

Influence of a New Growing Technology on Antioxidant Capacity and Phenolic Composition of Blackberry ' a anska Bestrna'

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SUMMARY

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Botrytis cinerea Pers.,
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The investigation was conducted in a ' a anska bestrna' blackberry orchard set up using the intensive cultivation technology, i.e. with pre-formed double-sloping eaves (rain-shield).

Considering that the Serbian blackberry yield suffers an annual loss of around 30% due to gray mould caused by the phytopathogenic fungi *Botrytis cinerea* Pers., introduction of more intensive blackberry cultivation systems is imperative in order to prevent adverse action of rain and other abiotic components, thus securing continuous harvest and supply of improved-quality fruits.

This cultivation technology contributes to a higher content of high-quality fruits, i.e. prevention of gray mould, while at the same time securing continual harvesting,

regardless of the environmental conditions.

Each blackberry sample was analysed for phenolic acids (protocatechuic, 4-hydroxybenzoic, vanillic, ellagic, gallic, *p*-coumaric, caffeic, and ferulic acids), flavonoids (quercetin), anthocyanidins (cyanidin), total phenolics, total anthocyanins, and Trolox-equivalent antioxidant capacity. The analysis was conducted using high-performance liquid chromatography (HPLC) and spectrophotometric techniques.

Regarding the identified phenolic compounds, the blackberries grown under the rain-shield recorded higher values of these components, with the exception of the ellagic acid. Significant higher value of the total phenolic and total anthocyanin content recorded in blackberries grown using the rain-shield was 396.44 and 75.85 mg/100 g FW. There was not significant effect of intensive growing technology on total antioxidant capacity in blackberries and ranged from 2.68 to 2.70 Trolox mmol/100 g FW.

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Key words: blackberries, cultivation techniques, phenolic acids, antioxidant capacity

INTRODUCTION

Berries (e.g., blueberry, blackberry, and strawberry) are well known as 'super fruits' for their potential in the nutraceutical and functional food markets (Ding et al., 2006; Tulipani et al., 2008).

Owing to the specific nature of the present phytochemicals, the low caloric value and the high contents of fibre and essential micro-nutrients, the fruits possess antioxidant qualities that help alleviate adverse effects of the oxidation stress in the cell, thus reducing the risk of chronic disease occurrence.

et al., 2010). (Sariburun et al., 2010). (Hager et al., 2008). (Reyes-Carmona et al., 2005). (Narayana et al., 2001; Liu, 2003). 12 000 t (15 000), 5 000 ha (Nikoli and Tanovi , 2011). " " " " " 95%, " " " " " " " (Nikoli et al., 2012). " " " 1997 . " " 50% (Nikoli et al., 2009). - (Botrytis cinerea).

- Blackberries are notable for their health benefits based on high nutritional contents of dietary fibre, vitamin C, vitamin K, folic acid, and the essential mineral manganese (Sariburun et al., 2010). Blackberries also rank highly among fruits for their antioxidant strength, particularly due to their high contents of phenolic compounds, such as ellagic acid, tannins, ellagitannins, quercetin, gallic acid, anthocyanins, and cyanidins (Hager et al., 2008).

- Polyphenols have potentially beneficial effects on health including anti-inflammatory, antiviral, antimicrobial, and antioxidant activity (Reyes-Carmona et al., 2005).

- Antioxidant activity is defined as the ability to reduce free radical formation and scavenge reactive oxygen species (ROS) (Narayana et al., 2001; Liu, 2003).

- As a consequence of the hyper-production on one side and the uncertain sales prospects on the other side, the average blackberry production in Serbia has declined in recent years to approximately 12,000 t (spread to app. 15,000 individual farms), with the total area under blackberry not exceeding 5,000 ha (Nikoli and Tanovi , 2011). The blackberry orchards in Serbia are dominated by the ' a anska Bestrna' and 'Thornfree' cultivars, with more than 95% share, followed by the 'Black Satin', 'Dirksen Thornless' and some more recent cultivars, such as 'Loch Ness', 'Chester Thornless', 'Triple Crown' (Nikoli et al., 2012). The ' a anska Bestrna' has been produced since 1997, and its current share in the cultivar assortment amounts to approximately 50 % (Nikoli et al., 2009). The comparatively low market demand for the blackberry has been partly caused by relatively lower fruit quality, with significant presence of grey mould (*Botrytis cinerea*). The intensification of the cultivation technology

(Tanovi et al., 2012).

(Scalzo et al., 2005).

1.

(2013–2014 .)

“ (Rubus subg. Rubus Watson).

2006 .

(43°53'N

20°20' E , 290 m . .)

3.0 m

1.5 m,

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2.

accompanied by the introduction of the rain shield resulted in increasing the share of quality fruits, i.e. prevention of the rot, but it has also made it possible to perform the harvesting in continuity, regardless of the external conditions (Tanovi et al., 2012). Apart from the strong impact that the species of fruit has on the anti-oxidant features of the fruit, the cultivation conditions of the plant (environmental and cultivation techniques) must not be neglected (Scalzo et al., 2005).

The research was aimed at establishing the indirect impact of the new cultivation technique (Rain shield) of blackberry on the biological activity of the fruit, i.e. its nutritive and anti-oxidant values.

MATERIAL AND METHODS

1. Plant material and experimental design

The investigation was conducted over a two-year period (2013–2014) in a orchard of ‘ a anka Bestrna’ cultivar of blackberry (Rubus subg. Rubus Watson). The experimental orchard was established in 2006 and was located at Gornja Gorevnica (43° 53'N latitude, 20° 20' E longitude, 290 m altitude) near a ak, Western Serbia.

The blackberry were planted in rows spaced 3.0 m apart with plants set at 1.5 m apart in the row, and trained as a four-wire trellis. Plastic arches were placed on the existing trellis structure in the blackberry. The arches were covered using 150 μ thick foil, forming the shape of an umbrella (Rain shield). The trial was conducted using a randomised block design and it included four replications of each treatment. Fertilization, weed control, and irrigation practices standard for the region were provided during both seasons.

2. Determination of Phenolic Acids, Flavonols and Anthocyanins

Samples were analyzed using an

HPLC (Agilent Technologies, Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, CA, USA) ChemStation, ZORBAX Eclipse Plus C18 (4.6 150 mm, 3.5 μm). Hertog et al. (1992). 5 μL 30 °C. 1% 0 10 min, 10% B A; 10 25 min, 15 50% B A; 25 30 min, 50–80% B A; 30 32 min, 10% B (0.5 ml/min)

HPLC 260 nm, 280 nm, 329 nm, 360 nm 520 nm. UV/Vis mg/100 g FW.

3. (TPH)

Folin-Ciocalteu (Singleton et al., 1999; Liu et al., 2002) 100 g (mg (4.0 g) 40 ml (80% v/v) 2 15 min 3500 rpm. Minisart 0.45 μm . 40 μL 3.16 ml

Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, CA, USA) linked to a ChemStation data handling system, using a ZORBAX Eclipse Plus C18 column (4.6 150 mm, 3.5 μm particles). Samples were prepared according to the method of Hertog et al. (1992). Injection volume was 5 μL and the temperature was set at 30 °C. Solvent A was 1% formic acid and solvent B was acetonitrile. The gradient used was as follows: 0 10 min, 10% of B in A; 10 25 min, 15 50% of B in A; 25 30 min, 50–80% of B in A; 30 32 min, 10% of B in A. By using this gradient (flow rate 0.5 ml/min), a good purity and separation was achieved in fruit samples. The HPLC equipment was used with a diode array detector (DAD). Phenolic compounds were detected at 260 nm, 280 nm, 329 nm, 360 nm, and 520 nm. Phenolic compounds were identified according to peak retention time and UV/Vis spectra by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as mg/100 g FW.

3. Determination of Total Phenolics (TPH)

The TPH content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Liu et al., 2002) and the results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW). Grinded sample (4.0 g) was stirred vigorously with 40 mL of extraction solution consisting of methanol and distilled water (80% v/v) and was kept for 2 hours in the dark at room temperature. The mixture was centrifuged in two sequential times for 15 min at 3500 rpm, and supernatant was filtered through a 0.45 μm Minisart filter before analysis.

