and trends. Zbornik referatov 4. slovenskega sadjarskega kongresa z mednarodno udeležbo, Krško, 20. – 21. januar 2017, 337-350.
6. Milivojević, J., Rakonjac, V., Fotirić Akšić, M., Bogdanović Pristov, J., Maksimović, V. (2013): Classification and fingerprinting of different berries based on biochemical profiling and antioxidant capacity. Pesquisa Agropecuaria Brasileira, 48(9):1285-1294.
7. Milivojević, J., Nikolić, M., Dragišić Maksimović, J., Radivojević, D. (2011a): Generative and fruit quality characteristics of primocane fruiting red raspberry cultivars. Turkish Journal of Agriculture and Forestry, 35, 3: 289-296.
8. Milivojević, J., Nikolić, M., Radivojević, D., Poledica, M. (2011b): Does harvest time influence fruit quality traits in primocane fruiting raspberry cultivars? Proceedings. 46th Croatian & 6th International Symposium on Agriculture. Opatija, Croatia: 1036-1039.
9. Milutinović, MD, Nikolić, M, Milivojević, J, Milutinović, MM, Đaković, G. (2008): Growing primocane raspberry cultivars in Serbia. Acta Horticulturae 777: 443-446.
10. Ochmian, I., Skupień, K. (2008): Comparison of field performance, fruit firmness, and nutritional value of yellow- and red-fruit polish raspberry cultivars. EJPAU 11: 19
11. Pritts, M. (2008): Primocane-fruiting raspberry production. HortScience 43 (6): 1640-1641.
12. Sønsteby, A., Heide, O.M. (2009): Effects of photoperiod and temperature on growth and flowering in the annual (primocane) fruiting raspberry (*Rubus idaeus* L.) cultivar 'Polka'. J. Hort. Sci. Biotechnol. 84:439–446.

CHARACTERISTICS OF FRUITING SHOOTS OF SOME PEACH AND NECTARINE CULTIVARS

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Abstract

Morphological characteristics of fruiting shoots: length, diameter, internode length, length of the basal part without flower buds, number of flower buds (per shoot, node and 1 m of shoot length) were studied in 12 peach and 12 nectarine cultivars. The study was conducted at the Experimental farm "Radmilovac" of the Faculty of Agriculture in Belgrade during the two–year period (2016-2017). Control cultivar for peaches was 'Redhaven', and for nectarines 'Stark Redgold'. For all studied traits statistically significant differences between cultivars were found. The average length of shoots varied from 62.4 to 76.8 cm, diameter from 5.9 to 8.0 mm, and internode length from 2.45 to 3.00 cm. The length of basal part of a shoot without flower buds was on average lower in nectarines (3.9 cm) than in peaches (9.1 cm). Peach cultivar 'Tardibelle' is characterized by the longest basal part of a shoot without flower buds (22.2 cm). All studied peach cultivars are characterized by lower flower bud density compared to control ('Redhaven'). In nectarines, most of the studied cultivars had flower bud density similar to control ('Stark Redgold'). Cultivars with higher flower bud density such as peaches: 'Redhaven', 'Maria Marta', 'Autumn Glo', and nectarines 'Rita Star', 'Maria Carla', 'Orion' require severe pruning in order to obtain better fruit size. On the other hand, cultivars with lower flower bud density e.g. 'Tardibelle', 'Flavorcrest', 'Royal Glory', 'Bolero', 'Maria Lucia' should be pruned slightly in order to obtain higher yield.

Keywords: *Prunus persica*, flower bud density, internode length, pruning.

Introduction

Achieving high yield and good quality of peach fruits requires the regular application of cultural practices. Among them, one of the most important is the pruning. In order to perform the pruning properly, it is necessary to know the characteristics of the fruiting shoots of different cultivars. The main fruiting shoots of peach are long shoots. They are also called „mixed" shoots because on the nodes they have both leaf and flower buds. These shoots are located on the periphery of the crown and give the best quality fruits. The length of shoots and arrangement of flower buds are cultivar-specific traits (Mratinić, 2012). Quality of peach shoots is affected by training system, planting density and rootstock (Loreti et al., 1991). Shoot quality is especially important in high density plantings because of the competition between vegetative and reproductive activity. Perez-González (1993) states that the flower bud density of peach can be expressed as a number of buds per node or per 1 m length of the shoot. The former is the better indicator because it allows the separation of standard genotypes from those with short internodes. The density of flower buds is strongly correlated with the density of flowers and the density of the fruits. Okie and Werner (1996) found that the density of flower buds is under the stronger influence of genetic factors than the environmental factors. In the conditions of the continental climate it is better to choose cultivars with a higher density of flower buds. This increases the survival of generative organs due to the frost occurence and ensures higher yields. Fournier et al. (1998) studied the arrangement of flower buds on peach shoots of different length. They found that, in the long shoots, in the middle part there is a zone in which usually two flower buds are formed. Above and below it are the zones in which one flower bud is formed, while the flower buds are not formed at the base and the top of the shoot. Milatović and Đurović (2010) studied morphological characteristics of fruiting shoots in 15 peach and 10 nectarine cultivars. For all traits statistically significant differences between the cultivars were

found. Some cultivars, such as 'Suncrest', 'Pegaso' and 'Carolina Belle' were characterized by high flower bud density, while other cultivars like 'Summerset', 'Sunprince' and 'Goldcrest' had low flower bud density. The study of the fruiting shoot characteristics of peach and nectarine is important because it can point to the potential productivity of cultivars. This research can also contribute to determining the intensity of the pruning in certain cultivars.

Material and methods

The study was conducted in the peach collection orchard at the Experimental farm "Radmilovac" of the Faculty of Agriculture in Belgrade during the two–year period (2016-2017). The training system is Sloping Leader, a new original system with high-density planting (Zec et al., 2013). This system has a central leader that is bent and follows the row direction at the angle of 25°. The rootstocks (vineyard peach seedlings) were planted in the orchard in June 2010, at a spacing of 3.5 m × 1 m (2800 trees ha^{-1}). The seedlings were budded at a height of 50 cm in September 2010. The study included 12 cultivars of peach and 12 cultivars of nectarine. Control cultivar for peaches was 'Redhaven', and for nectarines 'Stark Redgold'. All cultivars are represented by seven trees. Shoots were taken for examination in the spring, before flowering. Of all cultivars, 20 long („mixed") fruiting shoots were taken. The length of the shoots was measured by a measuring tape and the diameter was measured by a calliper at the base of the shoot (1 cm from the basal end). The number of flower buds was recorded on each node. The density of flower buds has been calculated per node (Werner et al., 1988) and per 1 m of a shoot length (Lombard et al., 1988). The coefficient of variation (CV) was calculated for the studied traits. The results were analyzed using the two-factorial analysis of variance. The significance of differences between means was determined using the LSD test for probabilities of 0.05 and 0.01.

Results and discussion

The length of shoots in peach cultivars was 68.0 cm in average, with a variation from 62.4 cm in the 'Maria Marta' cultivar to 76.8 cm in the 'Redhaven' cultivar (Table 1). Nectarine cultivars had a slightly smaller average length of shoots of 66.9 cm, which varied from 63.9 cm ('Maria Carla') to 70.2 cm ('Max 7'). Differences in the length of shoots between cultivars were statistically significant. The values obtained in our study were higher than the results of Mitreski (1984), who reported an average length of 53.2 cm for 42 cultivars of peach and nectarine. These differences can be due to different age of trees, training system, and pruning intensity. Our results on the length of shoots are in line with the results of Klenyán et al. (1998), who reported an average length of shoots of 57.6 – 73.8 cm in 36 cultivars of nectarine, as well as Milatović and Đurović (2010), who reported the average length of shoots of 53.7 – 78.3 cm in 25 cultivars of peach and nectarine. The diameter of the shoots varied in the interval from 5.9 mm ('Sirio') to 8.0 mm ('Flavorcrest') and in nectarines it was somewhat smaller than in peaches. The obtained values were higher than the results of Mitreski (1984), and similar to the results of Milatović and Đurović (2010). The average length of the internode ranged from 2.45 cm in the 'Bolero' cultivar up to 3.00 cm in the 'Max 7' cultivar. This parameter showed the lowest variability (CV = 15.0%). The length and the diameter of the shoots also showed a relatively small variation (18.1% and 19.5% respectively). At the base of peach fruiting shoots there is a shorter or longer part in which flower buds are not formed, but only incompletely differentiated leaf buds (Fournier et al., 1998). The shoot length without flower buds varied from 1.3 cm in the 'Pegaso' cultivar to 22.2 cm in the 'Tardibelle' cultivar (Table 2). Nectarines had a significantly smaller share of the shoot length without flower buds (6% of the total length in average) compared to peaches (13% in average). The largest share was found in the 'Tardibelle' cultivar (35%). Based on the classification of peach cultivars into three groups according to the arrangement of flower buds along the shoot, this cultivar belongs to the group "B", which has flower buds at 2/3 at the top of the shoot (Veličković, 2004). All other studied cultivars belong to the group "A" which has flower buds almost along the entire length of the shoot.

The average number of flower buds per shoot in peach cultivars ranged from 14.3 ('Tardibelle') to 29.8 ('Autumn Glo'). All studied peach cultivars are characterized by lower flower bud density compared to control ('Redhaven'). In nectarines, the average number of flower buds per shoot was lowest in the 'Maria Lucia' cultivar (22.3), and highest in the 'Rita Star' cultivar (39.1). Most of the studied cultivars had the flower bud density similar to control ('Stark Redgold'). The number of flower buds per shoot was about two times lower in 2016 compared to 2017. The reasons for this can be the absence of summer pruning and strong attack of *Monilinia laxa* in 2015.

Table 1. Characteristics of fruiting shoots of peach and nectarine cultivars

Cultivar	Length (cm)			Diameter (mm)			Length of internode (cm)		
	2016	2017	Mx	2016	2017	Mx	2016	2017	Mx
Peach cultivars									
Autumn Glo	72.4	68.1	70.3	7.3	7.5	7.4	2.75	2.35	2.55
Bolero	59.9	70.4	65.2	6.9	7.9	7.4	2.50	2.39	2.45
Flavorcrest	67.8	74.5	71.2	7.1	8.8	8.0	2.69	2.30	2.50
Maja	73.4	65.1	69.3	6.3	6.5	6.4	3.02	2.51	2.77
Maria Marta	66.5	58.3	62.4	6.1	6.1	6.1	2.70	2.23	2.47
Redhaven	71.8	81.8	76.8	6.7	8.2	7.5	2.90	2.60	2.75
Rich Lady	63.7	66.9	65.3	5.5	7.8	6.7	2.64	2.46	2.55
Romestar	66.9	59.8	63.4	6.2	8.5	7.4	2.93	2.30	2.62
Royal Gem	60.9	71.3	66.1	6.6	8.1	7.4	2.67	2.27	2.47
Royal Glory	65.2	83.0	74.1	5.3	7.2	6.3	2.61	2.54	2.58
Spring Red	64.8	71.2	68.0	6.6	9.0	7.8	2.67	2.29	2.48
Tardibelle	68.2	60.0	64.1	7.1	7.4	7.3	3.45	2.43	2.94
Average	66.8	69.2	68.0	6.5	7.8	7.1	2.81	2.42	2.62
Nectarine cultivars									
Caldesi 2000	67.6	68.1	67.9	6.3	8.8	7.6	2.74	2.51	2.63
Caldesi 85	68.4	60.4	64.4	6.6	6.1	6.4	3.12	2.34	2.73
Maria Carla	66.4	61.4	63.9	5.7	7.4	6.6	2.63	2.39	2.51
Maria Lucia	64.9	75.1	70.0	6.7	8.5	7.6	3.07	2.63	2.85
Max 7	73.8	66.5	70.2	6.9	5.7	6.3	3.38	2.61	3.00
Morsiani 51	60.1	72.0	66.1	5.9	8.6	7.3	2.54	2.68	2.61
Orion	64.6	65.4	65.0	6.1	6.7	6.4	2.57	2.53	2.55
Rita Star	63.2	71.9	67.6	6.7	8.1	7.4	2.38	2.59	2.49
Sirio	71.9	64.4	68.2	6.6	5.2	5.9	3.35	2.61	2.98
Stark Redgold	72.0	60.7	66.4	6.6	7.2	6.9	3.41	2.42	2.92
Venus	63.0	71.5	67.3	6.4	8.0	7.2	2.57	2.78	2.68
Vinčanka	68.0	61.9	65.0	7.2	7.5	7.4	3.12	2.48	2.80
Average	67.2	66.6	66.9	6.4	7.3	6.9	2.79	2.39	2.59
CV (%)			18.1			19.5			15.0
LSD 0.05			5.2			0.5			0.15
LSD 0.01			6.8			0.7			0.19

The number of flower buds per node varied from 0.64 in the 'Tardibelle' cultivar to 1.29 in 'Redhaven' and 'Rita Star' cultivars. This parameter had higher average value in nectarine cultivars compared to peach cultivars. Cultivars in which this parameter has values of about 1.0 or more can be characterized as potentially very productive, while cultivars with lower values (below 0.8) have a potentially lower productivity. The values obtained for the number of flower buds per node are consistent with the results of Werner et al. (1988) who studied this parameter in 36 cultivars of peach and nectarine in the United States and obtained values in the range from 0.35 to 1.59. They concluded that cultivars originating from the California breeding program had lower values of this indicator than cultivars originating from the eastern part of the United States. This indicates that in the eastern part of the United States, greater attention is paid to the flower bud density in the peach

breeding because of the higher risk of frost. Our results of the number of flower buds by node are approximate to those obtained by Klenyán et al. (1998) for nectarine cultivars in the conditions of Hungary (0.33 – 1.50), as well as Milatović and Đurović in the Belgrade area (0.41 – 1.28). The number of flower buds per 1 m of the shoot length is an important indicator of the potential yield of stone fruits (Peréz-González, 1993; Thurzó et al., 2006; Milatović et al., 2014; Milatović et al., 2015). The average number of flower buds per 1 m varied from 23.4 in the 'Tardibelle' cultivar to 51.5 in the 'Rita Star' cultivar. Our results are in line with those obtained by Szabó et al. (1998) that the number of flower buds per 1 m in peach cultivars was 22 – 65, and for nectarine cultivars 25 – 110. The same authors for the conditions of Hungary recommend the cultivars with higher density of flower buds, given the frequent exposure of peach trees to frost damage. In this study, a slightly smaller number of flower buds per 1 m of shoot length was obtained compared to the results of Milatović and Đurović (2010), where the average number for peach cultivars was 44, and for nectarine cultivars 46. The reason for this is a smaller number of flower buds in 2016 year. The largest variability of the tested traits was found in the length of the shoots without flower buds (CV = 94.8%). Also, high variability was found in the number of flower buds per shoot (CV = 42.4%).

Table 2. Distribution and number of flower buds on fruiting shoots of peach and nectarine cultivars

Cultivar	Shoot length without flower buds (cm)			No. of flower buds per shoot			No. of flower buds per	
	2016	2017	Mx	2016	2017	Mx	Node	1 m shoot length
Peach cultivars								
Autumn Glo	10.9	7.9	9.4	23.3	36.2	29.8	1.07	42.7
Bolero	9.2	4.2	6.7	16.0	24.3	20.2	0.75	30.6
Flavorcrest	19.6	6.5	13.1	11.1	31.1	21.1	0.73	29.1
Maja	11.1	5.2	8.2	15.5	27.2	21.4	0.85	31.4
Maria Marta	4.5	3.6	4.1	23.0	32.2	27.6	1.08	44.9
Redhaven	4.3	3.7	4.0	26.3	46.5	36.4	1.29	46.7
Rich Lady	12.5	1.9	7.2	17.0	37.3	27.2	1.06	41.2
Romestar	15.0	5.6	10.3	10.6	32.7	21.7	0.86	35.3
Royal Gem	11.2	5.3	8.3	11.6	31.8	21.7	0.80	31.8
Royal Glory	12.1	13.9	13.0	19.5	24.7	22.1	0.77	29.8
Spring Red	4.8	1.6	3.2	12.4	38.3	25.4	0.91	36.5
Tardibelle	38.6	5.8	22.2	4.1	24.5	14.3	0.64	23.4
Average	12.8	5.4	9.1	16.3	31.4	23.9	0.90	34.8
Nectarine cultivars								
Caldesi 2000	2.9	3.1	3.0	18.6	35.8	27.2	1.04	40.0
Caldesi 85	7.6	3.0	5.3	16.7	33.3	25.0	1.04	39.8
Maria Carla	7.0	2.5	4.8	18.6	36.3	27.5	1.08	43.6
Maria Lucia	3.9	2.7	3.3	14.5	30.7	22.6	0.90	31.6
Max 7	5.3	5.9	5.6	20.8	31.9	26.4	1.10	38.1
Morsiani 51	2.8	4.4	3.6	18.1	28.8	23.5	0.92	35.1
Orion	2.3	2.8	2.6	21.4	35.3	28.4	1.11	43.6
Rita Star	0.6	2.8	1.7	24.7	45.9	35.3	1.29	51.5
Sirio	2.8	4.4	3.6	21.6	28.9	25.3	1.09	37.5
Stark Redgold	5.2	1.8	3.5	19.0	34.6	26.8	1.15	41.7
Venus	1.3	3.0	2.2	21.4	30.4	25.9	1.03	38.2
Vinčanka	2.9	2.1	2.5	20.5	32.7	26.6	1.13	41.5
Average	4.5	3.2	3.9	15.9	32.2	24.1	1.07	35.3
CV (%)			94.8			42.4		
LSD 0.05			2.7			3.0		
LSD 0.01			3.6			3.9		

Conclusions

Cultivars with higher flower bud density (higher number of flower buds per node and per 1 m of shoot length) such as peaches: 'Redhaven', 'Maria Marta', and 'Autumn Glo', and nectarines 'Rita Star', 'Maria Carla', and 'Orion' require severe pruning in order to obtain better fruit size. In these cultivars, during the pruning a smaller number of shoots should be left on the tree. On the other hand, cultivars with lower flower bud density e.g. 'Tardibelle', 'Flavorcrest', 'Royal Glory', 'Bolero', and 'Maria Lucia' should be pruned slightly in order to obtain higher yield. In areas where there is a greater risk of frost, cultivars with higher density of flower buds should be grown, because they can provide a more secure productivity. In contrast, in areas where there is a small risk of frost could be grown cultivars with a lower density of flower buds which require less work for fruit thinning.

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References

1. Fournier, D., Costes, E. and Guédon, Y. (1998). A comparison of different fruiting shoots of peach tree. Acta Horticulturae, 465: 557–565.
2. Klenyán, T., Timon, B. and Szabó, Z. (1998). Morphological characteristics of nectarine varieties twigs and flowers. Acta Horticulturae, 465: 275–278.
3. Lombard, P.B., Callan, N.W., Dennis, F.G.Jr., Looney, N.E., Martin, G.C., Renquist, A.R. and Mielke, E.A. (1988). Towards a standardized nomenclature, procedures, values, and units in determining fruit and nut tree yield performance. Hort Science, 23: 813–817.
4. Loreti, F., Massai, R. and Morini, S. (1991). Effect of training system, planting density and rootstock on growth and quality of peach shoots. Advances in Horticultural Science, 5: 45–47.
5. Milatović, D. and Đurović, D. (2010). Karakteristike mešovitih rodnih grančica sorti breskve i nektarine. Voćarstvo, 44: 27–34.
6. Milatović, D., Đurović, D. and Zec, G. (2014). Morfološke osobine rodnih grančica sorti kajsije. Journal of Agricultural Sciences 59(3): 265–274.
7. Milatović, D., Đurović, D. and Zec, G. (2015). Karakteristike rodnih grančica sorti evropske šljive (*Prunus domestica* L.) i japanske šljive (*Prunus salicina* Lindl.). J. of Agr. Sci. 60(2): 149–158.
8. Mitreski, Z. (1984). Prilog proučavanju mešovitih rodnih grančica u nekih sorti bresaka. Jugoslovensko voćarstvo, 18: 39–44.
9. Mratinić, E. (2012). Breskva. Partenon, Beograd.
10. Okie, W.R. and Werner, D.J. (1996). Genetic influence of flower bud density in peach and nectarine exceeds that of environment. Hort Science, 31: 1010–1012.
11. Peréz-González S. (1993). Bud distribution and yield potential in peach. Fruit Varieties Journal, 47: 18–25.
12. Szabó, Z., Nyéki, J., Szél, I., Pedryc, A. and Szalay, L. (1998). Low temperature injury in peach and nectarine cultivars. Acta Horticulturae, 465: 399–404.
13. Thurzó, S., Drén, G., Dani, M., Hlevnjak, B., Hazic, V., Szabó, Z., Racskó, J., Holb, I.J. and Nyéki, J. (2006). Fruit bearing shoot characteristics of apricot and sweet cherry cultivars in Hungary. International Journal of Horticultural Science, 12: 107–110.
14. Veličković, M. (2004). Opšte voćarstvo I. Poljoprivredni fakultet, Beograd.
15. Werner, D.J., Mowrey, B.D. and Chaparro, J.X. (1988). Variability in flower bud number among peach and nectarine cultivars. Hort Science, 23: 578–580.
16. Zec, G., Vulić, T., Milatović, D., Đorđević, B. and Čolić, S. (2013). The influence of planting density on characteristics of one-year old shoots of peach and nectarine. Acta Horticulturae, 981: 249–253.