A 40 μL of fruit extracts or gallic acid standard solution was mixed with 3.16 mL of distilled water whereupon 200 μL of Folin-Ciocalteu reagent was added and

200 μ L
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Na₂CO₃.
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765 nm
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(Torre and Barritt, 1977; Liu et al.,
2002).
, 20 g e
40 ml
(95% /1.5 N HCl, 85:15).
30 ml
70 ml
2
200 mL.
510
700 nm
UV/VIS (PU 8740 UV/VIS,
) 1-
.
100 g (mg cyn-3-glu/100 g
FW),
26 900 L/cm/mol
449.2 g/mol.

5.
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ABTS
Arnao et al. (1999).
ml 4.9 mM
5 ml 14 mM
ABTS.
16
(25 \pm 1°).
,
0.700 \pm
0.02 734 nm,

allowed to stand for 8 min before 600 μ L
of 20% Na₂CO₃ solution was added.

Solution was well mixed and absorbance
at 765 nm against an appropriate blank
was determined after 2 hours. Data are
reported as means for at least three
replications.

4. Determination of Total Anthocyanin (TAN)

The monomeric anthocyanin
pigment content of the aqueous extracts
was determined using the previously
described pH-differential method (Torre
and Barritt, 1977; Liu et al., 2002). Briefly,
20 g of grinded fruit was blended with 40
mL of extracting solvent (95% ethanol/1.5
N HCl, 85:15). The extract was collected
by filtration with an additional 30 ml of
solvent washing. The residue was soaked
with 70 mL of extracting solvent, and the
extract was collected after 2h. The total
extracts were pooled and brought up to
200 mL. A UV/VIS spectrophotometer (PU
8740 UV/VIS, England) and a 1-cm path
length disposable cell were used for
spectral measurements at 510 and 700
nm. Pigment content was calculated as
milligrams cyanidin-3-glucoside per 100 g
of fresh weight (*mg cyn-3-glu/100 g FW*)
using an extinction coefficient of 26,900
L/cm/mol and molecular weight of 449.2
g/mol.

5. Determination of the Total Antioxidant Capacity

Antioxidant capacity (TAC) was
determined by the ABTS assays
according to Arnao et al. (1999). ABTS
solution was freshly prepared by adding 5
ml of a 4.9 mM potassium persulphate
solution to 5 ml of a 14 mM ABTS solution
and the resulting solution was kept for 16
h in dark at room temperature (25 \pm 1 °C).

This solution was diluted with methanol to
yield an absorbance of 0.700 \pm 0.02 at
734 nm and the same solution was used
for the antioxidant assay. One milliliter of

950 μ l of ABTS solution and 50 μ l of the samples. This solution was vortexed for 10 sec and the absorbance was recorded at 734 nm after 6 min using UV/VIS spectrophotometer (PU 8740 UV/VIS, England) which was compared with the control ABTS solution. The results were expressed as mmol Trolox equivalents per 100 g of fresh matter (mmol/100 g FW).

6. Statistical analysis
 All tests were performed in triplicate and the results are presented as mean \pm standard error of mean (SE). Differences between mean values were compared by Duncan's Multiple Range test in two-way analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). Differences with *p* values of 0.05 were considered insignificant.

reaction mixture of standard and extracts comprised 950 μ l of ABTS solution and 50 μ l of the samples. This solution was vortexed for 10 sec and the absorbance was recorded at 734 nm after 6 min using UV/VIS spectrophotometer (PU 8740 UV/VIS, England) which was compared with the control ABTS solution. The results were expressed as mmol Trolox equivalents per 100 g of fresh matter (mmol/100 g FW).

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RESULTS AND DISCUSSION

1. Phenolic Acids, Flavonols and Anthocyanins

Table 1 shows the profile of free phenolic acids determined in fruit of 'a anska Bestrna' blackberry.

The dimer of gallic acid-ellagic acid, four hydroxybenzoic acids, including protocatechuic, 4-hydroxybenzoic, vanillic and gallic acids, as well as three hydroxycinnamic acids, including p-coumaric, caffeic and ferulic acids, were identified and quantified in the fruit (Table 1).

1.

Table 1. Phenolic acids content in fruit of ' a anska Bestrna' blackberry

Phenolic acids (mg/100 g FW)	Rain shield		Standard		Mean of growing year		Mean of cultivation techniques	
	2013	2014	2013	2014	2013	2014	Rain shield	Standard
	Protocatechuic	1.52±0.17 a	1.82±0.15 a	1.29±0.71 a	0.97±0.21 a	1.67±0.12 a	1.13±0.34 b	1.41±0.33 a
4-hydroxybenzoic	0.36±0.01 a	0.46±0.09 a	0.46±0.09 a	0.41±0.06 a	0.41±0.05 a	0.43±0.05 a	0.44±0.04 a	0.41±0.05 a
Vanillic	1.04±0.54 a	1.02±0.33 a	0.78±0.14 a	0.39±0.02 a	1.03±0.28 a	0.58±0.11 b	0.91±0.26 a	0.70±0.20 a
/Ellagic	6.94±0.92 a	6.88±1.12 a	3.39±1.03 a	6.00±0.29 a	6.91±0.65 a	4.70±0.76 b	5.16±1.01 b	6.44±0.55 a
/Gallic	2.79±0.13 a	2.56±0.59 a	3.38±0.46 a	2.89±0.11 a	2.68±0.28 b	3.14±0.24 a	3.09±0.25 a	2.73±0.28 a
-	0.67±0.18 a	0.59±0.05 a	0.33±0.19 a	0.40±0.02 a	0.63±0.08 a	0.37±0.09 b	0.51±0.14 a	0.50±0.05 a
p-coumaric	0.37±0.02 a	0.33±0.04 a	0.43±0.05 a	0.41±0.01 a	0.35±0.02 a	0.42±0.02 a	0.40±0.03 a	0.37±0.03 a
/Caffeic	0.39±0.02 a	0.33±0.01 a	0.41±0.06 a	0.38±0.01 a	0.37±0.02 a	0.40±0.03 a	0.41±0.04 a	0.36±0.01 a
/Ferulic								

(P 0.05)

For each analysed compound mean values within each row (in the treatment and interaction) followed by the same small letter are not significantly different according Duncan's Multiple Range test (P 0.05)
FW – /fresh weight of fruit

Phenolic acids subjected to analysis of variance showed significant effect of growing year on content vanillic and ellagic acids.

The content of protocatechuic, 4-hydroxybenzoic, vanillic and gallic acids ranged from 0.97±0.21 to 1.82±0.15, 0.36±0.01 to 0.46±0.09, 0.39±0.02 to 1.04±0.54 and 2.56±0.59 to 3.38±0.46 mg/100 g FW, respectively.

The comparison of the different cultivation techniques treatments showed that contents of hydroxybenzoic acids were higher in Rain shield treatment. Higher contents of hydroxybenzoic acids, except protocatechuic and gallic acids were recorded in the second growing year.

The content of p-coumaric, caffeic and ferulic acids ranged from 0.33±0.19 to 0.67±0.18, 0.33±0.04 to 0.43±0.05 and 0.33±0.01 to 0.41±0.06 mg/100 g FW, respectively. Higher contents of hydroxycinnamic acids were recorded in blackberries subjected to the Rain shield cultivation techniques, in the second growing year. The growing year significantly affected content of cyanidin (Table 2).

2.

Table 2. Flavonols and anthocyanidins content in fruit of ' a anska Bestrna' blackberry

Treatment		Flavonols		Anthocyanidins (mg/100 g FW)	
		/Quercetin		/Cyanidin	
Growing year (A)	/First	0.29±0.04 a		3.65±0.07 b	
	/Second	0.32±0.03 a		5.06±0.62 a	
Cultivation techniques (B)	/Rain cap	0.34±0.04 a		4.87±0.68 a	
	/Standard	0.27±0.03 a		3.83±0.13 a	
ANOVA					
A		ns		*	
B		ns		ns	
A × B		*		ns	

p 0.05

Values within each column followed by the same small letter are insignificantly different at the *p* 0.05 by Duncan's Multiple Range test
 ns – / non significant differences
 FW – / fresh weight.

1).

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 -
 0.27±0.04
 0.34±0.03 3.65±0.07 4.87±0.68
 mg/100 g FW,
 -
 -
 -

The interaction effect of the growing year and cultivation techniques produced significant differences in the content of quercetin (Figure 1). The quercetin and cyanidin contents ranged from 0.27±0.04 to 0.34±0.03 and 3.65±0.07 to 4.87±0.68 mg/100 g FW, respectively and were higher in blackberries undergoing the Rain shield treatments. It was also observed that the quercetin and cyanidin contents were higher in the second growing year.

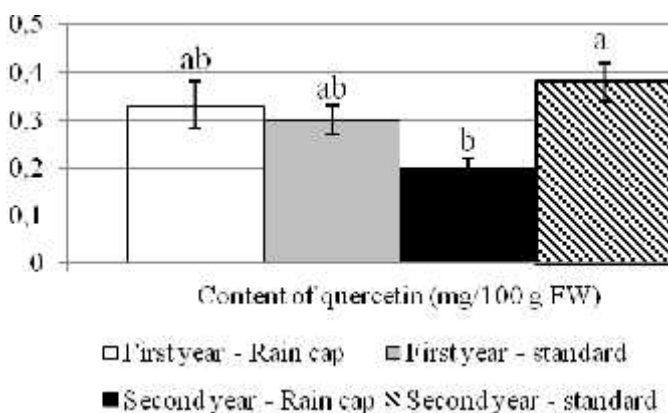


Fig. 1. Content of quercetin (A × B)

The analysis of the interaction

(0.38 ± 0.04 mg/100 g FW)

(0.20 ± 0.02 mg/100 g FW)

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(2).

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effect of the growing year and cultivation techniques inferred that the quercetin content was highest in standard cultivation techniques (0.38±0.04 mg/100 g FW) during the second year, and lowest with the Rain shield (0.20±0.02 mg/100 g FW) in the same year.

2. Total Antioxidant Capacity, Total Phenolics and Anthocyanin

Analysis of variance showed significant effect of growing year on TAC and TPH whereas the cultivation techniques significantly affected the TPH (Table 3).

The interaction effect of growing year and cultivation techniques showed significant differences among the TAC and TPH (Figure 2).

Table 3. Total antioxidant capacity, total anthocyanins and phenolic content in fruit of blackberry ' a anska Bestrna'

Treatment		TPH	TAN	TAC
		mg/100 g FW		Trolox, mmol/100 g FW
Growing year (A)	/First	373.99±32.12 a	70.22±2.55 a	2.92±0.05 a
	/Second	327.68±12.33 b	69.80±3.23 a	2.46±0.06 b
Cultivation techniques (B)	Rain cap	396.44±23.69 a	75.85±1.56 a	2.68±0.16 a
	Standard	305.24±2.29 b	64.17±0.92 b	2.70±0.05 a
A		*	ns	*
B		*	*	ns
A × B		*	ns	*

p 0.05

/ Values within each column followed by the same small letter are insignificantly different at the *p* 0.05 by Duncan's Multiple Range test

ns – / non significant differences

FW – / fresh weight.

The TAC, TPH and TAN in blackberries ranged from 2.46±0.06 to 2.92±0.05 Trolox mmol/100 g FW, 305.24±2.29 to 396.44±23.69 and 64.17±0.92 to 75.85±1.56 mg/100 g FW, respectively. The higher chemical

FW. -

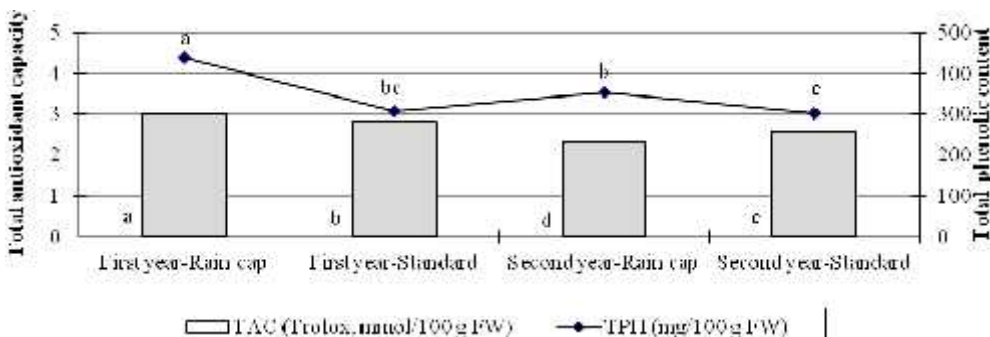
parameters, TAC, TPH and TAN were recorded in the first growing year.

Comparing the different cultivation techniques treatments, it can be concluded that higher TAC was recorded in Rain cap, whereas the standard cultivation techniques produced higher TPH and TAN.

The analysis of interaction effect of growing year and cultivation techniques (Figure 2) inferred that TAC and TPH were highest in Rain shield during first year (3.02 ± 0.13 Trolox, $mmol/100$ g FW; 439.41 ± 29.62 $mg/100$ g FW, respectively).

TAC was lowest in Rain shield during second year (2.33 ± 0.04 Trolox, $mmol/100$ g FW), whereas TPH was lowest in standard cultivation techniques in the same year (301.89 ± 3.54 $mg/100$ g FW).

(3.02 ± 0.13 , $mmol/100$ g FW;
 439.41 ± 29.62 $mg/100$ g FW).
 (2.33 ± 0.04 , $mmol/100$ g FW),
 (301.89 ± 3.54 $mg/100$ g FW).



. 2.

(A $\bar{1}$ B)

Fig. 2. Total antioxidant capacity and content of total phenolic (A $\bar{1}$ B)

* $p < 0.05$
 / The same small letters represents not significant differences at $P < 0.05$ by Duncan's Multiple Range test

Phenolic compounds are an essential daily dietary component of fresh fruit and vegetables which aids in the protection and function of essential cellular constituents against oxidative

(Scalbert et al., 2005).
Rubus
 (Kahkonen et al., 2001; Proteggente et al., 2002).
 4-
 Zadernowski et al. (2005),
 Sellappan et al. (2002).
 Clark e al. (2002)
 (Mullen et al., 2002).
 (Erlund, 2004)
 (Kris-Etherton et al., 2002).
 (Erlund, 2004; Kahkonen et al., 2001). Milivojevi et al. (2011)

damage associated with various etiologies of neurological and chronic diseases (Scalbert et al., 2005). Berry fruit (including *Rubus* species) contain high concentrations of several classes of phenolic compounds, including phenolic acids, anthocyanins, and flavonols (Kahkonen et al., 2001; Proteggente et al., 2002). A high variation of phenolic contents of blackberry cultivars was found in the literature.

The acids discovered in the blackberries in our study - the protocatechuic, 4-hydroxybenzoic, vanillic, ellagic, gallic, *p*-coumaric, caffeic and ferulic acids, represent a significantly lower number of phenolic acids than the one presented in the results obtained by Zadernowski et al. (2005), who identified twenty phenolic acids in blackberries. Our results show that ellagic acid, caffeic acid, and ferulic acid levels were lower than those reported in blackberry Sellappan et al. (2002). As opposed to our results, Clark e al. (2002) observed higher quantities of ellagic acid in blackberris. In general, free ellagic acid levels observed in blackberry are quite low in this study, and their detection is probably the result of acid hydrolysis products of ellagitannin breakdown (Mullen et al., 2002).

The term flavonoid refers to flavonols and flavones, with quercetin being the most abundant (Erlund, 2004) and widespread throughout the plant being found in fruit, vegetables, nuts, seeds, flowers, and bark (Kris-Etherton et al., 2002).