PRODUCTIVITY OF EARLY AND MEDIUM EARLY APRICOT CULTIVARS IN THE BELGRADE AREA

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Abstract

Characteristics of productivity and growth (yield per tree, trunk cross-sectional area, yield efficiency and fruit weight) were studied in 40 apricot cultivars of early and medium early maturity in the Belgrade area during the six-year period (2009 – 2014). The cultivar 'Hungarian Best' was used as a control for comparison. The period of study was divided to initial bearing (age of trees three and four years) and full bearing (age of trees from five to eight years). Significant differences in yield between cultivars and years were found. Average yield in the period of initial bearing ranged from 0.3 to 11.3 kg per tree, and in the period of full bearing from 5.6 to 24.8 kg per tree. Compared with the control cultivar, significantly higher yield was obtained in 17 cultivars. Adverse weather conditions resulted in significant reduction of yield in two out of six years of study. Cumulative yield efficiency varied from 0.17 to 0.87 kg/cm^2 and in 19 cultivars it was significantly higher compared with the control cultivar. Fruit weight ranged from 27.7 to 80.1 g. Compared with control, fruit weight was significantly higher in nine cultivars, and significantly lower in 11 cultivars.

Keywords: *Prunus armeniaca*, yield, trunk cross-sectional area, yield efficiency, fruit weight.

Introduction

When selecting cultivars of fruit trees for growing, one of the most important characteristics is their productivity. Apricot is characterized by irregular cropping and large variation of yields among years. This is primarily a result of freezing of flowers and fruitlets due to the occurrence of spring frosts (Rodrigo et al., 2006; Milatović et al., 2013) and, to a lesser extent, the freezing of flower buds due to strong winter frosts (Ozturk et al., 2006; Szabó et al., 2010). Taking into account the variability of weather conditions in particular years, a many-year evaluation is necessary to obtain objective results of productivity of apricot cultivars (Vachůn, 2002a). Apricot cultivar assortment in Serbia is characterized by a small number of cultivars and a short period of maturing. Most apricot fruits are harvested in the season of 'Hungarian Best', which is the most grown cultivar, or at a short time (about ten days) afterwards. There is particularly a lack of early-maturing cultivars of high quality fruit. The introduction of new foreign cultivars and their study in Serbian environmental conditions allow better choice of cultivars, and may improve production of apricots (Milatović et al., 2012). Vachůn (2002a) studied the yield of 24 apricot cultivars in a period of six years and it was in the range of 3 – 20 kg per tree. Yield efficiency (expressed per unit area of trunk cross-sectional area) is a better indicator of productivity than yield level and it provides easier comparability of results (Vachůn, 2002b). Milatović et al. (2006) studied yield of 35 apricot cultivars in the period of eight years. On the basis of the average yield per tree, they classified apricot cultivars into four groups: poor yielding (< 10 kg), medium yielding (10 – 20 kg), high yielding (20 – 30 kg) and very high yielding (>30 kg). The aim of this study was to examine the productivity of a large number of introduced apricot cultivars of early or medium early maturity over a six-year period. The results can help in choosing varieties for cultivation or their use for further breeding work.

Material and methods

Studies were carried out in the apricot collection orchard of the Faculty of Agriculture in Belgrade, at the Experimental Farm "Radmilovac", in the period 2009 – 2014. The orchard was planted in 2007,

and during the study period the age of trees was 3 – 8 years. The study included 40 introduced apricot cultivars of early and medium-early maturity. The cultivar 'Hungarian Best' was used as a control for comparison. All cultivars are grafted on seedlings of Myrobalan (*Prunus cerasifera* Ehrh.) and are represented in the collection orchard with five trees. Training system is central leader, and planting distance is 4.5 × 3 m. The yield was determined by measuring the weight of harvested fruits and is expressed in kg per tree. Trunk cross-sectional area (TCSA) was calculated from the trunk diameter measured at a height of 30 cm above the graft union. Cumulative yield efficiency (CYE) is calculated as a ratio of cumulative yield per tree for six years (2009 – 2014) and TCSA in the last year of study (2014), and is expressed in kg per cm^2. Fruit weight was determined on a sample of 25 fruits per cultivar. Data were analysed statistically by the method of two-factorial analysis of variance. The significance of differences between mean values was determined by LSD test for the probability of 0.05.

Results and discussion

The average time of maturation of tested cultivars ranged from 3 to 26 days before the control cultivar - 'Hungarian Best' (Table 1). Given that the average date of maturity of the cultivar 'Hungarian Best' is 3 July, tested cultivars matured from 7 to 30 June. Productivity of apricot cultivars during the six-year period was uneven, both among years and cultivars (Table 1). Differences in yield between cultivars and years were statistically significant. Although the first yield was obtained in the third year after planting (2009), the first economically significant yield (7.3 kg per tree on average) was obtained in the fourth year (2010). The average yield per tree in the period of initial bearing ranged from 0.3 to 11.3 kg. Early coming into bearing and high initial yield was found in cultivars: 'Sylred', 'Dunstan', 'Pinkcot', 'Palava', 'Bella d'Imola', 'Ninfa', 'Lebona', 'Goldrich', 'Bobcot' and 'Lenova'. On the other hand, late coming into bearing and low initial yield was found in cultivars: 'Moldavsky Olympic', 'LE 5959' and 'Radka'. The average yield per tree in the period of full bearing varied from 5.6 kg in the cultivar 'Moldavsky Olympic' to 24.8 kg in the cultivar 'Sylred'. Compared with the control, a significantly higher yield was found in 17 cultivars. Obtained results for yield are consistent with previous findings (Vachůn, 2002a; Milatović et al., 2006; Fajt et al., 2013). Adverse weather conditions resulted in significant reduction of yield in two out of six years of study. In 2012, there was a winter frost of –20.7°C on 9 February, and a spring frost of –3.0°C on 10 April, after the end of flowering. In tested 33 apricot cultivars the average damage of flower buds from the winter frost was 32.8%, and the average damage of fruitlets from spring frost was 61.4% (Milatović et al., 2013). In 2013, there was a spring frost of -3.4°C on 25 March, which could lead to freezing of flowers of cultivars with early flowering time, that were then in the beginning of the flowering. However, the main reason for lower yields in this year was the cold weather during the flowering, that resulted in weak bee fly and poor fruit set. In 2014 the flowering was extremely early and started from 23 February to 17 March. A frost of –3.0°C occured on 1 March, and caused damage to the cultivars of early flowering time. Nevertheless, the highest average yield (20.5 kg per tree) was recorded in this year. High yield (16.1 kg per tree on average) was also recorded in 2011. On the basis of classification of Milatović et al. (2006), during the period of full bearing, the majority of tested cultivars (27) had medium yielding, (10 – 20 kg per tree) or low yielding, less than 10 kg per tree (13 cultivars). High yielding (more than 20 kg per tree) was found only in the cultivar 'Sylred'. The average yield per hectare in the period of full bearing of all cultivars was 8.9 t, and it ranged from 4.1 to 18.4 t. In some cultivars grown in the Mediterranean area under good cultural practices, including irrigation, a yield of 40 – 50 t per ha can be achieved (Egea et al., 1995). However, in conditions of continental climate, the average yield for a multi-year period is significantly lower due to the occurrence of spring frosts, as well as the decline of trees due to "apoplexy" (Vachůn, 2001). The lowest vigour expressed through the trunk cross-sectional area was determined in the cultivar 'Veselka' - 83.2 cm^2, and the highest in the cultivar 'Radka' - 203.0 cm^2 (Table 2). Significantly higher vigour compared with the control cultivar was found in 14 cultivars.

Table 1. Yield (kg per tree) of apricot cultivars

Cultivar	TM*	Initial bearing			Full bearing				
		2009	2010	Mx	2011	2012	2013	2014	Mx
Aurora	-26	0.1	5.2	2.7	10.1	0.2	4.1	17.5	8.0
Ninfa	-24	5.1	9.3	7.2	18.4	3.6	20.7	15.3	14.5
Zorky	-21	0.2	3.5	1.9	8.4	5.3	1.9	21.1	9.2
LE 5959	-18	0.2	1.1	0.6	4.7	4.6	2.1	31.5	10.7
Leskora	-18	2.2	4.8	3.5	22.0	6.6	5.1	30.9	16.1
Dunstan	-18	6.6	12.3	9.4	33.7	0.1	12.2	22.4	17.1
NJA-2	-17	1.1	4.3	2.7	15.5	10.8	1.0	8.8	9.0
Radka	-17	0.1	1.2	0.7	16.9	1.5	3.2	17.2	9.7
Lejuna	-16	2.0	4.0	3.0	14.1	0.0	3.8	12.7	7.6
Mary Lady	-15	0.1	2.9	1.5	8.5	0.0	2.4	31.5	10.6
Strepet	-15	0.3	5.2	2.8	12.5	13.7	0.1	31.9	14.5
Veselka	-14	0.7	5.9	3.3	16.5	10.7	5.7	18.7	12.9
Vesna	-14	0.5	7.8	4.1	8.4	4.2	3.6	10.1	6.6
Velita	-13	1.5	2.3	1.9	10.9	0.0	11.0	7.3	7.3
Tomcot	-12	0.9	8.1	4.5	15.3	4.7	21.9	18.4	15.1
Lenova	-10	3.1	9.7	6.4	19.3	3.1	1.4	21.4	11.3
Lebona	-10	1.1	13.3	7.2	12.7	5.1	2.1	10.2	7.5
NJA 55	-10	0.1	5.9	3.0	16.2	0.7	11.6	14.4	10.7
Moldavsky Olympic	-9	0.4	0.2	0.3	9.6	0.3	1.3	11.1	5.6
Orangered	-8	1.4	9.7	5.6	25.9	2.3	8.9	34.6	17.9
Legolda	-8	0.8	7.3	4.0	18.5	0.0	6.8	35.7	15.3
Harcot	-8	1.2	6.4	3.8	15.5	16.4	1.3	30.6	16.0
Pinkcot	-7	3.3	14.9	9.1	20.1	6.2	15.4	20.0	15.4
Sylred	-7	3.7	18.9	11.3	33.3	6.7	24.1	35.1	24.8
Palava	-7	2.9	14.7	8.8	30.1	0.8	13.4	5.7	12.5
Velvaglo	-7	0.3	6.7	3.5	17.7	0.5	6.8	9.4	8.6
Lerosa	-6	1.1	9.4	5.2	19.4	2.2	7.9	27.9	14.4
Neptun	-6	1.8	6.1	4.0	13.0	5.7	8.4	32.7	14.9
Effect	-6	2.4	6.1	4.2	12.1	1.4	4.9	31.3	12.4
Bobcot	-6	1.9	11.5	6.7	22.7	10.7	1.1	5.7	10.0
Stark Early Orange	-5	0.3	3.0	1.6	8.7	8.6	4.4	18.0	9.9
Dacia	-5	2.0	4.2	3.1	23.7	9.4	9.0	22.2	16.1
Sundrop	-4	1.5	12.6	7.1	24.7	7.2	2.2	21.4	13.9
Bella d'Imola	-4	2.2	14.2	8.2	12.3	14.8	18.4	14.8	15.1
Velikij	-4	0.4	9.4	4.9	9.8	7.4	0.2	26.9	11.1
Goldrich	-3	1.7	12.5	7.1	15.0	5.8	4.8	18.9	11.1
Forum	-3	0.5	3.4	1.9	13.3	22.6	4.1	11.7	12.9
Laycot	-3	2.8	6.7	4.8	15.5	3.2	2.0	20.8	10.4
Robada	-3	0.9	8.2	4.6	9.2	1.2	7.4	19.8	9.4
Lemira	-3	3.0	7.7	5.4	10.5	0.2	5.8	26.8	10.8
Hung. Best (control)	0	0.3	2.1	1.2	10.8	2.0	3.0	10.3	6.5
LSD 0.05				3.4					6.1

*TM – Time of maturation: Number of days comparing with the cultivar Hungarian Best

Yield efficiency is an important indicator of productivity of apricot cultivars, that combines yield and vigour. Cumulative yield efficiency (CYE) in the six-year period ranged from 0.17 kg/cm^2 in the cultivar 'Moldavsky Olympic' to 0.87 kg/cm^2 in the cultivar 'Sylred'. Compared with the control cultivar, significantly higher CYE was determined in 19 cultivars. The rank of cultivars according to yield (in kg per tree) and CYE is not identical. On the basis of CYE, low vigorous cultivars such as 'Neptun', 'Veselka', 'Strepet', 'Lenova' and 'Lemira' are much better ranked than on the basis of yield. The opposite is case with vigorous cultivars, such as 'Radka', 'Mary Lady', 'NJA 2' and 'NJA 55'.

Table 2. Trunk cross-sectional area (TCSA) in 2014, cumulative yield efficiency (CYE) and fruit weight (average 2009-2014) of apricot cultivars

Cultivar	TCSA (cm^2)	CYE (kg/cm^2)	Fruit weight (g)
Aurora	147.2	0.25	51.1
Ninfa	149.3	0.49	43.4
Zorky	95.9	0.42	33.2
LE 5959	127.4	0.35	49.2
Leskora	143.8	0.50	38.9
Dunstan	175.9	0.50	75.3
NJA-2	160.0	0.26	33.3
Radka	203.0	0.20	62.0
Lejuna	92.0	0.40	27.7
Mary Lady	184.2	0.25	65.1
Strepet	89.4	0.71	64.7
Veselka	83.2	0.70	53.5
Vesna	121.1	0.29	46.8
Velita	137.9	0.24	41.9
Tomcot	116.5	0.60	43.1
Lenova	93.1	0.62	40.8
Lebona	154.8	0.29	49.3
NJA 55	168.5	0.29	50.7
Moldavsky Olympic	130.6	0.17	43.8
Orangered	171.0	0.48	48.2
Legolda	139.6	0.50	53.3
Harcot	106.1	0.67	49.4
Pinkcot	113.5	0.70	50.3
Sylred	140.4	0.87	59.7
Palava	154.1	0.44	51.0
Velvaglo	158.8	0.26	55.4
Lerosa	138.4	0.49	50.5
Neptun	84.6	0.80	80.1
Effect	161.2	0.36	76.0
Bobcot	122.3	0.44	43.3
Stark Early Orange	147.2	0.29	53.1
Dacia	100.3	0.70	61.9
Sundrop	116.8	0.60	42.8
Bella d'Imola	112.7	0.68	55.3
Velikij	131.7	0.41	52.6
Goldrich	129.3	0.45	61.8
Forum	137.1	0.41	47.8
Laycot	99.6	0.51	58.5
Robada	141.6	0.33	62.3
Lemira	88.0	0.61	49.6
Hungarian Best (control)	101.5	0.28	53.3
LSD 0.05	41.7	0.18	8.3

Our results of CYE are within the ranges reported by other authors: Ogašanović et al. (1991) 0.48 – 1.63 kg/cm^2, Vachůn (2002b) 0.22 – 1.36 kg/cm^2; Milatović et al. (2006) 0.07 – 0.96 kg/cm^2; Licznar – Malanczuk and Sosna (2013) 0.07 - 0.41 kg/cm^2, Milošević et al. (2013) 0.17 – 0.82 kg/cm^2. Fruit weight ranged from 27.7 g in the cultivar 'Lejuna' to 80.1 g in the cultivar 'Neptun'. Compared with the control, nine cultivars had significantly higher and 11 cultivars significantly lower fruit weight. Our results of fruit size are consistent with previous findings for some cultivars (Drogoudi et al., 2008; Krška et al., 2013; Licznar-Malanczuk and Sosna, 2013; Szalay et al., 2013).

Conclusions

Productivity of apricot cultivars in the Belgrade area was uneven, both among years and cultivars. Adverse weather conditions resulted in significant reduction in yield in two years. High yields (above 15 kg per tree) in the period of full bearing were found in cultivars: 'Sylred', 'Orangered', 'Dunstan', 'Leskora', 'Dacia', 'Harcot', 'Pinkcot', 'Legolda', 'Tomcot' and 'Bella d'Imola'. A large fruit size (over 60 g) was determined in the following cultivars: 'Neptun', 'Effect', 'Dunstan', 'Mary Lady', 'Strepet', 'Robada', 'Radka', 'Dacia' and 'Goldrich'. On the basis of high yield and large fruit size, for growing in the Belgrade area cultivars 'Sylred', 'Dunstan' and 'Dacia' can be recommended.

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References

1. Drogoudi, P.D., Vemmos, S., Pantelidis, G., Petri, E., Tzoutzoukou, C. and Karayiannis, I. (2008). Physical characters and antioxidant, sugar, and mineral nutrient contents in fruit from 29 apricot (*Prunus armeniaca* L.) cultivars and hybrids. Journal of Agricultural and Food Chemistry, 56(22): 10754–10760.
2. Egea, J., Garcia, J.E., Egea, L. and Berenguer, T. (1995). Productive behaviour of apricot varieties in a warm winter area. Acta Horticulturae, 384: 129–133.
3. Fajt, N., Komel, E., Usenik, V., Donik-Purgaj, B., Beber, M., Ambrožič-Turk, B., Viršček Marn, M. and Mavrič-Pleško, I. (2013). Proizvodnja kajsije u Sloveniji - problemi i perspektive. Zbornik radova IV Savetovanja „Inovacije u voćarstvu", 11 Februar 2013, Beograd, pp. 171–182.
4. Krška, B., Vachůn, Z., Nečas, T. and Ondrášek, I. (2013). Apricot breeding at the Faculty of Horticulture in Lednice. Zbornik radova IV Savetovanja „Inovacije u voćarstvu", 11 February 2013, Beograd, pp. 117–122.
5. Licznar-Malanczuk, M. and Sosna, I. (2013). Growth and yielding of the several apricot cultivars on the 'Somo' seedling and vegetative rootstock Pumiselect®. Acta Scientiarum Polonorum - Hortorum Cultus, 12(5): 85–95.
6. Milatović, D., Đurović, D. and Milivojević, J. (2006). Rodnost sorti kajsije u beogradskom području. Arhiv za Poljoprivredne Nauke, 67 (240): 69–77.
7. Milatović, D., Đurović, D., Nikolić, D. and Zec, G. (2012). Improvement of apricot cultivar assortment in Serbia. Acta Horticulturae, 966: 131–135.
8. Milatović, D., Đurović, D. and Zec, G. (2013). Osetljivost sorti kajsije na zimski i pozni prolećni mraz. Zbornik radova IV Savetovanja „Inovacije u voćarstvu", 11 February 2013, Beograd, pp. 239–247.
9. Milošević, T., Milošević, N. and Glišić, I. (2013). Neki pokazatelji uspešnosti gajenja kajsije (*Prunus armeniaca* L.) u polugustoj sadnji. Zbornik radova IV Savetovanja „Inovacije u voćarstvu", 11 February 2013, Beograd, pp. 225–238.
10. Ogašanović, D., Plazinić, R. and Papić, V.M. (1991). Results from the study of some early apricot cultivars on various interstocks. Acta Horticulturae, 293: 383–389.
11. Ozturk, K., Ölmez, H.A., Guloglu, U. and Kuden, A. (2006). Evaluation of the resistance of some apricot varieties growing in Malatya to winter hardiness and late spring frost. Acta Horticulturae, 701: 247–252.
12. Rodrigo, J., Julian, C. and Herrero, M. (2006). Spring frost damage in buds, flowers and developing fruits in apricot. Acta Horticulturae, 717: 87–88.
13. Szabó, Z., Veres, E., Soltész, M., Gregová, E., Benediková, D. and Nyéki, J. (2010). Flower density and winter damage of apricot and peach varieties. International Journal of Horticultural Science, 16(4): 53–56.
14. Szalay, L., Hajnal, V., Németh, S., Ficzek, G. and Vécsei, B. (2013). Fruit quality parameters of foreign apricot cultivars in Hungary. Acta Horticulturae, 981: 675–678.