Quercetin has been studied more thoroughly than other flavonoids, not only because of its abundance, but because it has been reported to exhibit antioxidative, anticarcinogenic, anti-inflammatory, anti-aggregatory, and vasodilating effects (Erlund, 2004; Kahkonen et al., 2001). Milivojevi et al. (2011) reported that

(Bilyk Sapers, 1986; Siriwoharn et al., 2004),

Clark et al. (2002).

(Clark et al., 2002). Wang Lin (2000)

(Rice-Evans and Miller, 1986). Garcia-Alonso et al. (2004)

TEAC) (406 $\mu\text{mol/g}$), (192 $\mu\text{mol/g}$), (187 $\mu\text{mol/g}$) (163 $\mu\text{mol/g}$). Pantelidis et al. (2007)

Moyer et al. (2002), Siriwoharn et al. (2004) Clark et al. (2002).

(Wang, 2007).

quercetin was not detected in any of the blackberry samples in their study, but several studies have already reported the presence of quercetin glycosides in blackberries (Bilyk and Sapers, 1986; Siriwoharn et al., 2004), which has also been confirmed by the results obtained in our study. Our results show that content of quercetin was higher than those reported in blackberry Clark et al. (2002).

The contents of quercetin and cyanidin were higher in second growing year, which may be related to genetic differences, maturity at harvest, cultural practices, different extraction and laboratory methods employed (Clark et al., 2002). Wang and Lin (2000) reported that delphinidin, cyanidin, pelargonidin, malvidin, and peonidin are the major anthocyanins found in berries.

Various phytochemical components, including flavonoids, phenylpropanoids, and phenolic acids are known to be responsible for TAC in fruits and vegetables (Rice-Evans and Miller, 1986). Garcia-Alonso et al. (2004) reported that the greatest TAC obtained by TEAC method were persimmon (406 $\mu\text{mol/g}$), blackberry (192 $\mu\text{mol/g}$), blueberry (187 $\mu\text{mol/g}$) and strawberry-tree fruit (163 $\mu\text{mol/g}$). Pantelidis et al. (2007) reported that blackberry 'Hull Thornless' gave the highest TAC of the examined cultivars of raspberries, blackberries, red currants, gooseberries and Cornelian cherries.

In this study the TAC of blackberries was generally lower than the reported by Moyer et al. (2002), Siriwoharn et al. (2004) and Clark et al. (2002). Namely, plants grown in cool day and night temperatures generally had the lowest antioxidant capacity (Wang, 2007).

The comparison of the differences in TPH and TAN related to the growing

(Sellapan et al., 2002).
Wang Lin (2000),
338 mg/100 g FW.
" Milivojevi
et al. (2011)
, Benvenuti et al. (2004)
192.8
351.7 mg/100 g FW,
(Skrede and Wrolstad, 2002), Wang Lin
(2000)
(Hosseinian et al., 2007).
Benvenuti
et al. (2004).
(Duthie et al., 2003).

year revealed that these were higher in the first than in the second year, suggesting that the growing season, climate and region have an influence on the antioxidant power of blackberries (Sellapan et al., 2002). In the study conducted by Wang and Lin (2000) the total TPH content of berries and leaves varied from 91 to 338 mg/100 g of FW.

Our results revealed a higher TPH content in ' a anska Bestrna' blackberry than that reported by Milivojevi et al. (2011) for the same cultivar under similar agro-ecological conditions.

On the other hand, Benvenuti et al. (2004) reported that content of TPH in some thornless blackberry cultivars grown in Italy ranged from 192.8 to 351.7 mg/100 g FW, which is similar to the results of our study.

The anthocyanin content of blackberries compares favourably with other fruits (Skrede and Wrolstad, 2002), and Wang and Lin (2000) have shown that TAC of blackberries is highly correlated with the anthocyanin pigment content. However, many factors such as genes, soil type, light, temperature, and agronomic conditions affect anthocyanin composition in plants (Hosseinian et al., 2007). Our results show that the TAN contents were similar to those reported in the thornless blackberry Benvenuti et al. (2004).

However, even when good experimental evidence exists, results need to be interpreted with caution in relation to human health benefits, as polyphenols may have limited bioavailability and may also be extensively metabolised (Duthie et al., 2003).

CONCLUSIONS

The present study indicates that blackberries are a rich source of natural

- antioxidants and that intensification of the cultivation technology of blackberry contributes to an increase in the polyphenol contents. On the other hand, total phenolic content and antioxidant activity varied among the different ecological conditions used in this study.

- Consumption of blackberries can provide a good source of antioxidants, and therefore they may have potential for use in the development of food ingredients that are beneficial to human health.

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(*Cornus mas* L.)

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Pomological Descriptions of Bulgarian Cultivars and Forms of Cornelian Cherry (*Cornus mas* L.)

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SUMMARY

- In the present pomological survey
 - are included five local forms, bearing
 - temporary names according to their
 - geographical origins – ‘Vratsa-Kastel’,
 - ‘Tsarigradski zhalt’, ‘Shandryan’, ‘Atkov
 - dryan’ and ‘Yaltenski’, as well as four of
 - the Bulgarian cultivars – ‘Kazanlashki
 - krushoviden’, ‘Pancharevski tsilindrichen’,
 - ‘Shumenski prodalgovat’ and ‘Zhalt
 - Hadzhiyski’. Up-to-date pomological
 - descriptions have been developed based
 - on new observations and data. The
 - pomology descriptions of the five large-
 - fruited forms are presented in this study
 - for the first time. The results of the
 - pomological studies showed that the fruits
 - of the f. ‘Vratsa-Kastel’ are the largest,
 - with an average weight of 9.46g, with
 - individual fruits reaching 14g.
 -
 - The fruits of ‘Shumenski prodalgovat’ –
 - 8.78g and ‘Yaltenski’ - 8.79g are also very
 - large. Smallest are the fruits of cv. ‘Zhalt
 - Hadzhiyski’ – 3.64g. Such is the average
- 9,46g,
14g.
- 8,78g
- 8,79g.
- 3,64g.

Cornus mas L.,

(150-200),

Leontyak (1984) Dudukal and Imaliyev (1985).

Ercycly (2004) Karadeniz et al. (2007).

mass of the fruits of the major part of the widespread wild forest forms.

The purpose of the present study is to examine the pomological characteristics of the new large-fruited forms compared to those of established and wide-spread Bulgarian cultivars and to recommend some of the new forms for breeding, official registration as new cultivars and their introduction into future tree orchards.

Key words: cornelian cherry, *Cornus mas* L., pomological characteristics

INTRODUCTION

Unlike most fruit species, cornelian cherry is represented by a limited number of cultivars (150-200 varieties), but with a wide variety of forms distributed in nature. The natural cornelian cherry deposits in Crimea and the whole Black Sea coast, the Caucasus, the Balkan Peninsula, Moldova, Turkey and other countries and regions in southern Europe are full of form diversity, including with large-fruited forms.

A number of studies from neighbouring as well as from more distant countries are devoted to the genetic diversity and selection of new cultivars of cornelian cherry.

In-depth biological research on the natural form diversity, growth characteristics and resistance of cornelian cherry to diseases and pests were conducted in Moldova by Leontyak (1984) and Dudukal and Hasmaliev (1985). Described were also the shape and colouring of the fruits in natural cornelian cherry populations.

The natural genetic variety of cornelian cherry in Turkey is also very large. Focused on this research were Ercycly (2004) and Karadeniz et al. (2007), they selected large-fruited cornelian cherry forms.

- The selection work and the pomological studies on natural populations of cornelian cherry have also been conducted in Serbia. A rich collection of local and introduced cornelian cherry cultivars and forms was created at the University of Novi Sad (Bijeli et al., 2009; Bijeli, 2011; Bijeli et al., 2008; Bijeli et al., 2015).
- Through the use of molecular technologies, the natural genetic diversity of the common cornelian cherry in Iran has been investigated. (Hassanpour et al., 2013).
- In Russia, studies have been conducted on agrobiological characteristics and the prospects for cultivation of large-fruited cornelian cherry forms in the Pricubanska fruit-growing area (Kravchuk, 2003).
- Rich genetic resources from cornelian cherry have been explored on the territory of Ukraine, where the largest number of large-fruited cultivars and shapes are created, including yellow-fruited. In Ukraine, the cornelian cherry is grown as a cultivated fruit species on vast areas (Mezhenskiy, 2005; Klimenko, 2007; Klimenko, 2013).
- The natural form diversity of cornelian cherry in Bulgaria is very large and represents an inexhaustible genetic source for the selection of new large-fruited forms and cultivars. As a cultivated plant, the cornelian cherry is represented by five officially registered Bulgarian cultivars and a variety of large-fruited forms, mass-grown as individual trees mainly in backyards and villas, fruit nurseries, mixed orchards and others. In recent years, there has been a tendency for increased interest in the creation of cornelian cherry orchards.
- In Bulgaria there is no official breeding program for the improvement of the cornelian cherry assortment, thus all registered Bulgarian cultivars and large-fruited forms have been selected from local resources by common folk or enthusiasts. In parallel, large-fruited forms

and cultivars have been introduced many times from neighbouring and more distant countries.

The first three most consolidated natural large-fruited forms of cornelian cherry are officially registered as Bulgarian cultivars in 1983 and bear local names 'Kazanlashki krushoviden', 'Pancharevski tsilindrichen' and 'Shumenski prodalgovat' (Tsolov, 1983; Lazarov and Zhivondov, 2014). Significantly later in 2007, officially registered as cultivars are another two natural forms of cornelian cherry, which are named as 'Pancharevski yaytseviden' and 'Zhalt Hadzhiyski' (Lazarov and Zhivondov, 2014).

Most extensive studies of Bulgarian cultivars and forms of cornelian cherry in Bulgaria were conducted by Tsolov (1983). The pomological characteristics established by the author are confirmed and corresponding to the later studies carried out by other Bulgarian researchers (Zhivondov, 2012; Zhivondov et al., 2007).

The increased interest in planting orchards of cornelian cherry in recent years and the rich natural form diversity, necessitated the need to look for new large-fruited forms, to study their pomological characteristics, comparing them with established and distributed Bulgarian cultivars. The final goals are to recommend some of the new large-fruited forms, registration as new cultivars and introduction into future cornelian cherry orchards. The ones bearing the largest fruits are to be recommended for use in the selection of new cultivars.

MATERIAL AND METHODS

The present pomological research includes five local large-fruited forms, found as a result of scientific expeditions carrying temporary names according to their geographical origin – 'Vratsa-Kastel',

‘Yaltenski’, ‘Tsarigradski zhalt’, ‘Shandryan’ and ‘Atkov dryan’. The study also includes four of the Bulgarian cultivars – ‘Kazanlashki krushoviden’, ‘Pancharevski tsilindrichen’, ‘Shumenski prodalgotat’ and ‘Zhalt Hadzhiyski’, which are described using new up-to-date observations and data.

The cultivars of ‘Kazanlashki krushoviden’, ‘Pancharevski tsilindrichen’ and ‘Shumenski prodalgotat’ are the most common and widespread throughout the country. The cultivar ‘Zhalt Hadzhiyski’ is poorly distributed, mainly in different regions of northern Bulgaria and less in the southern parts of the country. The same is less studied. The large-fruited form ‘Vratsa - Kastel’ was created by the fruit growing enthusiast Kostadin Todorov in Vratsa, by selecting of seedlings derived from large-fruited cultivars and forms to be used for the purpose of propagation of rootstocks. ‘Yaltenski’ was introduced at the Fruit Growing Institute - Plovdiv, without a name in 1964 from Nikita botanical garden near Yalta (Crimean Peninsula). In the past, ‘Tsarigradski zhalt’ has been transferred from Istanbul to the fruit nursery in the town of Kazanluk, where its propagation was limited. Preserved old trees from it were found in the yard of Yordan Koev from Skobelevo near the town of Kazanluk. ‘Shandryan’ is a local form, selected by Stanyu Ivanov, propagated and preserved in the village of Shanovo, Stara Zagora. ‘Atkov dryan’, large-fruited form, was discovered in a private yard in the city of Plovdiv.

All cultivars and newly discovered forms have been propagated and planted 16 years ago for cultivation and study, in a private collection orchard near Plovdiv.

Observations and studies were conducted during the period 2014-2018, according to the accepted methodologies for studying plant resources in the fruit-growing.

2014-2018 .

- Average data for the five-year period is presented. The pomological descriptions of the five large-fruited forms are presented in this study for the first time.

RESULTS AND DISCUSSION

- The main characteristics and the appropriate pollinators of each of the studied cultivars and forms are reflected in Table 1, 2 and 3.

1.

Table 1. Main pomological characteristics

Cultivar / Form	Tree vigor	Fruit ripening time	Colour of fruit skin	Fruit shape
'Atkov dryan'	medium	15 - 31 () 15 - 31 Aug (medium)	red	cylindrical
'Vratsa - Kastel'	medium to strong	25 - 31 () 25 - 31 Aug (late)	dark red	() cylindrical to pear-shaped (varying)
'Zhalt Hadzhiyski'	weak to medium	15 - 30 () 15 - 30 July (very early)	yellow	cylindrical
'Kazanlashki krushoviden'	medium to strong	01 - 10 () 01 - 10 Sept (very late)	dark red	pear-shaped
'Pancharevski tsilindrichen'	medium	15 - 31 () 15 - 31 Aug (medium)	red	cylindrical
'Tsarigradski zhalt'	medium	15 - 25 () 15 - 25 Aug (medium)	yellow	cylindrical
'Shandryan'	medium to strong	15 - 31 () 15 - 31 Aug (medium)	dark red	barrel-shaped
'Shumenski prodalgovat'	medium to strong	01 - 10 () 01 - 10 Sept (very late)	dark red	pear-shaped to bottle-shaped
'Yaltenski'	medium to strong	01 - 15 () 01 - 15 Sept (very late)	dark red	pear-shaped to bottle-shaped

2.

Table 2. Biometrical data

Cultivar / Form	Max fruit weight [g]	Average fruit weight [g]	Average stone weight [g]	Fruit / Stone weight ratio [%]
'Atkov dryan'	9.00	7.14	0.64	91.05
'Vratsa - Kastel'	14.00	9.46	0.56	94.13
'Zhalt Hadzhiyski'	4.30	3.64	0.38	89.65
'Kazanlashki krushoviden'	9.50	7.84	0.75	90.48
'Pancharevski tsilindrichen'	9.50	7.69	0.75	90.30
'Tsarigradski zhalt'	6.50	5.44	0.48	91.21
'Shandryan'	6.50	5.33	0.57	89.23
'Shumenski prodalgovat'	10.50	8.78	0.78	91.19
'Yaltenski'	10.50	8.79	0.81	90.84

3.

Table 3. Suitable pollinators

Cultivar / Form	Pollinators
'Atkov dryan'	'Shumenski prodalgovat'; 'Tsarigradski zhalt'; 'Zhalt Hadzhiyski'
'Vratsa - Kastel'	All other cultivars and forms
'Zhalt Hadzhiyski'	'Kazanlashki krushoviden'; 'Pancharevski tsilindrichen'; 'Shumenski prodalgovat'
'Kazanlashki krushoviden'	'Pancharevski tsilindrichen'; 'Shumenski prodalgovat'; 'Tsarigradski zhalt'; 'Zhalt Hadzhiyski'
'Pancharevski tsilindrichen'	'Kazanlashki krushoviden'; 'Shumenski prodalgovat'; 'Tsarigradski zhalt'; 'Zhalt Hadzhiyski'; 'Yaltenski'
'Tsarigradski zhalt'	All other red-coloured cultivars and forms
'Shandryan'	All other cultivars and forms
'Shumenski prodalgovat'	'Pancharevski tsilindrichen'; 'Kazanlashki krushoviden'
'Yaltenski'	'Kazanlashki krushoviden'; 'Pancharevski tsilindrichen'; 'Tsarigradski zhalt'

9,46g,
14g.