15. Vachůn, Z. (2001). The influence of tree decline on yields of new genotypes of apricots and some cultivars of the world collection (*Prunus armeniaca* L.). Horticultural Science (Prague), 28(4): 138–144
16. Vachůn, Z. (2002a): Production weight and its variability in 24 apricot genotypes over six years. Horticultural Science (Prague), 29(3): 105–113.
17. Vachůn, Z. (2002b). Specific productivity of selected apricot genotypes. Horticultural Science (Prague), 29(4): 125–132.

ROOTSTOCK INFLUENCE ON VIGOR AND GENERATIVE POTENTIAL OF YOUNG SWEET CHERRY TREES

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Abstract
The main goal of breeding and testing of rootstocks for sweet cherry is to obtain small and productive trees and to improve precocity. The objective of this study was to examine the influence of six rootstocks on vigor and productive characteristics of young sweet cherry trees. The experimental orchard is situated at the Experimental farm "Radmilovac", of the Faculty of Agriculture (near Belgrade). Three sweet cherry cultivars: 'Kordia', 'Karmen' and 'Regina' were grafted on six rootstocks: *Prunus mahaleb* L. seedlings, 'Colt', 'Ma×Ma 14', 'Gisela 6', 'Gisela 5' and 'Oblačinska' sour cherry. During a two-year period (2015-2016) the following characteristics were studied: scion diameter above the grafting union, rootstock diameter under the grafting union, length and diameter of shoots, height of the tree and the number of spurs per tree. The results showed different influence of rootstocks on the tree vigor, spur formation and precocity. The average diameter of the scion in all tested cultivars was the largest on the Mahaleb rootstock. The largest average number of spurs per tree in the second year was recorded on cherry trees grafted on the rootstocks 'Gisela 6' and 'Oblačinska' sour cherry (28 and 23 respectively). The lowest average number of spurs (6) was found on trees grafted on the rootstock 'Colt'.

Keywords: *Prunus avium*, clonal rootstocks, cultivar, vigor, spurs.

Introduction

Cherry production in Serbia is predominantly extensive and almost fully based on seedling rootstocks - Mazzard (*Prunus avium* L.) and Mahaleb (*Prunus mahaleb* L.). Trees have strong vigor that makes application of cultural practices, especially harvest, difficult and also decreases effectiveness of production (Milatović et al., 2011c). Furthermore, cherry trees grafted onto vigorous seedling rootstocks have slow coming into bearing. In the last decades, new low vigorous clonal rootstocks are taking dominance in cherry production since their influence on small tree habit and precocity. Intensification of sweet cherry production can be achieved by using the dwarf or moderate vigorous rootstocks such as 'Gisela 5' and 'Gisela 6'. These rootstocks induce precocity and decrease the vigor of scion. The low vigor of the 'Gisela 5' rootstock allows higher planting density, earlier coming into bearing and higher yield per unit area compared to seedling rootstocks (Lang, 2001). However, this rootstock has many negative features: requires fertile soil, irrigation and support, and is prone to over-cropping and small fruit size. In addition, cherry cultivars on this rootstock are susceptible to the freezing of flower buds during the winter (Lichev and Papachatzis, 2006; Milatović et al., 2011a). In Serbia, as a replacement for 'Gisela 5' rootstock, genotypes of 'Oblačinska' sour cherry are often used as a dwarf rootstock for sweet cherry (Milatović et al., 2011b). New achievements in the selection of rootstock that are suitable for high density orchards mostly improved cherry production (Miljković et al., 2002). The main goal of our research was the evaluation of different rootstocks on vigor and generative potential of young sweet cherry trees.

Material and methods

Trial was established in 2014 at the Experimental farm "Radmilovac", belonging to Faculty of Agriculture, situated near Belgrade. Three sweet cherry cultivars 'Kordia', 'Carmen' and 'Regina' were grafted onto six rootstocks: *Prunus mahaleb* L. seedlings, 'Colt', 'MaxMa 14', 'Gisela 6', 'Gisela 5' and 'Oblačinska' sour cherry. The central leader training system was applied. During a two-year period (2015-2016) the following characteristics were studied: scion diameter above the grafting union, rootstock diameter under the grafting union, diameter and length of shoots, height of the tree and the number of spurs per tree. Trial was established as a completely random block system and each cultivar was represented with three replications with three trees per replication. Statistical data analysis was performed using analysis of variance and LSD multiple range test at $P \leq 0.05$ to determine significance of differences between the means.

Results and discussion

The largest average diameter of the rootstock, as well as height, length and diameter of the shoot was found in cherry trees of the cultivar 'Carmen' on the Mahaleb rootstock (Table 1). These trees had statistically significantly higher values of the diameter of the rootstock (in both years) and the length of the shoot (in 2015) compared to other rootstocks. Hrotko et al. (2009) examined the vigor of the 'Carmen' cultivar on several rootstocks and concluded that the trees grafted on the Mahaleb had a significantly higher value of the diameter of the trunk than trees on the 'Colt' rootstock. The values obtained in this study are in accordance with these results. All investigated parameters of vigor had the least average values on the 'Carmen' trees grafted on the 'Gisela 5' rootstock. The values of rootstock diameter, tree height and shoot length on the rootstocks 'Gisela 5', 'Gisela 6' and 'Oblačinska' sour cherry in 2016 were statistically significantly lower compared to other three rootstocks, indicating their lower vigor.

Table 1. Values of tested parameters on the trees of the cultivar 'Carmen'

Year	Rootstock	Rootstock diameter (mm)	Scion diameter (mm)	Height of tree (cm)	Length of shoot (cm)	Diameter of shoot (mm)	Number of spurs
2015	*P. mahaleb*	38.7	29.3	168.7	33.0	5.9	1.7
	Colt	23.3	21.3	152.3	20.0	4.9	1.7
	Ma x Ma 14	30.0	29.0	160.0	20.7	6.0	3.7
	Gisela 6	25.7	29.3	116.3	21.3	6.0	12.0
	Gisela 5	22.7	25.0	98.3	16.7	4.7	8.3
	Oblačinska	29.0	30.3	145.3	21.7	5.8	13.3
	LSD 0.05	5.6	2.1	16.8	2.8	0.9	2.5
2016	*P. mahaleb*	63.7	56.3	266.7	68.3	9.5	11.3
	Colt	47.3	39.7	220.3	62.3	9.8	9.3
	Ma x Ma 14	52.3	40.0	217.3	60.0	9.4	10.3
	Gisela 6	31.0	28.7	124.3	35.7	8.4	15.3
	Gisela 5	22.0	25.0	113.7	29.3	7.3	13.7
	Oblačinska	25.7	27.7	163.3	45.0	9.4	15.0
	LSD 0.05	6.9	5.5	32.5	9.8	1.4	2.8

Highest average number of spurs in the 'Carmen' cultivar in 2015 was recorded on 'Oblačinska' sour cherry (13.3) and in 2016 on the 'Gisela 6' rootstock (15.3). In both years, trees of this cultivar on rootstocks 'Gisela 6', 'Oblačinska' sour cherry and 'Gisela 5' had statistically significantly higher number of spurs than trees on other rootstocks (except for Mahaleb in the second year). The smallest average number of spurs in both years was recorded on the 'Colt' rootstock (1.7 and 9.3). All investigated parameters of vigor (except the shoot diameter) in the 'Kordia' cultivar showed highest average values on the Mahaleb rootstock (Table 2). The values of the Mahaleb rootstock diameter were statistically significantly higher compared to rootstocks 'Gisela 5', 'Gisela 6' and

'Oblačinska' sour cherry. Comparing the trunk cross-sectional area of trees on Mazzard with that on 'Gisela 5' and 'Gisela 6' rootstocks after 7 seasons, Whiting et al. (2005) found lower values for 45% and 20% respectively. The results obtained in our study are in accordance with the above data. The highest average number of spurs in the first year (11) was recorded on the 'Kordia' cultivar on the 'Gisela 6' rootstock. In the second year the number of spurs was highest on the 'Oblačinska' sour cherry rootstock (23). This is in line with states of Milatović et al. (2011b) that the rootstocks 'Gisela 5', 'Gisela 6', and 'Oblačinska' sour cherry affect the precocity and increase the productive potential of grafted sweet cherry trees. The smallest average number of spurs in the first year was found on the 'Colt' rootstock (2.3), and in the second year on the 'MaxMa 14' rootstock (6.3).

Table 2. Values of tested parameters on the trees of the cultivar 'Kordia'

Year	Rootstock	Rootstock diameter (mm)	Scion diameter (mm)	Height of tree (cm)	Length of shoot (cm)	Diameter of shoot (mm)	Number of spurs
2015	*P. mahaleb*	34.0	32.0	167.0	47.7	6.5	3.0
	Colt	24.7	24.7	139.0	44.0	6.9	2.3
	Ma x Ma 14	25.0	22.3	155.7	39.3	6.3	3.0
	Gisela 6	23.3	24.3	163.7	34.0	6.0	11.0
	Gisela 5	20.7	22.0	136.3	25.0	5.7	10.3
	Oblačinska	24.7	25.3	163.7	30.0	6.3	10.3
	LSD 0.05	2.8	2.6	14.5	2.7	0.4	1.8
2016	*P. mahaleb*	62.3	57.0	286.7	80.3	10.1	14.3
	Colt	48.0	42.3	227.0	62.3	9.1	9.3
	Ma x Ma 14	49.7	45.7	239.0	71.3	10.1	6.3
	Gisela 6	34.3	41.0	210.3	58.3	8.6	16.3
	Gisela 5	36.0	36.0	182.7	47.7	7.7	20.0
	Oblačinska	47.7	52.0	243.0	65.3	9.9	23.0
	LSD 0.05	5.1	7.1	55.8	10.0	1.4	4.1

Highest average values of the vigor parameters were recorded on trees of the 'Regina' cultivar on the Mahaleb rootstock, while they were lowest on 'Gisela 5' rootstock (Table 3). The values of the rootstock diameter and the length of shoot in this cultivar on the rootstocks 'Gisela 5', 'Gisela 6' and 'Oblačinska' sour cherry were statistically significantly lower compared to rootstocks Mahaleb and 'MaxMa 14', which confirms their lower vigor. Baryła et al. (2014) examined the influence of Mazzard, Mahaleb and 'Gisela 5' on the vigor of the 'Regina' cultivar in the nursery. The study showed a significant effect of rootstocks on the trunk diameter of maiden sweet cherry trees. This cultivar had a significantly smaller diameter on 'Gisela 5' rootstock. This is in line with results of our study. The highest average number of spurs in the second year was recorded in the 'Regina' cultivar on the 'Gisela 6' rootstock (28). In both years, the number of spurs in the this cultivar was statistically significantly higher on rootstocks 'Gisela 6' and 'Oblačinska' sour cherry in comparison with other rootstocks. The smallest average number of spurs in both years of study was recorded on the 'Colt' rootstock (4 and 6 respectively). The trees of all three cultivars on rootstocks 'Gisela 5', 'Gisela 6' and 'Oblačinska' sour cherry had a larger diameter of the scion than that of the rootstock, which also classifies them as a weak to moderate vigorous.

The average number of spurs in all cultivars was smaller on the 'Gisela 5' rootstock compared to rootstocks 'Gisela 6' and 'Oblačinska' sour cherry. This can be explained with the smallest length and the smallest diameter of the shoot that was recorded in both years on this dwarf rootstock. Low vigor expressed through short and thin shoots is mostly genetically determined, but it may also be the result of inadequate soil properties and cultural practices. This is in accordance with the observation of Bassi et al. (2016) that the rootstock 'Gisela 5' performs well in good pedoclimatic conditions, while in suboptimal conditions it does not perform well, therefore it is advisable to use the 'Gisela 6' rootstock for such locations.

Table 3. Values of tested parameters on the trees of the cultivar 'Regina'

Year	Rootstock	Rootstock diameter (mm)	Scion diameter (mm)	Height of tree (cm)	Length of shoot (cm)	Diameter of shoot (mm)	Number of spurs
2015	*P. mahaleb*	41.3	37.7	191.0	47.7	6.8	11.0
	Colt	24.3	23.0	165.7	33.0	5.8	4.0
	Ma x Ma 14	31.7	30.3	173.0	32.7	5.9	11.0
	Gisela 6	25.7	28.7	167.7	30.3	5.9	20.0
	Gisela 5	23.7	25.7	138.0	27.3	5.3	11.3
	Oblačinska	26.0	28.3	173.0	31.3	5.9	23.0
	LSD 0.05	2.3	2.5	18.4	7.5	0.5	2.3
2016	*P. mahaleb*	62.0	59.0	297.3	72.7	9.5	12.0
	Colt	51.7	49.0	246.0	58.3	8.7	6.0
	Ma x Ma 14	56.3	53.3	245.7	64.7	9.0	12.3
	Gisela 6	45.3	54.0	226.0	53.0	8.1	28.0
	Gisela 5	38.3	46.3	182.0	43.0	8.1	20.0
	Oblačinska	43.3	49.3	210.0	51.0	8.1	20.7
	LSD 0.05	6.8	7.5	43.5	10.8	1.0	3.4

Conclusions

The vigor of the tested sweet cherry cultivars was under significant influence of the rootstock. It was highest on the Mahaleb rootstock, and lowest on 'Gisela 5' rootstock. The largest number of spurs in all cultivars was recorded on rootstocks 'Gisela 6' and 'Oblačinska' sour cherry. The obtained results indicate that the trees on rootstocks 'Gisela 6' and 'Oblačinska' sour cherry had a medium vigor and good initial generative potential. 'Oblačinska' sour cherry rootstock showed very good initial results that should encourage further examination of selected clones in multi-year trials. The smallest number of spurs, and therefore the lowest initial generative potential in all cultivars was found on the 'Colt' rootstock. The very low vigor, short and thin shoots resulted in poor initial generative potential of studied cultivars on the 'Gisela 5' rootstock.

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References

1. Bassi, D. (2005). Influenca dei portinnesti sulla produzione del ciliegio. L' Informatore Agrario, 24: 44-59.

2. Bassi, G., Fajt, N., Bisko, A., Donik Purgaj, B., Draicchio, P., Folini, L., Gusmeroli, F. and Steinbauer, L (2016). Vegetative and productive performances of 'Kordia' and 'Regina' sweet cherry cultivars grafted on four clonal rootstocks in the Alpe Adria region. Acta Horticulturae, 1139: 159-166.

3. Baryła, P. Kapłan, M. and Krawi, M. (2014). The effect of different types of rootstock on the quality of maiden trees of sweet cherry (*Prunus avium* L.) cv. 'Regina'. Acta Agrobotanica, 67(4): 43-50.

4. Hrotko, K., Magyar, L. and Gyeviki, M. (2009). Effect of rootstocks on growth and yield of 'Carmen' sweet cherry. Bulletin UASVM Horticulture, 66(1): 143-148.

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. and Papachatzis, A. (2006). Influence of ten rootstocks on cold hardiness of flowers of cherry cultivar 'Bigarreau Burlat'. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture, 25(3): 296-301.

7. Milatović, D., Đurović, D., Vulić, T., Đorđević, B. and Zec, G. (2011a). Osetljivost novijih sorti trešnje na podlozi Gizela 5 na zimske mrazeve. Zbornik radova III savetovanja „Inovacije u voćarstvu“, Beograd, pp. 231-238.
8. Milatović, D., Nikolić, M. and Miletić, N. (2011b). Trešnja i višnja. Naučno voćarsko društvo Srbije, Čačak.
9. Milatović, D., Đurović, D., Đorđević, B., Vulić, T. and Zec, G. (2011c). Pomološke osobine novijih sorti trešnje na podlozi Colt. Journal of Agricultural Sciences, 58(1): 61-72.
10. Miljković, I., Čmelik, Z. and Vrsaljko, A. (2002). Podloge za trešnju. Pomologia Croatica, 8: 115-134.
11. Ninkovski, I. (1998). Trešnja: savremeni načini podizanja, gajenja i iskorišćavanja. Potez Uno, Beograd.
12. Whiting, D.M., Lang, G. and Ophardt, D. (2005). Rootstock and training system affect sweet cherry growth, yield and fruit quality. Hort Science, 40(3): 582-586.

INFLUENCE OF SOME SOIL AND CLIMATE FACTORS OF THE REGION OF TROYAN OVER THE YIELD AND QUALITY OF PLUM FRUITS OF 'KATINKA', 'TEGERA', 'ELENA' CULTIVARS, IN NATURAL GRASS ESTABLISHMENT

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Abstract

Grey forest soils with heavy sandy clay structure and low nutrient availability are characteristic for the conditions of the region of Troyan. Climate conditions are the following: the average annual temperature is about 10°C and the annual rainfall is about 750 mm. The duration of vegetation period is 245 days. The experiment includes small-sized plum cultivars 'Tegera' and 'Elena', grafted on Mirobolan seedlings, and planted in 2000; and 'Katinka' grafted on 'Fereley' rootstock, planted in 2005, all are being grown under non-irrigated conditions. The aim is to determine the influence of soil and climate factors in the region of Troyan over the phenology and reproduction of the introduced German plum cultivars of 'Katinka', 'Tegera' and 'Elena'. The flowering, some vegetative and reproductive indicators were observed and chemical analysis of fruits was conducted for characterization of their taste qualities. It is found that in the foothill region of Troyan, plum cultivars find favourable conditions for growth and development, but they require regular agrotechnical measures and increased attention in extreme climate changes. In 2015, which was a favourable year, the plum harvest of 'Katinka' was about 40 kg per tree. Fruits had very small weight (15; 16; 21g), but the great quantity gave opportunity for high yield. The earlier ripening cultivars 'Tegera' and 'Katinka' accumulate less dry matter in comparison with the late ripening cultivar 'Elena'.

Keywords*:* Troyan region Bulgaria, agro-ecological conditions, plum, cultivars.

Introduction

The typical for plum production for the region of Troyan are agro-climatic conditions, which ensure the normal development and cultivation of the plum culture, meeting its biological requirements. The technologies being developed and tested in RIMSA include different elements for pre-ground preparation of the areas and different ways of maintaining the soil surface to ensure optimal yields and good quality of fruit production (Dinkova et al., 2006 b; 2010; Dinkova, 2009). The aim is to adapt to the requirements of sustainable agriculture. Sustainable farming technologies are aimed at: Not to destroy but to improve the natural environment, i.e. soil to be protected from erosion; To store the humus; Maximum moisture and nutrients to accumulate and economize; To better exploit the productive capacity of agricultural crops.