3,64g,
4,3g.

0,38g
0,81g

From the same it is evident that with weak to moderate growth are only the trees of the cultivar 'Zhalt Hadzhiyski'. Characterized by moderate growth are the trees of the cultivar 'Pancharevski tsilindrichen' and the forms 'Atkov dryan' and 'Tsarigradski zhalt'. The trees of the other cultivars and forms show moderate to vigorous growth. The largest fruits are those of the form 'Vratsa-Kastel', whose average mass is 9.46g, as some of the fruits could reach 14g. The fruits of the cultivar 'Shumenski prodalgotat' and the form 'Yaltenski' are also very large. The cultivar 'Zhalt Hadzhiyski' has the smallest fruits – only 3.64g as average and could reach 4.3g. Such is the average mass of the fruits of the majority of the natural forest forms. With a typical pear-shaped form are the fruits of the cultivar 'Kazanlashki krushoviden'. Cylindrical shape have the fruits of cultivars 'Pancharevski tsilindrichen', 'Zhalt Hadzhiyski' and the large-fruited forms 'Atkov dryan' and 'Tsarigradski zhalt'. Typical barrel-shaped fruits are only the fruits of 'Shandryan', and those of the other studied cultivars and forms demonstrate transient shapes of the fruits – cylindrical to pear-shaped.

The average mass of the stone ranged from 0.38g in the cultivar 'Zhalt Hadzhiyski' to 0.81g in the form 'Yaltenski'. The ratio between fruit flesh and the stone is the lowest in the form 'Shandryan' and the cultivar 'Zhalt Hadzhiyski', which also have the smallest fruits. That ratio is the highest in the fruits of 'Vratsa-Kastel'. The colouring of the fruit skin in the red-fruited cultivars and forms vary from red to dark red in different shades.

The ripening of the fruit covers not that short period of more than two months. Under the conditions of the Plovdiv region, the fruits of the cultivar 'Zhalt Hadzhiyski' ripen first – in the second half of July, and at the last to ripen are those of the form 'Yaltenski' – Mid-September.

- As a typical cross-pollinating plant, the relationships between pollination to fertilization in the cornelian cherry are extremely important. Studies in this direction showed that each red-fruited cultivar or form is well pollinated by any yellow-fruited and vice versa. Very well pollinate each other the cultivars 'Kazanlashki krushoviden', 'Pancharevski tsilindrichen', 'Shumenski prodalgovat' and 'Zhalt Hadzhiyski'. The Largest-fruited form 'Vratsa-Kastel' and 'Shandryan' are well pollinated by all other of the studied cultivars and forms. The cultivar 'Shumenski prodalgovat' and the form 'Yaltenski' do not pollinate well each other, as well as 'Pancharevski tsilindrichen' with 'Atkov dryan'.

Pomological descriptions of the studied cultivars and forms.

'Kazanlashki krushoviden'

The tree has medium to strong vigour. The shape of the canopy ranges from the semi-upright to spreading and it is moderately dense. The long shoots are moderately thick, with anthocyanin colouring to the top, with pubescence. The internodes are medium long (7-9cm with slightly protruding nodes. The leaf buds on them are peeled off from the long shoots, and on the short twigs are adherent. The flower buds are large, globular, with a little tip. The number of flowers in the inflorescence varies most often between 20 and 24.

The fruits ripen in the first ten days of September. They are large, with a typical pear-like shape, with an average weight of 7.84g and dimensions 31×17.5mm. The fruit skin is a dark red, shiny, medium thick, fragile, not separating from the fruit flesh. The flesh is tender, juicy, red, with a pleasant sour taste and 90.48% yield (fruit flesh to stone ratio). The fruit stalk is medium long (16-18mm, yellow green, with fine pubescence, sometimes with pear-shaped thickening close to the fruit. The stone is

24.

7,84g

31×17,5mm.

90,48%

(16-18mm),

19x8mm. 0,75g

small, with an average weight of 0.75g and dimensions 19x8mm. It is a light brown, sharpened at the base.

The fruits ripen almost at the same time and can be harvested in one hand (stage). The fruit drop is weak. The flowering is abundant and prolonged. Yields very good and regularly. It is rarely damaged by diseases and pests.

'Pancharevski tsilindrichen'

(8-9cm),

The tree has moderate vigour. The canopy is spreading, medium dense. The long shoots are medium thick, light green, quadrated to the top, with pubescence. The internodes are medium long (8-9cm) with slightly protruding nodes. The leaf buds on them are slightly peeled off, and on the weaker twigs are adherent. The flower buds are large, almost globular and contain most often from 22 to 28 flowers.

22 28

The fruits ripen in the second half of August. They are large with cylindrical form, with an average weight of 7.69g and dimensions 29x20.5mm. Fruit skin is bright red, shiny, medium thick, fragile, semi-detachable from the fruit flesh. The flesh is very tender, juicy, light red, with pleasant sour taste and 90.3% yield. The fruit stalk is a medium long (15-16mm), yellow green, with fine pubescence. The stone is small, with an average weight of 0.75g and dimensions 17x7.6mm. It is light brown, without sharp ends, semi-detachable.

7,69g

29x20,5mm.

90,3%

(15-16mm),

0,75g

17x7,6mm.

The fruits ripen almost simultaneously and can be harvested at the same time. The fruit drop is weak. The flowering is abundant and prolonged. Gives regular and heavy crops. It is rarely attacked by diseases and pests.

'Shumenski prodalgovat'

The tree has moderate to strong growth. The canopy is spreading to wide spreading, medium dense. The long

(8-9cm),
 30°.
 26
 8,78g 37,5×19,5mm.
 91,19%
 (19-20mm),
 0,78g 21,5×8,2mm.
 10
 18 24
 (5-6cm),

shoots are thick, light green, often with anthocyanin coloration on the sunny side, quadrated to the top, with pubescence. The internodes are medium long to long (8-9cm) with slightly protruding nodes. The leaf buds on them are peeled off from the shoot at an angle of 30°. As the length of the twig shortens, the leaf buds are increasingly more adherent to the twig. The flower buds are large, with a shape close to globular, with a small tip and contain most often between 22 and 26 flowers.

The fruits ripen in the first ten days of September. They are very large, elongated pear-shaped to bottle-shaped, with an average weight of 8.78g and dimensions 37.5×19.5mm. Fruit skin is a dark red, thin to medium thick, fragile, semi-detachable from the fruit flesh. The flesh and tender, juicy, dark red, with a fresh sour taste, dark red juice and 91.19% yield. The fruit stalk is a medium long (19-20mm), light green, with fine pubescence. The stone is medium-sized, with a mass of 0.78g and dimensions 21.5×8.2mm. It is light brown, with sharp ends, semi-detachable.

The fruits ripen for 10 days and are harvested on 2-3 hands (stages). The fruit drop is weak. The flowering is abundant and prolonged. Fruits regularly and plentifully. It is very rare to be attacked by diseases and pests.

'Zhalt Hadzhiyski'

The tree has weak to moderate growth. The canopy is dense, with the semi-upright to spreading habit. The long shoots are thin, light green, often with a violet tint and pubescence. The internodes are short to medium long (5-6cm) with slightly protruding nodes. The leaf buds are peeled off and on the short twigs are adherent. The flower buds are small, almost globular in shape, slightly pointed and contain between 18 and 24 flowers.