Scientific research shows that agrophysiological properties and, first of all, the structure have deteriorated sharply after extensive soil set-aside, especially without further introduction of organic matter (Merwin et al., 2004). The high aeration created as a result of soil cultivation leads to an intensive mineralization of the humus, which leads to soil degradation of organic matter. By bracing the interrow and maintained in the grass, it is possible to reduce the number of soil cultivation, at the expense of cultivation of green fertilization crops, which maintains the integrity of the soil surface and increases the content of organic matter, hence increasing the moisture holding capacity of the soil (Dinkova et al., 2006 a; Reeve et al., 2013). This reduces the surface runoff, the evaporation from the soil surface and the underground water reserves are increased (Savory and Butterfield, 1999; Devyatov et al., 2000). The cultivar is a major element in technology for growing and fruit production. It is required to be resistant to the abiotic factors of the ecosystem and to minimally apply chemical remedies. In this connection, for many years in RIMSA Troyan are tested

plum cultivars and among them are ‘Katinka’, ‘Tegera’ and ‘Elena’ (Dinkova, and Dragoyski, 2005; Dinkova et al., 2007; Dragoyski et al., 2009; 2010). The aim is to determine the influence of soil and climate factors in the region of Troyan over the phenology and reproduction of the introduced German plum cultivars 'Katinka', 'Tegera' and 'Elena'.

Material and methods

The experiment was conducted during the period 2014-2016 in the semi intensive plum plantation of RIMSA Troyan, founded in 2000 on a slope about 5-6º, with cultivars 'Katinka', 'Tegera' and 'Elena' and 'Stanley' for control, with a planting scheme of 5 × 3 m. The cultivation is under non-irrigating conditions, the interrow was maintained in grass.

Characterization of cultivars

Cultivars ‘Katinka’ and ‘Tegera’ ripen earlier (in the beginning of August) cv ‘Elena’ is the latest in our assortment – ripens at the end of September.Their fruits are small (‘Katinka’) to moderately large (‘Tegera’ and ‘Elena’). Placed under the same growing conditions they showed peculiarities in developmental and reproductive events, some of which require specific technological approach to cultivation. The very first results showed that the German cultivars ‘Katinka’, ‘Tegera’ and ‘Elena’ adapt well in terms of area. They bear fruit regularly and show satisfactory tolerance of viral disease Plum Pox.

Characteristics of soil

Prevailing soil type in the region is planosols with a pH 5.7, poor in nutrients. The area is maintained natural grass cover incurred, an agrochemical analysis for two consecutive years showed that organic matter concentrated in humus horizon 0-20 cm is slightly (1.72%) and decreases in depth horizon (20-40 cm) (Table 1). In 2015 reported higher values humus is stored, as they are not carried out soil cultivation. In 2015 the total nitrogen also reduced profile depth (0-20cm) by 42% compared to 2014. The low humus content and total nitrogen are the factors that determine poor preservation of the soil to absorb nitrogen. The content of assimilable phosphorus is low. Average stock is absorbed potassium. Soil reaction ranges from acidic to slightly acidic.

Table 1. Content of the main nutrients in the soil

Depth cm	pH H_2O	KCl	∑ $N\text{-}NH_4+NO_3$ mg/kg	P_2O_5 mg/100g	K_2O mg/100g	Humus %
2014						
0-20	4.8	3.6	28.2	2.7	20.9	1.72
20-40	5.7	4.7	20.2	3.7	16.6	1.21
2015						
0-20	6.1	5.6	15.0	18.0	25.3	3.26
20-40	5.0	4.1	11.5	1.1	14.9	1.17

Characteristics of climatic conditions

The average annual rainfall for the region of Troyan is 750 mm for the past 20 years, which is 20% more than for the country. Rainfall during the vegetation period is relatively evenly distributed. The average relative humidity is about 75%, the average annual temperature is about 10 °C. The length of the vegetation period is 245 days. Extreme abnormalities were observed during the study period, however, with an excessive amount of rainfall in the vegetation (1151 mm) in 2014. These extremely unfavorable climatic conditions during the flowering and the formation of harvesting compromised fruit harvest of all cultivars included in the study. In 2015, the cooler March and larger rainfall slowed the flowering phenophase. The highest rainfall is measured in June and September in 2015, and in 2016 in the spring months of April and May (fig. 1).

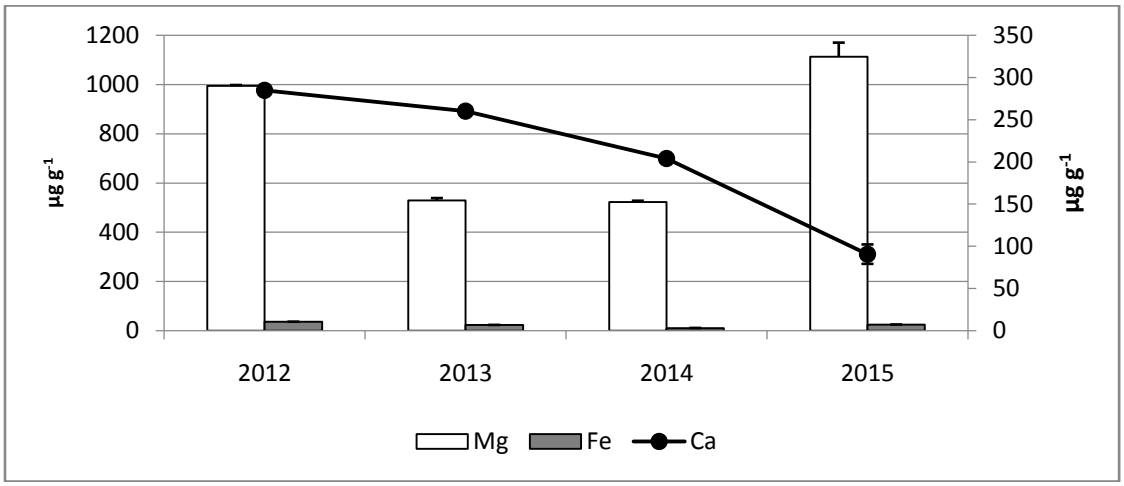

Figure 1. Climatic indicators 2014-2016

The following metrics were reported:

- Phenological data
- Vegetative parameters

Trunk cross-section area (cm^2); Crown volume (m^3); Projection of crown (m^2)

- Reproductive parameters:

Yield per tree (kg); Fruit weight (g)

- Chemical composition of fresh plum fruits:

- Soluble solids (refractometrically) – (%);
- Sugars according to Schoorl – (%);
- Acids, as malic, by titration with 0,1n KCl – (%);
- Tannins – according to Levental-Neubauer – (%);
- Antocianins – Filsky and Fransis (mg%)

The studies were conducted according to Methods for Studying Plant Resources (Nedev et al., 1979). The experimental data were subjected to statistical analysis by Fisher's single-factors ANOVA. The significance of differences between the mean values of the factors and the interaction means was determined by LSD test at significance levels of P≤0,05.

Results and discussion

The most important one is the sensitivity of the flowers of the variety to cool weather conditions at blooming time. For instance, 'Italian Prune' is very sensitive to bad weather conditions during flowering as well as 'Valjevka' and 'Cacanska najbolja', whereas 'Cacanska lepotica' and 'Katinka' are relatively robust (Neumüller, 2011). In 2014 the earliest flowering began in plum cultivar 'Tegera' and was the longest (15 days). The latest blooming cultivar was 'Katinka'. During full bloom, rain fell in high amounts that probably prevented the normal flow of pollination in plums. In 2015, there was a delay in starting dates of flowering. Overall the flowering phase passed in the period from 15 to 25.04 (last 10 days). High temperatures in late March in 2016, prompted by 10-12 days early awakening of buds bloom was extended in the period from 28.03 to 15.04. The terms of ripening of early cultivars 'Katinka' and 'Tegera' include 15-20 July. Later ripening cultivars 'Elena' and 'Stanley' (control) are ripe with 15 to 20 days earlier in 2016. A better price is usually realised at the end and especially at the beginning of the harvesting season. Therefore, an extension of the ripening time is desirable.

Table 2. Phenology phases 2014-2016

	Beginning of flowering	Full flowering	End flowering	Fruit ripening period
2014				
Katinka	5-6.04.	8-10.04.	11-14.04	10.07
Tegera	28.03-3.04	4-8.04	9-14.04	25.07
Elena	30.03-2.04	3-8.04	9-13.04	11.09
Stenley	30.03.-2.04	3-8.04	16-18.04	23.08
2015				
Katinka	14-16.4	17-24.04	25-29.4	15-20.07
Tegera	16-17.04	18-22.04	23-29.4	28-30.07
Elena	15-18.04	19-22.04	23-29.04	15-20.09
Stenley	14-16.04	17-23.04	25-29.04	1-6.09
2016				
Katinka	2-5.04	6-12.04	13-15.04	14-16.07
Tegera	31.03-04.04	5-8.04	9-10.04	20-25.07
Elena	29-31.03	1-4.04	5-6.04	1-5.09
Stenley	1-4.04	5-8.04	9-11.04	3-6.08

Table 3. Vegetative and reproductive characteristics 2015-2016

	Katinka	Tegera	Elena	Stenley	*LSD 0,05*
Trunk cross-section area (cm^2)					
2015	39.01	93.15	130.02	87.08	
2016	46.23	157.35	161.28	90.31	
Crown volume (m^3)					
2015	2.82	5.73	18.34	21.56	*8.32*
2016	5.29	22.96	27.69	12.81	*6.86*
Projection of crown (m^2)					
2015	3.04	4.19	11.39	13.04	*5.99*
2016	4.71	12.40	14.37	7.72	*3.17*
Yield per tree (kg)					
2015	40.00	25.00	35.00	6.00	
2016	6.40	3.50	8.00	1.50	
Fruit weight (g)					
2015	16.69	24.16	14.91	28.14	*2.47*
2016	18.95	27.97	21.63	37.68	*2.35*
Yield efficiency (kg cm^{-2})					
2015	1.025	0.268	0.269	0.069	
2016	0.138	0.022	0.050	0,017	

Studied plum cultivars have different strength growth, 'Tegera' and 'Elena' are more vigorous than control 'Stanley'. Parameters trunk cross-section area and crown volume for 2016 are similar in value significantly different from 2015 (table 3). Trees cv. 'Tegera' for one season increased the trunk cross-section area by 64 cm^2, while those of cv. 'Elena' 31 cm^2, i.e. increase in 'Tegera' is doubly stronger than cv. 'Elena'. The volume of crowns in cv. 'Tegera' increased 2 times more than cv. 'Elena' (table 3). The projections of the crowns are 12-14 m^2, and according to the scheme of planting, provided their nutritional area of 10 m^2, therefore after the winter season will apply slightly

stronger pruning to reduce the size of crowns. Yield efficiency is the highest in cultivar 'Katinka' in 2015, as the resulting yield per unit section is the greatest (table 3). In favorable 2015 reported satisfactory yields of plum harvest. The highest in cultivar 'Katinka', about 40 kg per tree, followed by 'Elena' (35 kg). Their fruits are of very small weight (15-16 g), but their abundant amount allows for high yield. Popski et al. (2015) determine the size fruits of cv. 'Katinka' as large, medium and small with scales according to 21.27-12,26g, respectively average weight 16.69g, similar to the actual investigation. In the control cv. 'Stanley' yield is extremely low and is not compensated by larger fruit with weight 28-38 g. In all cultivars there is a significant decrease in yield in 2016 compared to 2015 (table 3).

Table 4. Chemical composition of fresh plum fruits

	Soluble solids (%)	Total sugar (%)	Inverted sugar (%)	Sucrose (%)	Total acids (%)	Tannins (%)	Anthocyanins (mg%)
2015							
Katinka	14,5	8,5	5,5	2,90	0,38	0,104	21,94
Tegera	15,0	8,9	5,0	3,71	0,61	0,125	15,32
Elena	20,5	8,9	3,2	5,42	0,58	0,166	12,10
2016							
Katinka	15,0	10,6	6,9	3,56	0,45	0,229	23,71
Tegera	17,3	10,0	4,9	4,94	0,64	0,270	19,19
Elena	21,0	10,9	5,4	5,27	0,45	0,187	18,23

There have been chemical analysis of fruits to characterize the taste qualities. Early cultivars 'Katinka' and 'Tegera' had very small dry matter (%), later cultivar 'Elena' was able to accumulate a larger percentage (20,5 to 21,0%) as the dry summer and sunny days favor this process. Total sugars and acids were almost identical for all three cultivars, and in 2015 were around 9%, in 2016 about 10%. The acids ranged from 0,4 to 0,6%, regardless of the year. Cv. 'Katinka' contained very small amount of sucrose, making it suitable for consumption by diabetics and the highest amounts of anthocyanins (22-23%) (table 4).

Conclusions

The soil-climatic and agro-ecological conditions of the region of Troyan are favorable for the normal development and regular bearing of plums from the introduced plums of German cultivars 'Kantka', 'Tegera' and 'Elena'. In years with extremely different climatic conditions – severe over-humidification (abundant rainfall during the 2014 vegetation), the fruit harvest is completely compromised. Standard agro-technical measures can maintain optimal growth and yield regular and high yields as the studied cultivars have potential. Yield efficiency is the highest in cultivar 'Katinka' in 2015.

References

1. Devyatov, A. S. Kapichnikova N. G. Statskevich I. M. (2000). Soil maintenance systems in plum orchard on different rootstocks (Sistemy soderzhaniya pochvy v sadu slivy na raznykh podvoyakh), Plodovodstvo (Belarus), 13: 95-101.
2. Dinkova, H. and Dragoyski, K. (2005). New innovated plum cultivars and opportunities for their cultivation in Bulgaria. Plant Science, XLII, (1): 34-37
3. Dinkova, H. Mihaylova, P. Stefanova, B. Vitanova, I. Angelova, S. (2006 a.) Possibilities for growing of annual forage crops in a young plum plantation. Journal of Mountain Agriculture on the Balkan, 9, (5): 867-876.
4. Dinkova, H., Dragoiski, K. Mihaylova, P. (2006 b.) Planting in trenches – a promising method for establishing of orchards in the mountain regions. Vocarstvo, Vol. 40: 107-112.

5. Dinkova, H. Dragoiski, K. Stefanova, B. (2007). Tegera and Elena – new plum cultivars in Bulgaria, Vocarstvo, 41, (157-158): 25-30
6. Dinkova, H. (2009). New elements in plum cultivation technology. Habilitation work, Troyan, Bulgaria.
7. Dinkova, H. Dragoyski, K. Stefanova, B. Mihaylova, P. Vitanova, I. Rusenov, A. (2010). Possibilities for Organic Production of Plum Fruits from Cacanska Lepotica Cultivar by Applying Trench Planting. Acta Hort. (ISHS) 874: 113-119.
8. Dragoyski, K. Dinkova, H. Stefanova, B. Mihaylova, P. (2009). Evaluation of Plum Cultivars Suitable for Sustainable Fruit Production in the Mountain Regions of Bulgaria. Vocarstvo, 43: 37-43.
9. Dragoyski, K. Minev, I. Dinkova, H. Stoyanova, T. Minkov, P. (2010). Evaluation of some introduced plum cultivars at RIMSA Troyan in Bulgaria; Acta Hort. (ISHS) 874: 311-315.
10. Merwin, I. A. (2004). Groundcover management effects on orchard production, nutrition, soil, and water quality. New York Fruit Quarterly 12: 25-29.
11. Nedev N., Grigorov, Y. Baev, H. Serafimov, S. Strandzhev, A. Kavardzhikov, L. Lazarov, K. Nikolov, N. Djuvinov, V. Popova, L. Slavov, N. Iliev, P. Stoyanov, D. Kanev, I. Krinkov, H. Vishanska, Y. Topchiyska, M. (1979). Methodology for the Study of Plant Resources in Orchard Plants, Plovdiv
12. Neumüller M. (2011). Fundamental and Applied Aspects of Plum (*Prunus domestica*) Breeding Fruit, Vegetable and Cereal Science and Biotechnology, 5 (Special Issue 1): 139-156 ©2011 Global Science Books
13. Popski G., Stefanova B., Minev I**. 2015.** Structure and quality analysis of yield for 'Katinka' plum cultivar) Plant science (Bg), год. LII, (6): 58-61
14. Reeve, J. Black, B. Ransom, C. Culumber, M. Lindstrom, T. Alston, D. Tebeau, A. (2013). Developing organic stone-fruit production options for utah and the intermountain west United States. Acta Hort. (ISHS) 1001: 65-72. http://www.actahort.org/books/1001/1001_5.htm
15. Savory, A. and Butterfield, J. (1999). Holistic Management. Island Press. Washington, D. C: 616

PRIMARY EFFECTS OF GAMMA RADIATION (Cz^{137}) ON THE MORPHOLOGY OF LEAVES AT SOME SWEET CHERRY VARIETIES

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Abstract

II. The study has been conducted based on the presence of atypical leaf morphology, in relation to leaf shape, size, color, leaf nervation, leaf stem length and leaf stem thickness, as a primary effect product in the first MV1 generation after the gamma radiation with Cz^{137} on Bigareau Burlat, Pobeda Krimska and Kozerska cherry varieties**.** The selected leaf radiomorphosys were used as conservative and accurate signs for early detection of somatic mutations caused by radiation. Graft branches were exposed with dosages of 25Gy, 35Gy and 45Gy at the Institute of Radiobiology and Radio-preservation in Sofia. The grafting was performed with dormant buds onto Prunus mahaleb rootstock. The dosage augmentation caused average reduction of leaf dimensions and leaf stem lengths in all of the tested varieties. Also, with the augmentation of the radiation dosage, the percentage of chlorophyll deficiency leaves increased from 1,1% at 25Gy to 7,4% at 45Gy. An average of 47,5% of the analyzed leaves have an atypical shape, without significant differences between the varieties, and 14,5% of the leaves have an atypical leaf nervation . The highest percentage of leaves with atypical shape and leaf nervation are represented in plants treated with a dose of 45Gy.

Keywords: *Prunus avium* L., ionizing dosage, leaf shape, leaf size and color, leaf stem length.

Introduction

The primary effects of gamma radiation on fruit species are noticed as a visible phenotypic changes in the first MV1 generation following the treatment. The radiation usually results with lethal or sub-lethal damages of the cells and tissues and with atypical growth and development of plants. Appearance of the modification changes at fruit plants can be used as a diagnostic measure for somatic mutations (Nybom, 1961), (Bishop, 1967), (Равкин,1973), (Миленков, 1974,1979), (Donini,1975,1991), (Lapins, 1983). The effects are result of the direct damage of the apical meristem of the leaf buds, as well as the secondary effect of the physiological disbalance which emerges in the affected cells (Guncke and Sparrow, 1961). The authors mention that the regeneration area of the affected cells emerges in the primary brunches with an atypical position of the buds, significantly fattened nodal regions, and an atypical brunch color, presence of furcations and fasciations and atypical leaves morphology. These changes present a promising starting material for further selection and creation of new interesting genotypes. For the appearance of various morphological changes in the leaves as a result of the effect of ionizing radiation in the fruit species adduce Zwintcher (1955, 1967), (Жуков,1963), (Петров и Сермяжко, 1963), (Шепотъев, 1968), (Колесникова, 1970), (Lapins and Hough,1970), (Миленков, 1974). The authors of the available literature refer to the various changes in the leaf color, size, leaf-forms, the length of leaf stem, changes in the leaf serration and in the leaf nervature. The selected leaf radiomorphosys are conservative and accurate indications for early detection of somatic mutations. Zwintcher (1955) even declare that the propagation of leaf buds with radiomorphosys is more efficient and more economical than the propagation of all buds located in the plant's foundation. Considering the fact that there are not enough data for this scientific area in the available new literature, the aim of this

paper is to analyze the presence of leaf radiomorphosys, as a primary effect product of radioactive treatment and indicators for early detection of somatic mutations on three cherry varieties with different dosages of Cz^{137}.

Material and methods

Dormant buds from Bigareau Burlat, Pobeda Krimska and Kozerska cherry varieties were treated with radioactive Cz^{137} in doses of 25Gy, 35Gy and 45Gy. Graft branches were exposed to radiation at the Institute of Radiobiology and Radiopreservation in Sofia. Prunus mahaleb L. was used as a rootstock for grafting the buds in two following seasons, right after the treatment on 30th of August. Untreated buds from each variety were used as a control variant. Each variant was grafted onto two hundred rootstocks. An early diagnosis of the primary effects of the radiation was made in the first MV_1 generation following the treatment. Basic criteria for first choice was made according to the appearance of plants with decreased vigorousness and irregularly positioned leaf buds, presence of furcations (bi-, three- and polyfurcations), atypical leaves and expressive outspread of the plants (Popovska et al.,2011). A study has been conducted on the presence of atypical leaf morphology, in relation to leaf shape, leaf nervation, size, color, leaf stem length and leaf stem thickness, from total number of leaves at 195 selected plants, in the first year after the treatment of dormant buds. The measures were made in august, when leaves were properly differentiated. The leaf size is classified according to the leaf length in five groups: very small (< 5cm); small (5-8cm); medium size (8-12cm); large (12-16cm) and very large leaves (>16cm). Leaf stem length is classified in three groups: short (<3,5cm), medium length (3,5 – 5cm) and long leaf stem (>5cm). Control variants have dark green leaf- color. Atypical leaf color is noticed as various shades of green color, leaves with deficiency of chlorophyll and dark green leaves with yellow leaf-nerves. The research was performed at the experimental field in the Institute of Agriculture in Skopje. The soil type is silt - clay loam, suitable for cherry production, with moderate alkaline pH according to its reaction in water and neutral according to its reaction in KCl, carbonate, with a low amount of humus, with a good amount of hydrolyzing nitrogen and optimal amount of easily obtainable phosphorous and potassium. The trial was watered with a drop irrigation system.

Results and discussion

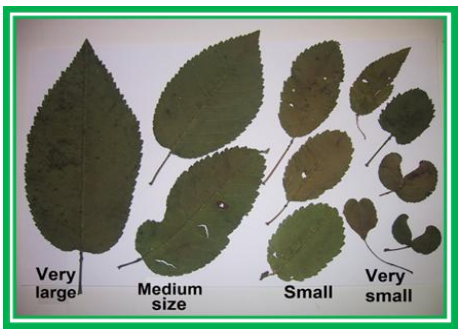
According to the basic criteria for early diagnostic of the effects of treatment with radioactive Cz137, 195 or 46,9% of the total number of plants received after the treatment, have been selected in MV1 generation (Popovska et al.,2011). The decreased vigorousness of the selected plants has the most participation of 29,3% from all present primary effects, followed by furcations (23%), then plants with atypical leaves (6,1) and then with expressive outspread (4,3%) (Popovska et al., 2011). Respective to the variety, 88 plants are selected from Bigareau Burlat, 54 plants from Pobeda Krimska and 53 are from Kozerska. Related to the dosage, 83 are selected from radiation of 25Gy, 58 from 35Gy and 54 from 45Gy (Popovska et al., 2011). The control variants are all with large and very large leaves. An average dimensions of 15,8 cm in length and 8 cm in width are present at Bigareau Burlat. This variety is by far the most versatile in terms of leaf size in comparison to the remaining control varieties (Table 1). All leaves are with a dark green hue, are smooth and have thick and mostly medium length leaf stems (Table 1-3), of about 3,6 cm. Pobeda Krimska has the most uniformed as well as the largest leaves, 18,5 cm long and 9,5 cm wide, out of all the varieties. The leaves are consistently smooth, dark green although slightly brighter than those of the remaining two varieties, while their leaf stems are of a medium thickness and long (about 5,5 cm in length). The Kozerska variety is comprised of very large leaves (16,8 cm long and 9 cm wide) with a smooth texture, dark green, and with thick and long leaf stems (5,2 cm). The data from Table 1., clearly indicate that the gamma rays more or less consistently lower the size of the leaves. This is easily confirmed by the 75,9% of large leaves with the control variants that has plummeted to 4,3% within the leaves of selected plants. The medium sized and small leaves fall 2-3 times away from the dimensions of controls, with an inconsistent percentage throughout the tested variants, but they are

still characterized with an average rise that accompanies the increased dosage with the values being at their highest at 45 Gy. The Kozerska variety is the most bountiful in small leaves while Pobeda Krimska is characterized by having very small leaves.

Table.1. Leaf size and leaf roughness of selected plants, %

Variety	Dose	leaf size, %					leaf roughness, %	
		very small	small	medium size	large	very large	smooth leaves	rough leaves
Bigareau Burlat	Control			13,3	56,7	30,0	100,0	
	25Gy	9,3	12,4	47,9	28,0	2,3	90,8	9,2
	35Gy	2,5	16,7	44,7	26,1	10,1	65,2	34,8
	45Gy	11,3	20,5	38,0	23,5	6,6	73,0	27,0
	25-45Gy	7,6	15,7	43,3	27,1	6,2	79,2	20,8
Pobeda Krimska	Control					100,0	100,0	
	25Gy	7,1	24,7	48,4	17,8	2,2	72,2	27,8
	35Gy	9,0	16,7	55,5	12,7	6,2	63,2	36,8
	45Gy	7,3	27,7	43,8	17,5	3,6	59,1	40,9
	25-45Gy	7,8	21,9	48,1	17,2	5,0	68,2	31,8
Kozerska	Control				16,7	83,3	100,0	
	25Gy	5,7	14,1	49,2	29,5	1,5	81,1	18,9
	35Gy	5,0	35,3	36,7	18,3	4,8	83,7	16,3
	45Gy	6,4	27,4	52,2	12,4	1,7	96,2	3,8
	25-45Gy	5,0	22,1	45,8	24,5	2,6	83,6	16,4
Average of controls				4,4	21,7	73,9	100,0	
Average 25 Gy		7,4	17,1	48,4	25,1	2,0	81,4	18,6
Average 35 Gy		5,5	22,9	45,6	19,0	7,0	70,7	29,3
Average 45 Gy		8,3	25,2	44,7	17,8	4,0	76,1	23,9
Average 25 - 45 Gy		7,1	21,7	46,3	20,6	4,3	76,0	24,0

Picture 1. Tipical leaf of controls (very large with tick and long stem)



Picture 2. Different leaf sizes, stem length and thickness and atypical leaf shapes

Leaves with sizes lower than 4 cm in length, 2 cm in width, and 1,5 cm in leaf stems were also noticed by (Шепотъев *(*1968) with units established by radiation of cherry seeds in a 0,5; 1,5; 2,0; 2,5, 3,0 and 3.5 kR variety of dosage with the control variety being of 12,5 cm long, 6 cm wide and leaves with 3 cm long leaf stems. Миленков (1974), had also noticed leaves two or three times the size of the control variety and attributed such changes to the larger radiation dosages. With the equal number of leaves (85) between a control and a 20Gy treated variety the Droganova Zolta exhibited an assimilate surface of 5323,55 cm^2 and 2721,35 cm^2 respectively. The samples with tiny leaves exhibited and assimilated surface of only 397,95 cm^2. According to the data in Table 1, all of the control variants leaves are of a smooth texture, while 24% of the treated material exhibits a rough leaf texture especially with the 35 Gy dosages. The leaf stem is a conservative asset to the

morphological description of the varieties and it consistently thick with the Bigareau Bulrat and Kozerska controls, and medium-thick with Pobeda Krimska control variant (Table 2, Picture 1-2).

Table 2. Thickness and length of leaf stem , %

Variety	Dose	leaf stem (thickness and length), %					
		thin	medium thickness	thick	short	medium length	long
Bigareau Burlat	Control			100,0		66,7	33,3
	25Gy	13,7	58,1	28,2	9,6	68,0	22,4
	35Gy	19,0	45,8	35,2	19,3	61,4	19,3
	45Gy	27,8	47,7	24,5	13,4	73,3	13,4
	25-45Gy	19,8	48,8	31,4	13,4	66,7	19,9
Pobeda Krimska	Control		100,0				100,0
	25Gy	45,4	37,3	17,3	10,9	59,9	29,2
	35Gy	24,6	60,3	15,1	11,3	66,4	22,3
	45Gy	51,1	23,4	25,5	29,2	26,3	44,5
	25-45Gy	35,8	45,6	18,6	13,3	59,0	27,7
Kozerska	Control			100,0			100,0
	25Gy	25,7	29,0	45,3	17,9	56,3	25,8
	35Gy	20,8	48,8	30,4	17,7	55,5	26,8
	45Gy	22,5	52,2	25,3	12,9	48,4	38,7
	25-45Gy	20,5	37,8	41,7	16,1	55,1	28,8
Average of controls			33,3	66,7		22,2	77,8
Average 25 Gy		28,3	41,4	30,3	12,8	61,4	25,8
Average 35 Gy		21,5	51,6	26,9	16,1	61,1	22,8
Average 45 Gy		33,8	41,1	25,1	18,5	49,3	32,2
Average 25 - 45 Gy		27,9	44,7	27,4	15,8	57,3	26,9

The selected plants are characterized mostly by a medium-thick leaf stem (44,7%) which closely follows the large sized leaves (Table 2, Picture 2). The small and tiny leaves exhibit thin leaf stems while the large and very large leaves are accompanied by thick leaf stems (Picture 2). The percentage of leaves with medium-thick leaf stems with Rana Bulratova closely follows the control variant. The number of leaves with long leaf stems has been decreased, and the percentage with short leaf stem has significantly risen with the highest value at 35Gy dosage (Table 2). All the leaves from the control variants of Pobeda Krimska and Kozerska are with long leaf stems whereas all the selected plants are characterized by leaves with medium-long leaf stems (55-59%). According to the data, the rise in dosage raises the short leaf stem percentage from 12,8% with 25Gy to 18,5 % with 45Gy radiation dosage. The change of leaf color is quite amusing. The gamma rays treatment can directly influence the synthesis of the biogenic elements which, in turn, directly influence the plants nourishment. The disruption of this synthesis most commonly manifests itself as a chlorophyll deficiency which reveals a certain discoloration with the leaves (Gunckel,1957). This deficiency can also occur as a side-effect from ionized radiation that inhibits the DNA, RNA and protein synthesis (Lapins,1983). The discoloration, as a modification, may disappear with following generations or be permanently adopted if the mutagen treatment directly inhibits the genetic material that controls chlorophyll synthesis in which case it is a matter of chlorophyll mutation (Петров и Сермяжко, 1963), (Lapins and Hough, 1970), (Миленков,1974). The control variants within this research refer to the dark-green leaves. There has been a myriad of hues detected with the treated material. The most characteristic ones were the light-green, chlorophyll deficiency leaves (Picture 3) and the dark-green leaves with a yellowish leaf nerves (Picture 4).

Picture 3. Chlorophyll deficiency (Bigareau Burlat 35 Gy) yellowish leaf nerves

Picture 4. Dark green leaves with a (Pobeda Krimska 45 Gy)

Table 3. Leaf color, %

Variety	Dose	leaf color, %			
		dark green	light green	chlorophyll deficiency leaves	dark green with yellowish nerves
Bigareau Burlat	Control	100,0			
	25Gy	92,2	5,3	2,5	
	35Gy	88,1	7,7	4,2	
	45Gy	93,1	0,8	6,2	
	25-45Gy	91,3	4,5	4,2	
Pobeda Krimska	Control	100,0			
	25Gy	98,5	1,5		
	35Gy	89,9	3,5	6,6	
	45Gy	72,3	2,9	16,1	8,8
	25-45Gy	90,3	2,7	5,6	1,4
Kozerska	Control	100,0			
	25Gy	96,4	2,7	0,9	
	35Gy	87,5	12,5		
	45Gy	90,3	9,7		
	25-45Gy	92,8	6,5	0,7	
Average of controls		100,0			
Average 25 Gy		95,6	3,3	1,1	
Average 35 Gy		88,5	7,9	3,6	
Average 45 Gy		85,2	4,5	7,4	2,9
Average 25 - 45 Gy		89,8	5,2	4,0	1,0

Approximately 5,2% of the tested leaves have displayed a light green hue (Table 3). This type of discoloration was most persistent with the 35Gy dosage with all the varieties especially with Kozerska (12,5%). On average, about 4% of the leaves are with deficiency of chlorophyll, with a yellowish tint which is not bound to any particular area of the leaf. The Bigareau Burlat 35Gy sample is characterized with a yellow tint between the leaf nerves while the nerves themselves are intensely green (Picture 3). These symptoms are very similar to that portraying iron deficiency in leaves. These types of leaves are also closely related to the radiation dosage in terms of them being more prominent with the rise of dosage (1,1% with 25Gy and 7,4% with 45Gy). The Pobeda Krimska variety has the most leaves with chlorophyll deficiency. Only four samples of the same variety displays an intriguing yellow hue along the lead nerve line (Picture 4) which has not yet been discovered in thus far available literature. Миленков (1974), noticed such discoloration of leaves acquired with a 50Gy radiation over mature cherry cuttings and 30Gy radiation over cherry green cuttings. The discoloration varied from light green, greying-green to dark green. The author analyzed the discoloration according to Krumel's table (1936) which distinctly clarifies the discoloration. The

discolored surface reached from 30 to 80 mm^2 and, with some samples, it even covered over 50% of the leaf surface. The yellowish discoloration was either present over the entire leaf surface or solely covered the edges of the leaf. All the symptoms resembled a nourishment microelement deficiency however, no matter how similar, these changed could not be attributed to any such changes for there were perfectly normal and green leaves along the same branch. The buds were further clipped and the same mutation was kept for the following three generations which points towards the mutation of the genetic material in charge of the organic matter synthesis. They can cover entire leaf surfaces or a selected area while the agrochemical analysis hinted at a certain Mn deficiency (Петров, Самяжко (1963), (Шепотъев, 1968), (Колесникова, 1970), (Lapins and Hough, 1970). They also mention of different hue discoloration ranging from light green to burgundy and purple. Петров и Самяжко (1963) noticed a mutagenic change in terms of a chlorophyll deficiency which went on to be transferred onto vegetative progeny. The ionizing radiation in our trial influenced on shape of the leaves from the treated material. The shape of the leaves is a conservative and accurate sign used to determine the morphological description of the varieties. The control variants are of unified slightly elongated elliptically shaped leaves with Bigareau Burlat displaying a more distinct narrowing at the tip of the leaf (Pictire 1).

Table 4. Atypical leaf shape and leaf nervation %

Variety/ Dose	leaf shape				leaf nervation			
	25 Gy	35Gy	45Gy	Average	25 Gy	35Gy	45Gy	Average
Bigareau Burlat	46,5	49,6	46,1	47,0	6,4	11,6	10,3	8,9
Pobeda Krimska	46,7	44,6	59,9	47,8	10,4	16,8	21,9	16,9
Kozerska	49,3	49,3	44,6	47,6	24,5	11,2	18,0	17,6
Average	47,5	47,8	50,2	47,5	13,7	13,2	16,7	14,5

Some of atypical forms include: oval leaves, extremely linear leaves, elliptical leaves without a distinguished tip, asymmetrical leaves, heart shaped leaves, wedge shaped, leaf stems with multiple leaves in different shapes, double tipped leaves, a double petioled leaf with an additional leaf attached to it, leaves with different sorts of cuts and different depths of cuts along the surface of the leaf. These myriads of anomalies make it difficult for each atypical shape to be accurately described. Approximately 47,5% of the analyzed leaves deviate in shape from the control varieties with no major differences between the varieties and dosages (Table 4). The control variants display a typical nervation with a central nerve symmetrically branched out into secondary fishbone nerves along the entire surface of the leaf, and more intensive tertiary nerves towards the edge of the leaf. A leaf's nervation is a pretty distinct mark of the fruit variety. About 14,5% of the leaves from the selected plants display atypical leaf nervation which does not correspond to that of the control variants. The atypical nervation was greatly prevalent under a 45Gy dosage and, in terms of variety, within Kozerska at 25Gy (24,5%) (Table 4.). Most commonly, an atypical nervation closely follows an atypical leaf shape. Some type of nervation are quite difficult to describe, for instance, there has been a case where the secondary nerves branch out upwards and parallel to the central nerve, a case where the central nerve splits in two at the very tip of the leaf, a case of absence of the central nerve, parallel nervation turned upward or down, all sorts of intense branching of the secondary nerves throughout the entire leaf surface or towards the edge of the leaf, parallel secondary nerves on the opposite side of the leaf, extreme nerve branching on the opposite side of the leaf, secondary nerves with an acute angle at the lower part of the leaf while the top part nervation is typical and follows the elongated and asymmetrical leaves etc.

Conclusions

Gamma radiation with doses of 25 Gy, 35 Gy and 45 Gy of Cz137, causes primary effect in relation of changes in the morphological properties of the leaves in the examined cherry varieties Bigareau Burlat, Pobeda Krimska and Kozerska, in the first MV1 generation after the radiation. The dosage augmentation causes average reduction of leaf dimensions and leaf stem lengths, and increasing of

the percentage of leaves with chlorophyll deficiency, in all of the tested varieties. The Kozerska is the most bountiful in small leaves while Pobeda Krimska is characterized by having very small leaves. With the augmentation of the radiation dosage, the presence of leaves with short leafy steam increased from 12,8% at 25Gy, up to 18,5% at 45Gy, and the presence of leaves with chlorophyll deficiency from 1,1% at 25Gy to 7,4 % of 45 Gy. The effect of ionizing radiation is reflected on leaves shape and on the leaf nervation. An average of 47,5% of the analyzed leaves have an atypical shape, without significant differences between the varieties, and 14,5% of the leaves have an atypical leaf nervation . The highest percentage of leaves with atypical shape and leaf-nervation are represented in plants treated with a dose of 45Gy.

References

1. Bishop C.J. (1967). Radiation induced mutations in vegetative propagated fruit trees. Proceedings of the XVII International Horticultural Congress, Vol 1:83-95.
2. Donini B. (1975). Improvement of vegetative propagated plants through induced mutations. Proceedings of Research Coordination Meeting, Tokai, Vienna. IAEA, Vol.1:35-51.
3. Donini *et.al*. (1991). Mutation breeding programmes for the genetic improvement of vegetative propagated plants in Italy. Proceedings of an International Symposium on the Contribution of Plant Mutation Breeding to Crop Improvement. Vienna. IAEA., (1): 237-254.
4. Gunchel, J.E. Sparrow A.H. (1961). Ionizing radiations:biochemical, physiological and morphological aspects of their effects on plants. Encyclopedia of Plant Physiology, 16, 555-611.
5. Жуков О.С. (1967). Изменения в характере роста и цветения костичковых култур под десйтвием рентгеновских лучей. Труды ЦГЛ им.Мичурина, N°9.
6. Колесникова А.Ф. (1970). Индуцированный мутации вишни. Селъскохозяйственная биология., No 4. Москва.
7. Lapins K.O., Hough L.F. (1970). Effects of gamma rays on apple and peach leaf buds at different stages of development.II. Injury to apical and axillary meristems and regeneration of shoot apices. Radiation Botany, 10: 59-68.
8. Lapins K.O. (1983). Mutation breeding. Methods in fruit breeding. Edited by James N. Moore and Jules Janick. Purdue University Press West Lafayette. Indiana.
9. Миленков М.М. (1974). Индуцированный мутагенез у черешни. Диссертация на соискание ученой степени кандидата биологических наук. Академия наук СССР, Сибирское отделение. Обединенный ученый совет по биологическим наукам. Новосибирск.
10. Миленков М., Георгиев С. (1979). Някои биологични особености на мутантни форми от черешовите сортове Хеделфингенска и Дроганова жълта. Постиженuа на научноисследователския Институт по овощтарство Кюстендил през периода 1969-1979. Издателство Хр. Данов, Пловдив, 61-67.
11. Nybom, N. (1961).Theuse of induced mutations for the improvement of vegetative propagated plants. Symposium on mutation and plant breeding. Washington, pp. 252-294.
12. Петров Д.Ф., Сермяжко И.А. (1963). К вопросу о значении воздействия ионизирующей радиации на черенки для получения новых сортов яблони, устойчивх в условиях Сибири. Новосибирск.
13. Popovska M., Popovski B., Gjamovski V. (2011). Criteria for early diagnostics of primary effects of the treatment with gamma rays (Cz137) at some cherry varieties. Plant Science, 48: 47-52.
14. Равкин А.С.(1973). Исполъзование ионизирующей радиации и химических мутагенов в селекции плодовых и ягодных култур. Москва.
15. Шепотъев Ф.Л. (1968). Опыты по мутагенезу у дрвесных ратений. Применение эксперименталъных мутаций в селекции растений. Киев.
16. Zwintzscher M., (1955). Die Auslösung von Mutationen als Methode der Obstzüchtung I. Die Isolierung von Mutanten in Anlehnung an primäre Veränderungen. Züchter, 25, 290 – 302.
17. Zwintzscher M., (1967). On variability of isolated the sour cherry variety Schatenmorelle for a mutation treal. Induziere Mutationen und ihre Nutzung. Berlin. N°2.