			-	The fruits ripen very early in the second half of July. They are small, elongated, with an average weight of 3.64g and dimensions 21×17mm. Fruit skin is light yellow, thin, fragile, semi-detachable from the fruit flesh. The flesh is very tender, juicy, light yellow, with pleasant slightly sour taste and low 89.65% fruit flesh to stone (f/s) ratio. The fruit stalk is short (10-11mm), thin, yellow green. The stone is small, narrow, with an average weight of 0.38g and dimensions 13.5×6.1mm. It is light brown, semi-detached from the fruit flesh.
			-	Fruits ripen together and can be picked at the same time. Fruit drop is weak. The blooming is early, but overlaps with other cultivars. The variety is small-fruited and with lower yields than other cultivars. Fruits are not resilient to transport, manipulation and storage. Recommended only for enthusiasts.
				'Vratsa - Kastel'
			-	The tree has moderate to strong vigour. The canopy is medium dense, with oval-globular to wide globular shape. The long shoots are thick, green, with anthocyanin colouring, with pubescence. The internodes are long (7-8cm), with medium protruding nodes. The leaf buds on them and on the short twigs are adherent. The flower buds are very large, almost globular, with a small tip and contain between 22 and 26 flowers.
			-	The fruits ripen relatively simultaneously at the end of August. They are very large – average weight 9.46g with size of 34.5×22.5mm. The fruit shape varies even on fruits from the same tree from close to pear-shaped to almost cylindrical. The fruit skin is dark red, shiny, thin, detachable from the fruit flesh. The flesh is dark red, tender, juicy, with weak, typical aroma, refreshing, slightly sour taste and highest f/s ratio – 94.13%. The fruit stalk is a medium long (12.5 mm), yellow green, with fine pubescence.
89,65%. 11mm),	3,64g	21×17mm.	(10-	
0,38g		13,5×6,1mm.		
8cm),			(7-	
22 26				
–				
34,5×22,5mm.	9,46g			
– 94,13%. (12.5mm),				

17,4x8,1mm. 0,56g

20

(8-9cm),

22 26

7,14g

27,5x19,5mm.

91,05%.

- 14mm,

- 0,64g 17,4x7,9mm.

The stone is small, with an average weight of 0.56g and dimensions 17.4x8.1mm. It has a fusiform, creamy light brown, semi-detachable from the flesh.

The yielding of this form is regular and heavy. The fruits can be harvested at the same time. Can be stored in controlled conditions to up to 20 days. 'Vratsa-Kastel' has the largest fruits and this is the most important characteristic in cornelian cherry. It has the highest fruit flesh to stone (f/s) ratio. It is advised to occupy the highest proportion of the areas in the new cornelian cherry orchards and is the most valuable donor for the selection of new large-fruited cultivars.

'Atkov dryan'

The tree has moderate growth. The canopy is medium dense, globular to spreading. Long shoots are medium thick, light green, with a violet tint, Finely hairy. The internodes are medium long to long (8-9cm), the nodes are slightly to medium protruding. The leaf buds are slightly peeled off, and on the short twigs are adhered to. The flower buds are large, with a close to globular form, with a small tip and contain between 22 and 26 flowers.

The fruits ripen in the second half of August. They are large, with an average weight of 7.14g and measuring 27.5x19.5mm. The shape of the most fruits is cylindrical, but seldom pear-shaped fruits can be found. The fruit skin is bright red, shiny, thin, separating from the flesh. The flesh is tender, juicy, light red, with pleasant sour taste, weak aroma and 91.05% f/s ratio. The fruit stalk is medium long – 14mm, yellow green, medium thick, with fine pubescence. The stone is small – 0.64g and dimensions 17.4x7.9mm. It is light brown, slightly tapered to the base, Semi-detached.

The yielding of the form is regular and good. The fruits ripen almost at the same time. The flowering is abundant. It

				22 26.
22,5×18,3mm		5,33g,		
	89,23%.			
	- 14,5mm,			
15,5×8,3mm.		0,57g		
			(7,5-8cm),	
				24 28

has good resistance to biotic and abiotic stressors.

'Shandryan'

The tree has moderate to strong vigour. The canopy is medium dense, globular to spreading. The long shoots are very thick, light green, with violet tint, with fine pubescence. The internodes are long (9-11cm), the nodes are medium convex. The leaf buds are large, peeled off on the long, and adhering on the shortest twigs. Flower buds are the largest in comparison to the other cultivars and forms, with oval to globular shape and pointed tip. The number of flowers in one inflorescence varies between 22 and 26.

The fruits ripen in the second half of August. They are medium large, average weight 5.33g, dimensions 22.5×18.3mm, barrel-shaped. The fruit skin is dark red, shiny, thin, non-separable from the fruit flesh. The flesh is red, juicy, with a sour taste, weak aroma and with a yield of 89.23%. The fruit stalk is a medium long - 14.5mm, yellow green, medium thick, with fine pubescence. The stone has an average weight of 0.57g and dimensions 15.5×8.3mm. It is light brown, with oval-elongated shape, semi-detachable.

It gives good yield, but the fruits are not large enough. The ripening is simultaneous. It is rarely and slightly damaged by biotic and abiotic stressors.

'Tsarigradski zhalt'

The tree has moderate growth. The canopy is medium dense, oval to globular in shape. The long shoots are medium thick, light green, slightly anthocyanin coloured, with pubescence. The internodes are medium long (7.5-8cm), with medium protruding nodes. Leaf buds are adherent to the shoots regardless of their size. Flower buds are large, almost globular, with a small tip and contain most often between 24 and 28 flowers.

15-25
 5,44g 24,5×16,5mm.
 - 91,2%.
 13,5mm,
 0,48g 16,5×6,8mm.
 2g.
 (8-9cm),
 30°.
 22 26.
 10
 8,79g, 37,5×20,5mm

The fruits ripen during the period 15-25 August. They are medium-large, elongated, with an average weight of 5.44g and dimensions 24.5×16.5mm. The fruit skin is yellow, shiny, thin, peeling from the flesh. The flesh is light yellow, translucent, tender, juicy, with a faint aroma and refreshing, pleasant taste. Has high f/s ratio - 91.2%. The juice is light yellow. The fruit stalk is a medium long - 13.5mm, yellow green, with fine pubescence. The stone is small, with an average weight of 0.48g and dimensions 16.5×6.8mm. It is creamy-light brown, fusiform, semi-detachable.

The fruiting is regular and hearty. The ripening is simultaneous and allows the harvest of one hand (stage). The fruits harvested on time can endure relatively well manipulations and transportation. Rarely and weakly damaged by biotic and abiotic stressors. The fruits outweigh those of the cultivar 'Zhalt Hadzhiyski' with 2g.

'Yaltenski'

The tree has moderate to strong growth. The canopy is medium dense, with spreading habit. The long shoots are thick, light green, with anthocyanin colouring, with pubescence. The internodes are medium long (8-9cm) with slightly protruding nodes. The leaf buds on them are peeled off from the shoot at an angle of 30°. With decreasing the length of the twigs, the buds are increasingly adherent. The flower buds are large, with shape similar to globular and with small tip. The number of flowers in one inflorescence varies most often between 22 and 26.

The fruits ripen late, unsimultaneously, with a difference of about 10 days, in the first half of September. They are very large, with an average weight of 8.79g, measuring 37.5×20.5mm and a transient pear-shaped to bottle-shaped form. Fruit skin is dark red, shiny, thin, semi-detachable

- 90,8%.
 - 17,5mm,
 0,81g,
 21,5×8,2mm,
 20

from the flesh. The latter is red to dark red, tender, juicy with pleasant slightly sour taste and a faint aroma. The juice is dark red and the f/s ratio is high – 90.8%. The fruit stalk is quite long – 17.5mm, medium thick, light green, with pubescence. Often at the end of the stalk, a short fleshy thickening with a conical shape is formed close to the fruit. The stone is medium large with a mass of 0.81g, measuring 21.5×8.2mm, light brown, fusiform, with sharp ends, semi-separable from the fruit flesh.

Fruiting is regular and heavy. Its fruits are the latest to ripen and spreads over a period of time. Can be stored in controlled conditions for up to 20 days. The fruit drop is weak. The flowering is abundant and prolonged. It is rarely damaged by biotic and abiotic stressors.