PRELIMINARY RESULTS OF TESTING OF NEWLY INTRODUCED APPLE CULTIVARS IN BULGARIA

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Abstract
The results of the testing of three new Polish apple varieties grown under the conditions of Kyustendil, Bulgaria during the first six years after planting (2012-2017) are presented. Earliest flowering time was recorded in 'Gold Milenium' (15 April) and the latest in 'Melfree' (20 April). Average ripening date was between 30 August ('Melfree') and 25 September ('Free Redstar'). The trees of Gold Milenium' had stronger growth. No significant differences were found in the productivity of the cultivars. Fruit weight range from 168.2 ('Melfree') to 198.0 g ('Gold Milenium'). With the most harmonious flavor were the fruit of 'Gold Milenium'. No symptoms of apple scab and powdery mildew were found. Preliminary data showed that all three cultivars are suitable for growing in the Kyustendil region.

Keywords: 'Gold Milenium','Free Redstar', 'Melfree', flowering, yield, chemical composition.

Introduction

Each cultivar is created under specific environmental conditions and in this area it perform the best its potential The right chois of the most appropriate cultivars for the different places of growth is a major factor for the successful productoin of apples. Very often, however, cultivars introduced to habitats with conditions other than those in which they are selected do not develop their potential. Therefore introduction and testing of new cultivars that have shown valuable biological and economic characteristics is one of the main tasks of many research teams in the world (Blagov et al., 2009; Kiprijanovski et al., 2009; Sotirov and Dimitrova, 2014). Apple scab and powdery mildew are the main diseases on apple worldwide and currently are managed mainly by scheduled applications of fungicides. That's why the creation and selection of resistant cultivars is one of the most important objectives of the apple breeding programs that has been conducted for many years in different countries (Blažek, 2004; Sasnauskas et al., 2006; Bryk and Broniarek-Niemiec, 2008; Dinkova et al., 2009; Czynczyk et al., 2011; Żurawicz et al., 2013). The objective of this study was to evaluate three new Polish scab-resistant apple cultivars under agro-ecological conditions of Kyustendil region (Bulgaria). In the present paper are showed the firs results for the development of the cultivars to the end of the 6th year after planting.

Material and methods

In the period 2014 – 2017 at the Institute of Agriculture in Kyustendil three new apple cultivars - 'Gold Milenium' 'Free Redstar' and 'Melfree', introduced from Research Institute of Horticulture in Skierniewice, Poland were studied. The cultivars were grafted on MM 106 rootstock and the trees were planted in the spring of 2012 at distances of 4.50 x 2.50 m. Each cultivar was represented with five trees and each individual tree was treated as a repetition. Trees were trained as free-growing crowns. The soil in the experimental orchard is Chromic Luvisols, slightly sandy-loamy with a neutral reaction. The space between the rows was maintained in clean cultivation and within the rows by manual digging around the trees and use of herbicides. The trees were fertilised annually with 9 g/tree nitrogen in the form of ammonium nitrate. The experimental plantation was grown under standard agro-technology. The yearly recorded parameters included: dates of flowering and

harvesting, trunk diameter (30 cm above the grafting zone) and from the data was calculated trunk cross-sectional area (TCSA, in cm^2), length of one-year old shoots (cm), average and cumulative yields (kg/tree), yield efficiency (kg/cm^2 of TCSA), fruit weight (g), fruit sizes (mm), fruit flesh firmness (kg/cm^2) - evaluated by a penetrometer. Soluble solids of the fruit (%) were determined by a hand refractometer (Pocket PAL-1, Atago, Japan), the total sugars by Luff-Schoorl method (%), and the titratable acids (%) - titrimetrically with 0.1 N NaOH. The susceptibility to apple scab (*Venturia inaequalis* (Cke) Wint) and powdery mildew (*Podosphaera leucotricha* (Ellis et Everh.) Salm.) were determined too. The results were statistically evaluated by analysis of variance (ANOVA). The significance of differences between the cultivars was evaluated by Duncan's multiple range test at p= 0.05.

Results and discussion

For the first time blooming in the studied apple cultivars, grafted on the MM 106 rootstock, was observed in the 3rd year after planting. Flowering in different years depend on average daily temperatures and relative air humidity during the January-April period. 'Gold Milenium' began to bloom comparatively earlier than the other two cultivars. The phenophase beginning of flowering in this cultivar was 15 April, and 3 to 5 days later in 'Free Redstar'and 'Melfree', respectively (Table 1). With regard to flowering, 'Free Redstar'and 'Melfree' had some advantages. Their trees began of full flowering 3-4 days after those of 'Gold Milenium', which is valuable quality - this can reduce damages from possible late spring frosts.

Table 1. Average dates of flowering and ripening of fruit of three scab-resistant apple cultivars (2014-2017)

Phenological phases	'Gold Milenium'	'Free Redstar'	'Melfree'
Beginning of flowering	15.04	18.04	20.04
Beginning of full flowering	18.04	21.04	22.04
End of full flowering	25.04	28.04	29.04
End of flowering	28.04	30.04	01.05
Duration of flowering (days)	14	13	11
Fruit ripening: from - to	26.08 - 03.09	22.09 - 28.09	21.09 - 25.09
Average ripening date	30.08	25.09	23.09

The end of flowering was earliest in 'Gold Milenium' (28 April), while latest in 'Melfree' (01 May). According to these data the flowering period continued for 11-14 days. The averaged data for the studied period showed that the calendar flowering times of these cultivars coincide with those of most apple cultivars cultivated in the Kyustendil region found in other studies (Blagov, 2011; Dimitrova and Sotirov, 2014, 2016). The earliest maturation period had the fruits of 'Gold Milenium', which ripened between 26 August and 3 September, under the conditions of the region. The average ripening date of the other two cultivars was 23 and 25 September for 'Melfree' and 'Free Redstar', respectively (Table 1). The tree sizes of the studied cultivars are expressed as a trunk cross-sectional area (TCSA) and average annual shoot length. The results obtained showed that in the period 3^{th}-5^{th} year after planting the trees of 'Gold Milenium' had significantly stronger growth compared with the other two cultivars (Figure 1). Between 'Free Redstar'and 'Melfree' the differences were minimal and insignificant. Our results with respect to the growth of these three cultivars are in agreement with the results reported by Czynczyk et al., 2011). The cultivars started to bear fruit in the third year after planting, but the obtained fruit quantity was negligible (Table 2). Over the next two years, a higher yield per tree and cumulative yield for the period were registered from 'Gold Milenium', followed by 'Melfree' and 'Free Redstar', but the differences were insignificant. In 2017 no fruit were obtained because late spring frosts on 22 and 25 April injured 100% the blossoms.

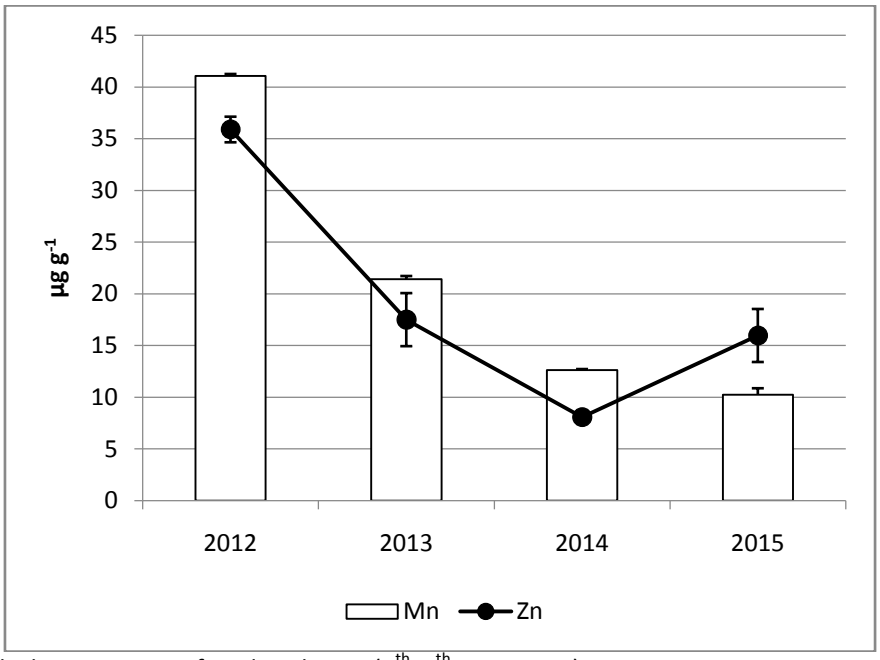

Figure1. Growth characteristics of apple cultivars (3th -5th vegetation).

Table 2. Productivity of apple cultivars for the period 3th -5th vegetation

Cultivar	Yield (kg/tree)				Yield efficiency (kg/cm^2 of TCSA)
	2014	2015	2016	Cumulative	
'Gold Milenium'	1,2 a	5,6 a	7,5 a	14,3 a	0,37 a
'Free Redstar'	0,6 a	3,8 a	6,2 a	10,6 a	0,33 a
'Melfree'	0,9 a	4,5 a	6,7 a	12,1 a	0,44 a

The productivity of the trees, expressed by the cumulative yield efficiency (kg/cm^2 of TCSA) was the highest for 'Melfree', followed by 'Gold Milenium'and 'Free Redstar', without significat differences among them. It was found a significant positive correlation between the trunk-cross sectional area and the yield (Figure. 2).

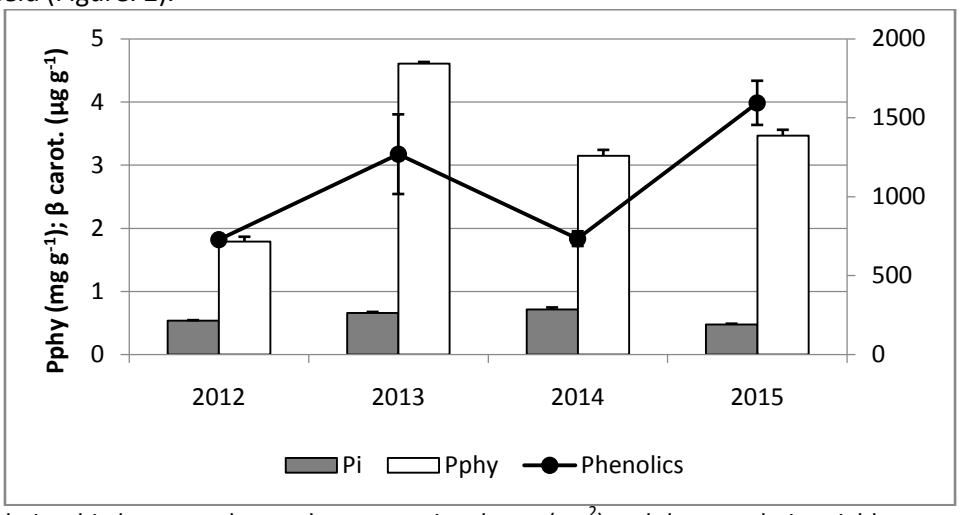

Figure 2. Relationship between the trunk-cross sectional area (cm^2) and the cumulative yield per tree

'Gold Milenium' had the largest fruit (average fruit weight 198.0 g), followed by 'Free Redstar' (185.5 g) and 'Melfree' (168.2 g) (Table 3). The fruit of all three cultivars had a diameter more than 70 mm, and according to this indicator, they refer to class 'Extra' according to the National ordinance №.108/2006 for the quality of the fresh fruit. Fruit flesh firmness was the highest in 'Free Redstar' (10.5 kg/cm^2) and the lowest in 'Melfree' (7.2 kg/cm^2).

Table 3. Physical and chemical characteristics of apple cultivars (2014-2016)

Cultivar	'Gold Milenium'	'Free Redstar'	'Melfree'
Fruit weight (g)	198.0 a	185.5ab	168.2 b
Flesh firmness (kg/cm^2)	9.7 a	10.5 a	7.2 b
Fruit diameter (mm)	76.8 a	73.9 a	70.9 a
Fruit height (mm)	63.9 a	67.3 a	62.4 a
Soluble solids (%)	16.5 ab	13.0 b	19.8 a
Total sugar (%)	8.7 a	7.5 b	9.0 a
Titratable acids (%)	0.34 b	0.49 a	0.63 a
Sugar/Acids index	25.6	15.3	14.3

Fruit analysis showed some significant differences in the chemical composition of the fruit. The content of soluble solids and total sugars were the highest 'Melfree', and the lowest in 'Free Redstar' (Table 3). The fruit of 'Gold Milenium' had the lowest titratable acid content and their taste was the sweetest. Fruit of this cultivar had the highest sugar-acid index (25.6) and this gives them the most harmonious flavor. For establishing the reaction of the trees to scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) annually were carried out observations and readings during the vegetation. In 2014-2017 the conditions for developing of deseases were extremely favorable. In the period April to September, there were from 26 to 32 days were favorable for infections from *V. inaequalis*. In 2017, the lack of apple fruits due to injured from spring frosts gave us the opportunity to restrict fungicide treatments in the apple plantation. This year we only had one fungicide treatment. Our goal was to better assess the response of the apple trees from the available gene pool, including and the studied cultivars. In the visual observations until the end of the sixth vegetation, there were no symptoms of apple scab and powdery mildew diseases, regardless of the number of plant protection treatments. Our results confirmed the results of other authors for the resistance of these cultivars to apple scab (Bryk and Broniarek-Niemiec, 2008; Czynczyk et al., 2011; Żurawicz et al., 2013).

Conclusions

The first results of the testing of the cultivars 'Gold Milenium', 'Free Redstar' and 'Melfree' showed that they are suitable for the soil-climatic conditions of the Kyustendil region. The phenological phases of flowering were within the established time for the most spreaded apple cultivars in the region. The cultivars started to bear fruit in the 3rd year after planting. The trees of 'Gold Milenium' produced the highest average yield and the largest fruit with the most harmonious flavor. 'Melfree' was the most yield efficient cultivar. The tolerance of the cultivars to the main economic diseases - apple scab and powdery mildew was confirmed and they can enrich the list of cultivated apple cultivars in our country. They can be successfully included in the organic apple production schemes.

Acknowledgements

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References

1. Blagov, A., N. Christov, D. Sotirov, An. Stoyanova (2009). Comparison of some new apple and sweet cherry cultivars under the environmental conditions of Kyustendil, Bulgaria. Acta Hortic., 825: 89-96.

2. Blagov, A. (2011). Some results of agrobiological investigation of apple cultivars and Hybrids in Kyustendil region. Plant Science, (48): 14-19.

3. Blažek,J., (2004). Response to diseases in new apple cultivars from the Czech Republic. J. Fruit Ornam. Plant Res. Special ed. Vol. (12): 241-250.

4. Bryk, H., A., Broniarek-Niemiec. (2008). Three years of experience with the apple disease control in an organic orchard. Zemdirbyste -Agriculture, Vol. 95 (3): 395–400.
5. Czynczyk, A., P., Bielicki, A., Mika, A., Krawiec. (2011). A nine-year evaluation of several scab-resistant apple cultivars for organic fruit production. J. Fruit Ornam. Plant Res. Vol. 19 (2): 87-97.
6. Dimitrova S., D. Sotirov. (2016). Biological and economic qualities of some apple hybrids. Acta Hortic. 1139: 37-42.
7. Dinkova., H., K., Dragoyski., B. Stefanova. (2009). Possibilities for organic production of apple fruits in the region of central Balkan mauntains. Plant Science, (46): 6-9.
8. Kiprijanovski, M., Arsov, T., Gjamovski, V., Damovski, K., (2009). Study of certain introduced apple cultivars in the Prespa region. Acta Hort., 825: 125-132.
9. Sasnauskas, A. Gelvonauskiene, D., Gelvonauskis, B., Viskelis, P., Duchovskis, P., Bobins, C., Siksnianieneand, J., Sabajeviene, G. (2006). Productivity and fruit quality of scab resistant apple cultivars and hybrids. J. Fruit Ornam. Plant Res. 14, (Suppl. 2): 247-255
10. Sotirov.D., S. Dimitrova. (2014). Comparative study of new apple cultivars inder the conditions of Kyustendil region. Journal of Mountain Agriculture on the Balkans,Vol. 17 (5): 1318-1329.
11. Żurawicz, E., M., Lewandowski, K. P. Rutkowski, K., Pruski. (2013). Productivity of selected polish scab resistant apple cultivars grown on different rootstocks. Acta Hortic. 976: 141-146.

THE INFLUENCE OF SOME PHYTOHORMONES ON THE ROOTING OF FIG CUTTINGS

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Abstract

The fig is a subtropical fruit species. In the Republic of Macedonia it is grown mainly, in the form of individual plants. Lately there is interest for establishing orchards from this fruit species. The purpose of this research was to determine the influence of some phytohormones on the rooting of hardwood cuttings from fig. The research was conducted with cuttings from 3 domestic populations of figs, "Bela smokva,, "Crna smokva,, and "Petrovka,,. The research was performed on an experimental field, property of the "Faculty of Agricultural Sciences and Food,, in Skopje. In the research, two phytohormones were used, indolebuteryc acid 0,1% and 1-Naphthaleneacetic acid 0,1%. In the end of the vegetation period the following parameters were evaluated: percentage of rooting, diameter and length of the shoots, the total mass of the roots, the length and nubmer of roots with a diameter above 3mm. On the basis of our research we determined that all of the cuttings had a satisfactory, high, percentage of rooting (86,2%). In the population of "Petrovka,, the treatments did not show any statistical significant differences on the evaluated characteristics, regarding the control treatment. With the cuttings from "Bela smokva,, treated with NAA, statistical significant difference was found in the parameter mass of the roots (12,2g), while regarding the nubmer and length of roots, this treatment, had statistical significant difference just with the control but not with the IBA treatment. With "Crna smokva,, the cuttings treated with IBA had the highest values with statistical significant difference with the number (4,5), the mass (24,3 g) and the length of the roots (179,3 cm).

Keywords: *Ficus carica*, IBA, NAA, propagation.

Introduction

The fig tree (*Ficus carica* L.) has gained more interest in recent years for its economic importance and medicinal virtues. It is considered to be a rustic plant, a fruit species of great economic importance and in expansion worldwide, with good adaptation to different climates and soil types (Rodrigues *et al*. 2012). In the Republic of Maceodnia the fig is grown on a limited scale, in the form of individual fruit plants and rarely in a commercial orchard. Lately there is interest for establishing orchards from this fruit species. Improvement of farming techniques (Fraquas *et al.*2004), the sanitation of local varieties (Mars *et al*., 2008), and the large scale production of good quality and healthy fig plants seem to be basic requirements for successful commercial orchards. This requires the ability of rapid propagation of local figs. Almost all grown cultivars are the result of old selection and maintained by vegetative propagation (Mars, 2003). The fig has a great ability for vegetative propagation, which is by micropropagation, layering, aerial layering, rooting of adventitious shoots, with hardwood and softwood cuttings. The fig tree is usually propagated by hardwood cuttings that are collected from the basal and median portions of the branches at the time of winter pruning, undertaken during the period of dormancy (Karedeniz, 2003; Sousa *et al*., 2013). Some cultivars have low rooting potential. Low rooting of hardwood cuttings may be related to factors inherent to the plant material, such as age of the tissue, time of collection of cuttings, and phytohormone concentration, or exogenous factors, such as the growing conditions of the cutting (Han *et al*., 2009). The use of auxins has shown excellent results in increasing the root formation in fig tree cuttings (; Lajus *et al*., 2007; Pio *et al*., 2008; Ramos *et al*., 2008). Theoretically, nowadays there are studies regarding the regulatory role of phytohormones in the process of root formation. In practice, many

efforts are made in order to find optimal conditions for rooting the cuttings of different species, especially those, where the rooting formation is hard (Stefančič *et al*., 2007) However, variation in the rooting potential of cuttings is a characteristic inherent to the genetic potential. This study aimed to determine the rooting potential of tree promising domestic populations of figs "Bela smokva,, "Crna smokva,, and "Petrovka,, with the use of two auxin phytohormones NAA and IBA.

Material and methods

The cuttings from "Bela smokva,, and "Crna smokva,, were obtained from the region of Valandovo and those from "Petrovka,, from the Skopje region. The cuttings were collected in January. They were kept in a plastic bag in a refrigerator on a temperature of 2^{o}C until the end of February. The cuttings were prepared from the middle and basal portion of the branch. The cuttings were about 30 cm long, the bottom part of the cutting is cut flat under the node and the top slightly obliquely in the opposite direction of the bud. Then, the cuttings were treated with individual phytohormones in the following variants:

1. NAA 0,1% dipped 1 min
2. IBA 0,1% dipped 1 min
3. Control (not treated)

After the treatment the cuttings were put in a black plastic bag and were kept in a dark room for a period of 7 days. After this period the cuttings were planted in a nursery at a distance of 10 cm inside the row and 100 cm between the rows, on a light soil. For the research, 30 cuttings were used for each variant, divided by 3 repetitions for each variant. During the vegetative period, the nursery was grown using standard technology. At the end of the vegetative period the cuttings were dug out after the leaves have dropped and the following parameters were evaluated: percentage of rooting, diameter and length of the shoots, the total weight of the roots above 3 mm, the length and nubmer of roots with a diameter above 3 mm. The percentage of received cuttings was determined according the number of received cuttings and the total number of cuttings set. The diameter of shoots was measured with a caliper. The length of the shoots and roots was measured with a measuring tape. The mass of the roots was determined with a scale (KERN EMB 600-2, d=0,01 g). The differences were evaluated by ANOVA analysis through general linear model procedure. After the GLM analyses, post hoc comparison of the means were calculated by LSD. Results were expressed at the $p < 0,05$ level of significance. Statistical analyses were performed using SPSS (IBM SPSS Statistics 19).

Results and discussion

In figure 1 we can observe that the highest percentage of rooted cuttings was obtained in the second variant (88,5%) while the lowest percentage of rooting was observed in the first variant (83,9). In figure 2 we can see that all of the populations of figs investigated have a high percentage of rooting. The highest percentage of rooting can be seen in the second variant in Bela smokva with a 92,3 % of rooting and in the control variant in Crna smokva with the same percentage. The lowest percentage of rooting can be observe in the first and third variant at the population Petrovka with a percentage of rooting of 81,3. In table 1 we can observe that the highest values, regarding the studied morphometric characteristics, with statistical significant difference with the control variant, were obtained in the second variant, length of the shoots was 48,5 cm, length of the roots 103,3 cm and number of roots above 3 cm 3,0. With the exception of the mass of the roots which was highest in the first variant (16,0 g).

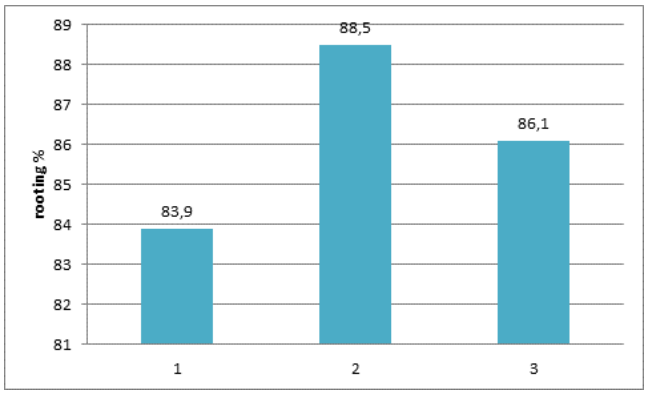

Figure 1. Percentage of rooting by variants

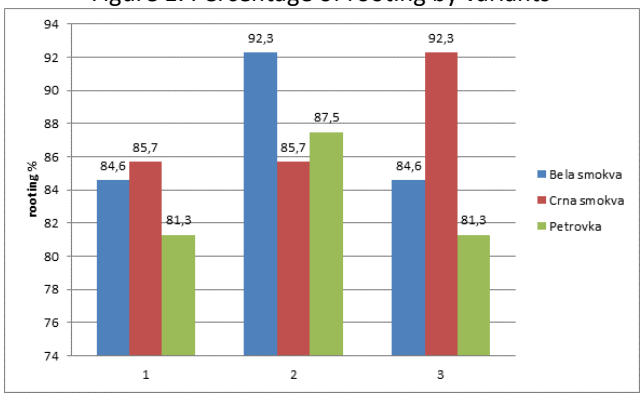

Figure 2. Percentage of rooting by fig population and variant

Table 1. Morphometrical characteristics of the rooted cuttings by variants

Variant	Diameter of the shoots (cm)	Length of the shoots (cm)	Mass of the roots (g)	Length of the roots (cm)	Number of roots (above 3cm)
1	7,0	44,8 b	16,0 a	74,9 ab	2,0 ab
2	7,1	48,5 b	15,0 a	103,3 b	3,0 b
3	6,6	38,3 a	13,3 a	33,3 a	1,3 a
$LSD_{0,05}$	5,4	4,4	31,1	1.2	

* Values followed by the same letter in the column were not statistically different.

Table 2. Morphometric characteristics of the rooted cuttings from Bela smokva

Population	Variant	Diameter of the shoots (cm)	Length of the shoots (cm)	Mass of the roots (g)	Length of the roots (cm)	Number of roots (above 3cm)
Bela smokva	1	7,5	54,1 b	16,2 b	69,7 a	1,7 b
	2	6,7	52,0 b	8,6 a	58,3 ab	2,2 b
	3	5,5	26,3 a	6,1 a	20,3 b	0,4 a
$LSD_{0,05}$		30,7	7,2	45,0	1,1	

* Values followed by the same letter in the column were not statistically different.

In table 2 we can see that in the population of Bela smokva the highest values for length of the shoots (54,1 cm), mass of the roots (16,2 cm) length of the roots (69,7 cm) with the exception of number of roots (1,7) were obtained with the use of NAA 0,1%. All of the examined morphometric characteristics were statistically significantly different from the control variant.

In the population of Bela smokva best results were obtained with the use of the second variant. The length of the shoots (67,1 cm), mass of the roots (24,3 g), length of the roots (179,3) and number of roots (4,3) (Table 3). All of the mentioned characteristics were statistically significantly different in

comparison with the control variant. In table 4 we see that in the population of Petrovka there is no statistical significant difference between the studied characteristics regarding the variants.

Table 3. Morphometric characteristics of the rooted cuttings from Crna smokva

Population	Variant	Diameter of the shoots (cm)	Length of the shoots (cm)	Mass of the roots (g)	Length of the roots (cm)	Number of roots
Crna smokva	1	6,2	48,8 b	13,4 bc	90,3 b	2,5 b
	2	8,0	67,1 a	24,3 a	179,3 a	4,3 a
	3	6,2	43,7 b	11,4 b	29,3 b	1,2 b
$LSD_{0,05}$		14,2	8,3	71,3	1,5	

* Values followed by the same letter in the column were not statistically different

Table 4. Morphometric characteristics of the rooted cuttings from Petrovka

Population	Variant	Diameter of the shoots (cm)	Length of the shoots (cm)	Mass of the roots (g)	Length of the roots (cm)	Number of roots
Petrovka	1	7,2	31,5 a	18,4 a	64,7 a	1,7 a
	2	6,6	26,5 a	12,0 a	72,3 a	2,4 a
	3	8,1	44,9 a	22,4 a	50,3 a	2.3 a
$LSD_{0,05}$		27,1	16,7	55,1	3,1	

* Values followed by the same letter in the column were not statistically different

Conclusions

On the basis of our research we can make several conclusions:

Regarding the treatments, the highest averige percentage of rooting was observed in the second variant (88,5) and the lowest percentage was achieved with the first variant (83,9). The highest values regarding the studied morphometric characteristics, with statistical significant difference with the not treated cuttings, were obtained with the use of IBA 0,1%, length of the shoots was 48,5 cm, length of the roots 103,3 cm and number of roots above 3 cm was 3,0. With the exception of the mass of the roots which was highest in the plants treated with NAA 0,1% (16,0 g). All of the studied variants of the populations of “Bela smokva,, “Crna smokva,, and “Petrovka,, had a high percentage (above 81,3) of rooting. Even though the studied populations when not treated have a natural high percentage of rooting, the use of the phytohormones showed statistical significant difference in: length of the shoots, mass of the roots, length of roots and number of roots in two of the populations. For the rooting of hardwood cuttings from the population of “Bela smokva,, the use NAA 0,1% can be recommended. In the population of “Crna smokva,, best results were obtained with the use of IBA 0,1% regarding the studied characteristics. In the populations of “Petrovka,, however, the phytohormones did not have an statistical significant difference regarding the studied parameters.

References

1. Fraquas, C. B., Paqual, M. and Pereira, A. R. (2004): Multiplicaçao In vitro de Ficus carica L.: Efeito da Cinetina e do Acido Giberélico. Ciênc. Agrotec., 28: 49-55.

2. Han, H., Zhang, S., Sun, X. (2009): A review on the molecular mechanism of plants rooting modulated by auxin. African Journal of Biotechnology, 8(3), 348-353

3. Karedeniz, T (2003): A study on some fruit characteristics and propagation of these by hardwood cuttings of local fig cultivars grown in ordu (Turkey). Acta Horticulturae, 605(1), 107-112.

4. Lajús, C. R. et al. Ácido Indolbutírico no Enraizamento de Estacas Lenhosas de Figueira (Ficus carica L.).Revista Brasileira de Biociências, v. 5, v. 2, p. 1107-1109, 2007. Suplemento.

5. Mars, M., Chatti, K., Saddoud, O., Salhi- Hannachi, A., Trifi, M. and Marrakchi, M. (2008): Fig Cultivation and Genetic Resources in Tunisia: An Overview. Acta Hort., 798: 27-32.

6. Mars, M. (2003): Fig (Ficus carica L.) genetic resources and breeding. Acta Horticulturae, 605(1), 19-27.
7. Ramos, D. P.; Leonel, S.; Damatto Júnior, E. R.(2008): Avaliação da época de estaquia e uso de bioregulador no enraizamento de estacas de figueira. Revista Brasileira de Fruticultura, v. 30, n. 3, p. 748-753.
8. Rodriques, D.N.B., Viana, T. V. A., Marinhho, A. B., Ferreira, T.T.S., Azevedo, B. M., Gomes Filho, R. R. (2012): Fertirrigação potássica na cultura da figueira no semiárido Cearence. Revista Brasileira de Agricultura Irrigada, 6(3), 176-183.
9. Pio, R. et al (2008): Enraizamento de estacas radiculares de figueira 'roxo de valinhos' tratadas com IBA e dois métodos de imersão. Scientia Agraria, v. 9, n. 1, p. 111-115.
10. Sousa, C. M., Bisquet, R. N., Vasconcellos, M. A. S., Miranda, R. M. (2013): Effects of auxin and misting on the rooting of herbaceous and hardwood cuttings from the fig tree. Revista Ciência Agronômica, 44(2), 334-338.
11. Štefančič M., Vodnik D., Štampar F., Osterc G. (2007): The effect of a fogging system on the physiological status and rooting capacity of leafy cuttings of woody species. Trees, 21: 491-496.

PRODUCTIVITY PER LEAF AREA IN SOME INTRODUCED STRAWBERRY VARIETIES IN THE SKOPJE REGION

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Abstract
The study presents the results of the investigation on productivity per leaf area of fifteen strawberry varieties: Idea, Camarosa, Belrubi, Evita, Honeoye, Tethis, Chandler, Onda, Miranda, Paros, Elsanta, Eris, Madeleine, Favette and Marmolada, as well as two standard varieties: Pocahontas and Senga Sengana. The experimental orchard was performed on open field in the Institute of Agriculture – Skopje, on black polyethylene foil in form of double file set on banks and at the distance of 40x30cm between the plants, irrigated by control drip irrigation system. The study has been conducted on fruit number, fruitage and yield of soluble solid matter per unit of leaf area. Also, the effective leaf area for production of 100 number of fruits, for 1 kg of fruitage and for 100 g yield of soluble solid metter in fruis of each variety were examined. The investigated parameters related to productivity per unit of leaf area are ranged from 54,0 at Miranda to 125,1 at Pocahontas for number of fruits, from 0,49 kg/m^2 in Favette and Elsanta , to 0,96 kg/m^2 in Honeoye for fruitage and from 41,2 g/m^2 in Elsanta, to 92,9 g/m^2 in Pocahontas for the yield of soluble solid metter in fruits. Leaf area of 1,37 m^2 is effective for 100 number of fruits production, as well as 1,47 m^2 for 1kg of fruitage and 1,75 m^2 for production of 100 g soluble dry matter fruits-yield. The productivity per leaf area of the investigated varieties is less dependent on the influence of leaf area per plant and fruit mass , but it is more dependent of the influence of number of fruits per plant, fuitage per plant and yeld of soluble solid matter per plant. The values of correlation coefficient between productive parameters per plant and effective leaf area parameters are with similar strength, but they are with an opposite dependency in relation to the corresponding correlations with productive parameters per leaf area.

Keywords: Number of fruits, fruitage, soluble solid matter, effective leaf area, productivity parameters.

Introduction

The fruit production is highly dependent on leaf area and its physiological activity (Ристевски и сор.1994). The number of fruits in a unit of leaf area is an important indicator for determining the leaf productivity (Bulatović 1970), (Blagojević, 1983), (Поповски, 2008). The yield is in direct correlation with plant leaf area, brightness and health of the leaves (Bulatović 1970), (Blagojević, 1983), (Walsh, 1992), (Кипријановски, 2001) (Поповски, 2008). The leaves that are in the peripheral parts of the plant which are exposed in direct sunlight have a 3 to 5 times more intense assimilation compared to the other leaves which are present in the interior of the plant (Sarić i sar., 1989). The photosynthesis in interior leaves even drops under compensation point. Having in mind the relevance of the leaf area for gaining big and quality yields in fruit production, it is of high importance to pay attention to the plant density and the system for shaping and forming the canopy in order to achieve optimal and highly illuminated leaf area (Ристевски и сор., 1994). Also, the specifically applied agro-technical measurements on the various varieties have the high influence on the leaves activity (Blagojević, 1983). Productivity per leaf area oscillates among strawberry varieties cultivated on different climatic and soil conditions (Dénes, 1997). Considering the mentioned facts, the aim of this paper is to examine the productivity on the unit of leaf area related to number of

fruits, fruitage and yield of soluble solid matter with 17 strawberry varieties cultivated in Skopje region.

Material and methods

The experimental orchard was performed on open field in the Institute of Agriculture –Skopje on black polyethylene foil in form of double file set on banks and at the distance of 40x30cm between the plants, irrigated by control drip irrigation system. It was established in the second half of September 2011, with a frigo virus-free planting matterial. The analysis was performed during 2012–2014. The soil was homogeneous, alluvial, possessing a good water-air regime, suitable for strawberry growing. The agrochemical composition of the soil consisted of 0,93-2,05% hummus, 9,32-10,38mg/100g N, 14,3-21,1mg/100g P_2O_5, 10,06-22,2 mg/100g K_2O, 6,49-7,.25% $CaCO_3$, pH 7,93-8,19 in H_2O and 7,4-7,63 in KCl. The soil has been ameliorative fertilised with mineral fertiliser and organic fertiliser from California worms. According to data for meteorological parametres from the Hydrometeorological Station Petrovec, the climate of the Skopje Region featured warm dry summers and foggy cold winters. Productivity per leaf area was observed on 15 strawberry varieties: Idea, Camarosa, Belrubi, Evita, Honeoye, Tethis, Onda, Chandler, Miranda, Paros, Elsanta, Eris, Madeleine, Favette and Marmolada, as well as two standard varieties: Pocahontas and Senga Sengana. Varieties production is presented per unit of leaf area by analyzing the following parameters: number of fruits per leaf area (NF/LA), fruitage per leaf area (F/LA) (kg/m^2) and yield of soluble solid matter per leaf area (YSSM/LA) (g/m^2). Also, the production of varieties is presented through the effective leaf area for production of 100 numbers of fruits (ELA100NF) (m^2), effective leaf area for 1kg fruitage (ELA1kgF) (m^2) and effective leaf area for 100 g of soluble solid matter (ELA100gYSSM) (m^2). The values of the parameters are obtained by calculating the data for leaf area per plant (LA/P) (cm^2) and productive parameters: fruit mass (FM) (g); number of fruits per plant (NF/P), fruitage per plant (F/P) (g) and yield of soluble solid matter per plant (YSSM/P) (g). Analyses of variance were performed for statistical analisis of the results. The results were processed using LSD-test to prove the statistical significance of the differences between the varieties, with a significance levels of 0,05 and 0,01. The interconnectedness of certain properties is expressed with correlation analysis by determining the correlation coefficient (R). The strength of the correlation between the properties is determined according to the classification of Chebyshev (cit. by Најчевска, 2002).

Results and discussion

The leaf area and productive parameters per plant of the investigated strawberry varieties are presented in Table 1. LA/P is ranged from 3051 cm^2 at Chandler to 5497 cm^2 at Miranda, while the average is 4530 cm^2. The FM has an average value of 10,3 g, ranges from 6,4 g at the control Senga Sengana to 12,8 at Onda. The Pocahontas control is characterized by the highest NF/P, F/P and YSSM/P. The lowest fruitage has Elsanta. Besides having the smallest leaf area, Chandler has the lowest values for NF/P (19,9) and for YSSM/P (13,0 g). NF/P for all varieties is in average of 35,10, while the F/P is 314,15g and YSSM/P is 27,0 g respectively. Our results are several times higher than the results obtained by Bulatović (1970) in the region of Belgrade, where the values for investigated parameter is ranged between 641 do 1402 cm^2 per plant. The average NF/LA for all varieties in the examined period is 78,8 (Table 2). The standard variety Pocahontas produces the highest amount of fruits (125,1 fruits/m^2), followed by Honeoye and second standard Senga Sengana. The lowest amount of fruits is noticed at Miranda (54,0 fruits/m^2). It is noticed that the varieties with larger fruits (Table 1) are characterized by smaller NF/LA (Table 2). From an annual point of view, the highest average NF/LA is present in 2014 (135,3) i.e. in the year with the highest fruitage per plant. On the other hand, the smallest average of NF/LA is present in 2012 (9,1), when the total fruitage was at its lowest. The medium strong negative correlation between the NF/LA and the FM was determined (R= -0,569) (Table 5). The medium positive correlation between the NF/LA and F/P and with YSSM/P was determined (R= 0,524 and R= 0,516).

Table 1. Leaf area and productive parameters per plant

No.	Variety	Leaf area per plant (cm^2)	Fruit mass (g)	Number of fuits per	Fruitage per plant (g)	Yeld of soluble solid matter per plant (g)
1	Idea	3589	9,2	35,41	303,4	30,0
2	Camarosa	5353	12,3	32,72	341,6	28,0
3	Belrubi	4988	11,4	29,11	341,7	30,2
4	Evita	4936	9,5	49,14	381,2	34,6
5	Honeoye	3792	9,1	42,89	362,5	33,6
6	Tethis	3219	11,6	29,48	303,4	20,5
7	Chandler	3051	9,2	19,90	245,9	13,0
8	Onda	5434	12,8	34,82	372,5	26,2
9	Pocahontas	4285	8,8	53,62	396,9	39,8
10	Senga Sengana	4973	6,4	52,48	297,5	27,9
11	Miranda	5497	12,3	29,68	305,4	30,4
12	Paros	3752	11,4	28,49	280,0	24,3
13	Elsanta	4719	11,4	29,73	231,9	19,4
14	Eris	5154	9,6	46,04	355,6	23,7
15	Madalene	4663	7,4	27,53	308,6	28,8
16	Favette	4720	10,7	26,34	233,1	21,8
17	Marmolada	4892	12,2	29,28	281,8	26,4
	AVERAGE	***4530***	***10,3***	***35,10***	***314,3***	***27,0***
		LSD 0.05=1083		LSD 0.05=22,88	LSD 0.05=218,20	LSD 0.05=16,10
		LSD 0.01=1456		LSD 0.01=30,76	LSD 0.01=293,40	LSD 0.01=21,70

Table 2. Number of fruits and effective leaf area for 100 fruits production (m^2)

No.	Variety	Number of fruits per m^2 leaf area				Effective leaf area for 100 fruits (m2)
		2012	2013	2014	Average	
1	Idea	30,9	100,0	165,2	98,7	1,01
2	Camarosa	0,6	71,3	111,5	61,1	1,64
3	Belrubi	7,0	36,3	131,8	58,4	1,71
4	Evita	4,5	106,6	187,6	99,6	1,00
5	Honeoye	4,5	110,9	224,0	113,1	0,88
6	Tethis	19,8	89,5	165,5	91,6	1,09
7	Chandler	24,6	30,9	140,2	65,2	1,53
8	Onda	7,7	56,4	128,2	64,1	1,56
9	Pocahontas	7,1	180,2	188,1	125,1	0,80
10	Senga Sengana	5,1	169,2	142,3	105,5	0,95
11	Miranda	2,9	52,3	106,8	54,0	1,85
12	Paros	3,6	87,8	136,4	75,9	1,32
13	Elsanta	1,3	100,1	87,7	63,0	1,59
14	Eris	3,3	128,2	136,5	89,3	1,12
15	Madalene	0,0	85,9	91,2	59,0	1,69
16	Favette	12,7	85,2	69,5	55,8	1,79
17	Marmolada	9,6	82,9	87,0	59,8	1,67
	AVERAGE	***9,1***	***92,6***	***135,3***	***78,8***	***1,37***
			LSD 0.05=19,99		LSD 0.05=47,58	LSD 0,05= 0,82
			LSD 0.01=2,74		LSD 0.01=63,97	LSD 0,01= 1,11

Since NF/LA has a weak negative correlative dependence with LA/P (R= -0.355), the increase in the value of NF/LA depends largely on the increase in NF/P, so the strong positive correlation between them was determined (R= 0,820) (Table 5). According to data in Table 2, Idea had the highest NF/LA (30,9) and Camarosa displayed the lowest amount (0,6) in 2012. It is noticed that Madeleine did not produce any fruit in 2012, because the trial was planted around 30 days later than the usual planting for the Skopje region, which caused weaker differentiation of flower buds. Therefore, the data on productive parameters in 2012 are lower compared to the other two years. The controls Senga Sengana and Pocahontas produced the highest NF/LA in 2013, while Belrubi as the variety characterized with large fruits had the lowest amount of fruits (36,3). Variety with the highest NF/LA

area is Honeoye (224,0) followed by control Pocahontas and Evita, in 2014. The lowest number was noticed at Favette. Кипријановски (2001), analyzed this property in Pocahontas and Red Gauntlet varieties in the region of Skopje. He identified an average of 136,2 NF/LA for Pocahontas. This value varies between 83,4 and 207,2 NF/LA in the duration of the study, which are a bit higher compared to the results present in our paper. For the Red Gauntlet variety, he identified an average of 126,9 NF/LA that varies from 75,6 up to 183,9 fruits. Statistical high significant differences were determined for the average values of NF/LA between the Pocahontas and Marmolada, Camarosa, Madeleine, Favette, Miranda and Belrubi. Significant differences were determined between Pocahontas and Paros, Chandler, Onda and Elsanta. Another set of significant differences were found between Honey and Chandler, Onda, Elsanta, Camarosa, Marmolada, Madeleine, Belrubi, Favett and Miranda, as well as between Senga Sengana and Favette and Miranda varieties. According to annual data, high significant differences between the average values of this parameter for all investigated years were found. The effective leaf area for production of the unit yield depends on the genotype and it presents the photosynthetic potential of the variety, i.e. the leaf efficiency for synthesis of organic matter. The ELA100NF of the investigated strawberry varieties, amounts to an average of 1,37 m^2 (Table 2). The varieties with lower values for this parameter are more productive. Therefore the Pocahontas variety with the lowest value for this parameter (0,8 m^2) produces the highest NF/LA (125,1),while Miranda which has the highest value for ELA100NF (1,85 m^2) produces the lowest NF/LA (54,0). The values for these two properties are inversely proportional. Together with the abundance of the variety, they have practical significance in determining the planting distance, the breeding system and the yield estimation for strawberry production. The LA/P has small dependence on the ELA100NF (R= 0,450). The data for F/LA are presented in Table 3. The average values for all examined varieties during the study period is 0,71 kg/m^2. The highest yield is identified for Honeoye the Tethis (0,96 and 0,94 kg/m^2), followed by the standard variety Pocahontas (0,93 kg/m^2), statistically different from more varieties. The lowest fruitage is mentioned at Elsanta and Favette (0,49 kg/m^2), statistically different from Paros, Evita, Chandler and Idea. The standard Senga Sengana is characterized with an average fruitage of 0,60 kg/m^2 leaf area, which is significantly lower than Honeoye, Tethis, Pocahontas and Idea. From an annual point of view, the highest average for F/LA is noticed in 2014, while and the smallest in 2012.

Table 3. Fruitage (kg/m^2) and effective leaf area for 1 kg of fruit production (m^2)

No.	Variety	Fruitage per leaf area (kg/m^2)				Effective leaf area for 1kg of fruits (m^2)
		2012	2013	2014	Average	
1	Idea	0,37	0,64	1,52	0,85	1,18
2	Camarosa	0,01	0,58	1,33	0,64	1,57
3	Belrubi	0,11	0,24	1,70	0,68	1,46
4	Evita	0,06	0,64	1,61	0,77	1,30
5	Honeoye	0,05	0,66	2,16	0,96	1,05
6	Tethis	0,33	0,54	1,97	0,94	1,06
7	Chandler	0,54	0,17	1,71	0,81	1,24
8	Onda	0,15	0,41	1,50	0,69	1,46
9	Pocahontas	0,09	0,90	1,79	0,93	1,08
10	Senga Sengana	0,04	0,83	0,93	0,60	1,67
11	Miranda	0,05	0,52	1,10	0,56	1,80
12	Paros	0,05	0,71	1,47	0,75	1,34
13	Elsanta	0,02	0,66	0,79	0,49	2,04
14	Eris	0,05	0,89	1,13	0,69	1,45
15	Madalene	0,00	0,89	1,10	0,66	1,51
16	Favette	0,19	0,62	0,67	0,49	2,03
17	Marmolada	0,17	0,62	0,94	0,58	1,74
	AVERAGE	***0,14***	***0,62***	***1,38***	***0,71***	***1,47***
			LSD 0.05=0,15		LSD 0.05=0,24	LSD 0.05= 0,49
			LSD 0.01=0,21		LSD 0.01=0,35	LSD 0.01= 0,73

The F/LA ranges between 0,01kg/m^2 at Camarosa to 0,54 kg/m^2 at Chandler, in 2012. Standard Pocahontas had the highest fruitage in 2013, followed by Eris, Madeleine and second standard Senga Sengana. The other 13 varieties had the lower fruitage then Senga Sengana (from 0,71 at Paros to 0,17 at Chandler). In 2014, the highest values was present at Honeoye (2,16 kg/m^2), followed by Tethis and standard Pocahontas. Senga Sengana is characterized by almost equal values for fruitage (0,93 kg/m^2), as 2014. The smallest F/LA in 2014 was noticed at Favette, and with Elsanta thay are the only two varieties with smaller fruitage than standard Senga Sengana. According to annual data, high significant differences between the average values of this parameter for all investigated years were found. In two different locations in Hungary which differ in climate and soil, Dénes (1997) examined numerous strawberry varieties. The F/LA differed a lot and varied from 1,19 kg/m^2 for Elvira variety to 2,0 kg/m^2 for Elsanta variety. Senga sengana had yield of 1,74 kg/m^2 in environment with moderate continental climate. The Melsada variety had exhibited the lowest yield (0,74 kg/m^2) while the highest yield was with the Tago variety (3,06 kg/m^2). The quoted values are significantly higher than the values in our examinations. Kiprijanovski (2001), while investigated this parameter at Pocahontas variety, indicated the F/LA of 1,01kg/m^2, ranged from 0,67 to 1,56 kg/m^2 during the examined period, similar in our data. According to Благојевиќ (1998), the average F/LA for 10 strawberry varieties range from 2,98 kg/m^2 per leaf area at Sequoia to 5,29 kg/m^2 for Red Gauntlet. These are higher values compared with our research. Also, the fruitages of Senga Sengana was in average of 3,17 kg/m^2, (range from 2,19 to 4,24 kg/m^2), while it was 3,29 kg/m^2 for Belrubi (from 2,71 to 3,98 kg/m^2) leaf area.

Table 4. Yield of soluble solid matter (g/m^2) and effective leaf area for 100 g of solid matter production (m^2)

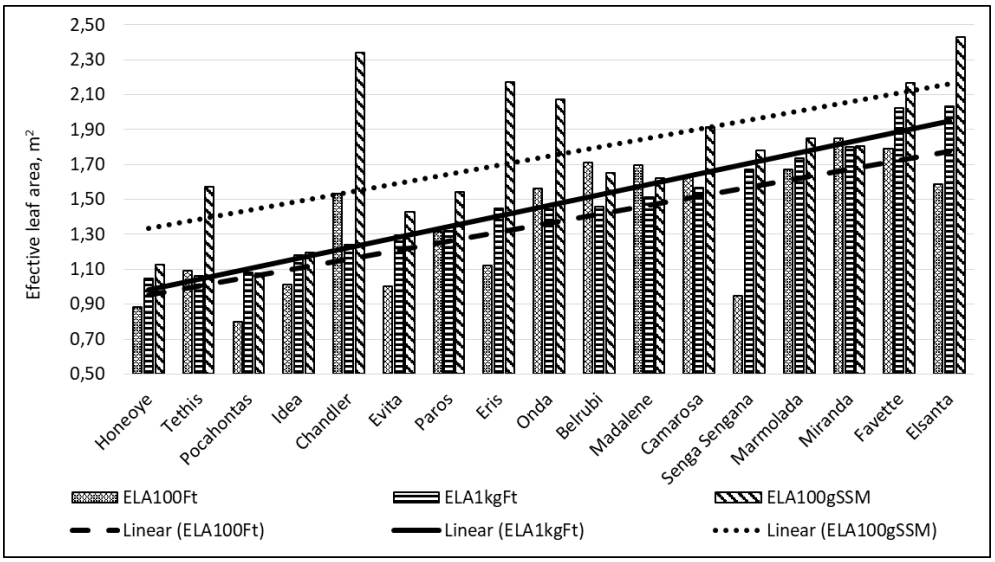
No.	Variety	Yield of soluble solid matter (g/m^2)				Effective leaf area for 100g of solid matter production (m^2)
		2012	2013	2014	Average	
1	Idea	35,8	61,0	154,1	83,6	1,20
2	Camarosa	0,8	45,7	110,2	52,2	1,91
3	Belrubi	9,6	23,0	149,2	60,6	1,65
4	Evita	5,7	54,4	150,1	70,1	1,43
5	Honeoye	5,2	63,0	197,6	88,6	1,13
6	Tethis	22,8	31,9	136,3	63,7	1,57
7	Chandler	22,9	26,3	78,9	42,7	2,34
8	Onda	10,2	28,2	106,2	48,2	2,07
9	Pocahontas	8,6	89,0	181,1	92,9	1,08
10	Senga Sengana	3,6	76,6	88,2	56,1	1,78
11	Miranda	4,8	52,1	109,1	55,3	1,81
12	Paros	4,9	60,7	129,0	64,9	1,54
13	Elsanta	2,0	55,2	66,3	41,2	2,43
14	Eris	3,0	59,9	75,1	46,0	2,17
15	Madalene	0,0	81,4	103,6	61,7	1,62
16	Favette	17,9	57,0	63,6	46,2	2,17
17	Marmolada	16,4	60,1	85,8	54,1	1,85
	AVERAGE	***10,9***	***54,4***	***116,7***	***60,5***	***1,75***
			LSD 0.05=19,99		LSD 0.05=42,23	LSD 0.05= 1,22
			LSD 0.01=2,74		LSD 0.01=56,77	LSD 0.01= 1,64

The medium strong negative correlation between the F/LA and LA/P was determinate. Its values are increased with the increase in the NF/P and Frg/P. Fruit mass has an very small negative influence on F/LA (Table 5). The average ELA1kgF is 1,47 m^2. The values range from 1,05 m^2 at Honeoye to 2,04 m^2 at Elsanta. The F/LA and ELA1kgF are inversely proportional parameters. Physiological activity and productivity of the plants can be expressed accurately from a net-mass i.e. through the production of YSSM/LA (Walsh, 1992). The data for YSSM/LA are presented in Table 4. The average yield for investigation period is 60,5 g/m^2. The highest average for YSSM/LA for the study period is the standard Pocahontas (92,9 g/m^2), which is statistically significantly higher than the average of

Onda, Favette, Eris, Chandler and Elsanta. It is followed by Honeoye (88,6 g/m2) and Idea (83,6 g/m^2).

Table 5. Correlations between examination parameters

Corelations		Productivity parameters per plant					Prouctivity parameters per leaf area		
		LA/P	FM	NF/P	F/P	YSSM/P	YSSM/LA	F/LA	NF/LA
Productivity parameters per leaf area	NF/LA	-0,355	-0,569	0,820	0,524	0,751	0,711	0,711	/
	F/LA	-0,681	-0,260	0,627	0,504	0,740	0,740	/	/
	YSSM/LA	-0,362	-0,353	0,504	0,552	/	/	/	/
Effective leaf area parameters	ELA100NF	0,450	0,548	-0,781	-0,586	-0,684	-0,684	-0,723	***-0,983***
	ELA1kgF	0,613	0,268	-0,580	-0,567	-0,698	-0,698	***-0,979***	-0,684
	ELA100gYSSM	0,292	0,314	-0,569	-0,567	-0,970	***-0,970***	-0,692	-0,677



Graph 1. Effective leaf area parameters of the examined varieties.

The average of Honey is statistically significantly higher than Favette, Eris, Chandler and Elsanta, as well as the average of Idea is higher than Elsanta.The values for this parameter are the lowest in 2012, and they are within a wide range of 0,8 g/m^2 in Camarosa, up to 35,8 g/m^2 in Idea. The highest values for this parameter were determined in 2014 (116,7 g/m^2), range from 63,6 g/m^2 of Favette to 197,6 g/m^2 at Honeoye. According to annual data, high significant differences between the average values of this parameter for all investigated years were found. According to Благојевиќ (1998), the average YSSM/LA for 10 strawberry varieties ranges from 0,026 kg/m^2 at Sequoia, up to 0,046 kg/m^2 for Red Gauntlet. These are twice lower values compared with our research. Also, the YSSM/LA of Senga Sengana was in average of 0,026 kg/m^2 (ranged from 0,022 to 0,041 kg/m^2), while it was 0,032 kg/m^2 for Belrubi (ranged from 0,022 to 0,041 kg/m^2). The average ELA100gYSSM is 1,75 m^2. Its values range from 1,08 m^2 at Pocahontas to 2,43 m^2 at Elsanta. The YSSM/LA and ELA100gYSSM are inversely proportional parameters. There is a low negative correlation between the YSSM/LA with LA/P and with FM, medium strong positive correlation with a NF/P and with F/P and strong positive correlation with the YSSM/P. The analyzed productive parameters per leaf area (NF/LA, F/LA and YSSM/LA) are interdependent with a medium strong positive correlation (Table 5). The values for correlative coefficients are very similar (from 0,711 to 0,751). Analyzing the dependence of the productive parameters per leaf area on the effective leaf area parameters, it can be noted that there is an medium strong and very strong negative correlation dependence between them. Very strong correlation is expected given that the corresponding data for both groups of parameters are inversely proportional (Table 5. The effective leaf area parameters of the examined varieties are

presented in Graph.1 It is noticed that the varieties with lower ELA100NF values are also characterized by lower values for ELA1kgF and ELA100gYSSM, and vice versa.

Table 6. Correlations between effective leaf area parameters.

ELA100Ft	ELA1kgFt	0,691	ELA100Ft	ELA100gSSM	0,621	ELA1kgFt	ELA100gSSM	0,678

Through the effective leaf area parameters lines tendencies (Graph 1), a medium strong positive correlation was established between all investigated parameters (Table 6).

Conclusions

The investigated parameters related to productivity per unit of leaf area are ranged from 54,0 at Miranda to 125,1 at Pocahontas for number of fruits, from 0,49 kg/m^2 in Favette and Elsanta , to 0,96 kg/m^2 in Honeoye for fruitage and from 41,2 g/m^2 in Elsanta, to 92,9 g/m^2 in Pocahontas for the yield of soluble solid metter in fruits. Leaf area of 1,37 m^2 is effective for 100 number of fruits production (from 0,8 m^2 at Pocahontas to 1,85 m^2 at Miranda), as well as 1,47 m^2 for 1kg of fruitage (from 1,05 m^2 at Honeoye to 2,04 m^2 at Elsanta) and 1,75 m^2 for production of 100 g soluble dry matter fruits-yield (from 1,05 m^2 at Honeoye to 2,04 m^2 at Elsanta). The productivity per leaf area of the investigated varieties is less dependent on the influence of LA/P and FM, but it is more dependent of the influence of NF/P, F/P and YSSM/P. A low to medium strong negative correlation between LA/P and FM with all productive parameters per leaf area was determined. While, medium strong to strong positive correlation between NF/P, F/P and YSSM/P with all productive parameters per leaf area was also determined. The values of correlation coefficient between productive parameters per plant and effective leaf area parameters are with similar strength, but they are with an opposite dependency in relation to the corresponding correlations with productive parameters per leaf area. Varieties characterized with higher assimilation activity, i.e. higher photosynthetic capacity, use a smaller leaf surface to produce unit yield. These varieties have lower values for effective leaf area parameters and have more productive leaf surface than the varieties characterized with higher values for these parameters.The varieties with the highest productivity per leaf area are Honeoye, Tethis and control variety Pocahontas. The smallest productivity is noticed at Elsanta, Favette and Miranda.

References

1. Blagojević R. (1983). Uticaj lisne površine na krupnoću i kvalitet plodova nekih sorti jagode. Magisterski rad, Poljoprivredni fakultet, Novi Sad.
2. Благојевиќ Р. (1998). Проучување на биолошките и технолошките особини кај поважните сорти јагоди. Докторска дисертација, Универзитет „Св. Кирил и Методиј“ - Скопје, Земјоделски факултет - Скопје.
3. Bulatović S. (1970). Važnija pomološka obeležja nekih sorti jagoda gajenih u ekološkim uslovima Radmilovca. Jugoslovensko voćarstvo, 11-12, Čačak
4. Dénes F., (1997). Performance of new strawberry cultivars under Hungarian conditions Proc. Third Ict. Strawberry Symp., Acta Hort. 439 Vol. 1: 285-289.
5. Киприjановски М. (2001). Влијание на начинот на одгледување на јагодите врз вегетативниот прираст и приносот. Докторска дисертација. Универзитет „Св. Кирил и Методиј“- Скопје, Земјоделски факултет -Скопје.
6. Најчевска Ц. (2002). Експериментална статистика применета во земјоделските и биолошките истражувања. Бона. Скопје.
7. Поповски Б. (2008). Биолошки и производни карактеристики на некои перспективни сорти јагоди. Докторска дисертација. Универзитет „Св. Кирил и Методиј“- Скопје, Земјоделски факултет -Скопје.
8. Ристевски Б., Колеќевски П., Георгиев Д. (1994). Подкултури во овошните насади. Зборник на трудови Средба „Факултет - стопанство ’94“, Земјоделски факултет, Скопје, II, 109-116.

9. Sarić M., Stanković Ž., Krstić B. (1989). Fiziologija biljaka. Naučna knjiga. Beograd.
10. Walsh C. (1992). Anoverwiew of peach training system and application of pruning techniques. Acta Horticultrae 322, 93-98.