CONCLUSIONS

The most significant pomological characteristic of the cornelian cherry is the size of the fruit. Superior in this this indicator is the form 'Vratsa - Kastel', which fruits are of average weight 9.46g, as individual fruits reach 14g. Very large are the fruits of the cultivar 'Shumenski prodalgovat' and the form 'Yaltenski'. Large are also the fruits of the most common cultivars 'Kazanlashki krushoviden' and 'Pancharevski tsilindrichen', as well as those of the form of the 'Atkov dryan'. The fruits of the forms 'Tsarigradski zhalt' and 'Shandryan' are of medium size, as the ones often found in the natural habitas. The smallest are the fruits of the cultivar 'Zhalt Hadzhiyski', similar to the fruit size of most forms naturally found in forests.

We recommend that in the structure of the new cornelian cherry orchards, the form 'Vratsa - Kastel' to be planted with the highest share, together with the cultivar 'Shumenski prodalgovat' or the form 'Yaltenski'. The growing of 'Kazanlashki krushoviden' and 'Pancharevski tsilindrichen' is also

2g

justified. From the yellow-coloured cultivars and forms, the form 'Tsarigradski zhalt' deserves more attention since its fruits are 2g larger than the cultivar 'Zhalt Hadzhiyski'.

For breeding new cornelian cherry cultivars, we recommended to include in the hybridisation scheme mainly the form 'Vratsa-Kastel', combined with the cultivars 'Shumenski prodalgovat', 'Pancharevski tsilindrichen' and the form 'Yaltenski'. For breeding of yellow-coloured large-fruited forms, it is preferable to use the form 'Tsarigradski zhalt'.

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Chemical Composition of Peaches Fertilized with Different Levels of Organic Products under the Conditions of Integrated Fruit Production

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SUMMARY

2015-2017 .
:
(0,6kg; 1,2kg 1,8kg/tree),
(0.5L 1,0L/da);
120 ml 150ml/da)
Brix, %

- In the period 2015-2017, the effect
- of different fertilization rates of organic
products on the chemical content in the
fruits of 'Glohaven' cultivar was studied in
a fruit-bearing peach orchard on the
territory of the Fruit-Growing Institute of
Plovdiv. Three variants of fertilization
- were investigated with increasing doses:
- soil nutrition with Biohumus (0,6 kg, 1,2 kg
and 1,8 kg/tree), soil nutrition with Agrifull
applied as water solution (0,5 L and 1,0
L/da); foliar nutrition with Humustim (100
ml, 120 ml and 150 ml/da) and untreated
- control. More pronounced differences
- were reported between the separate
fertilization rates rather than between the
bioproducts used. The increase of the
fertilization rate of the applied organic
products resulted in an increase of the dry
matter content by Brix and the percentage
and content of total sugars. The dry
- matter content by Brix was in the range of

10,0% 14,0%.
13,7%; 13,1% 14,0%

Brix -

1L/da – 13,8%.
, 5%
9,4 %
1L/da .
2,7
- ,
-
08% 7,14% 7,
.
(, kg)
-
:
, ,

10.0% to 14.0%. The best results (13,7%; 13,1% and 14,0%) were established in the variants treated with Humustim at all the applied rates and those with Agrifull at the rate of 1 L/da – 13,8%. Sugar content ranged from 5% in the untreated control to 9.4% in the variant with the application of 1 L/da of Agrifull. Sucrose values were about 2,7 times higher on average than the values of inverted sugar. The best sucrose values of 7,14% and 7,08% were established in the variant with fertilization with Agrifull at the two studied rates.

The average fruit weight was mainly influenced by tree loading (yield, kg) and by the meteorological conditions – air temperature and rainfall, and not so much by the different fertilization rates of the applied bioproducts.

Key words: peaches, bioproducts, fertilization, fruit quality

(*Prunus persica* L.)

(Génard et al., 1991).

INTRODUCTION

Peach fruits (*Prunus persica* L.) are highly valued for fresh consumption because of their attractive colour and taste. They are also a good source of sugars, vitamins and minerals, and that is why good management practices should be sought, leading to the production of top-quality fruit with a high market value. There are many factors influencing fruit quality – the cultivar, tree age, pruning, fruit thinning, soil and climatic conditions and agro-technical approaches applied (Génard et al., 1991).

Total sugars, organic acids and amino acids are natural components of the fruit and they play an important role for fruit quality and their nutritional value. The concentration of those ingredients is of scientific interest due to their effect on the organoleptic properties of fruits and hence on the market quality of production (Wrolstad, 1981; Ashoor and Knox, 1982).

(Wrolstad, 1981; Ashoor and Knox, 1982).
 ()
 () (Souty
 and André, 1975; Génard and Souty, 1996).

Peach flavour depends on the ratio of
 - sugars (sucrose, glucose and fructose)
 - and organic acids (malate and citrate),
 (Souty and André, 1975; Génard and
 Souty, 1996).

There is a necessity to update
 - some technological elements related to
 - fertilization for the protection of soil fertility
 - and the improvement of the fruit nutritional
 - value. Ecological solutions are sought for
 - maintaining the plant nutritional regime,
 - the preservation of soil fertility and the
 - production of healthy foods (Stoynev,
 2004). Despite the growing interest in
 organic products, the knowledge of how
 different levels of fertilization affect
 nutritionally relevant components is still
 limited. Roth et al. (2005, 2007) found out
 that there are no differences in flavour,
 volatile substances and other quality
 characteristics of apple fruits from three
 different regions of Belgium in
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The aim of the present study was to
 estimate the effect of different fertilization
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 integrated fruit production.

MATERIAL AND METHODS

The experimental work was carried
 out on the territory of the Fruit-Growing
 Institute in Plovdiv in the period 2015-
 2017 in a fruit-bearing peach plantation of
 'Glohaven' cultivar grafted on the
 vegetative rootstock GF 677. The soil is
 alluvial-meadow with a neutral reaction,
 pH of 7.10, with a good phosphorus
 content of 22 mg/100 g and potassium
 content of 26 mg/100 g of soil. The
 following fertilization variants were
 studied: soil enrichment with Biohumus;
 soil nutrition with water solution of Agrifull;
 leaf-feeding with Humustim; untreated

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 content of 26 mg/100 g of soil. The
 following fertilization variants were
 studied: soil enrichment with Biohumus;
 soil nutrition with water solution of Agrifull;
 leaf-feeding with Humustim; untreated

2015 2016 ;
 0,600kg; 1,200kg 1,800 kg :
 , 0,5 1L/da 100,
 120 150 ml/da. 2017 .
 100%.
 15-20
 Brix; - (%)
 (%)- ;
 (pH) - ;
 (mg) ;
 g 30
 (Koumanov, 2011).

control, without soil and leaf nutrition. In 2015 and 2016 the fertilization rates of the applied bio-products were: Biohumus – 0,600 kg; 1,200 kg and 1,800 kg per tree; Agrifull – 0,5 and 1 L/da and Humustim – 100, 120 and 150 ml/da. In 2017 the applied rates were increased by 100%. Each variant was in three replications. Treatment with the bio-products was carried out five times during the vegetation period, every 15-20 days from April to July inclusive.

The average samples for the chemical analyses were collected randomly at the time of consumption maturity of the fruit, by fertilization variants. The following characteristics were studied: dry matter content – Brix refractometrically; sugars – by Shoorl-Regenbogen; acid content – titrimetrically; active acidity (pH) – potentiometrically; and vitamin C (mg) – by the method of Tilmes. The weight of 30 peaches was measured in g by variants of fertilization (Koumanov, 2011).

RESULTS AND DISCUSSION

Data obtained on the chemical composition of peach fruits are presented in Table 1.

More pronounced differences in the studied characteristics were observed between the different fertilization rates than between the type of the organic products used. The dry matter content (Brix, %), and the content of total sugars increased with the increase in the fertilization level of the imported organic products. The introduction of a 100% higher fertilization rate of all the organic products in 2017 had a negative impact on the sugar content of the fruits of 'Glohaven' cultivar.

The meteorological conditions during the three years of the study had an effect on the chemical composition. Rainfall data and average daily temperatures during the vegetation period are presented by years in Figure 1.

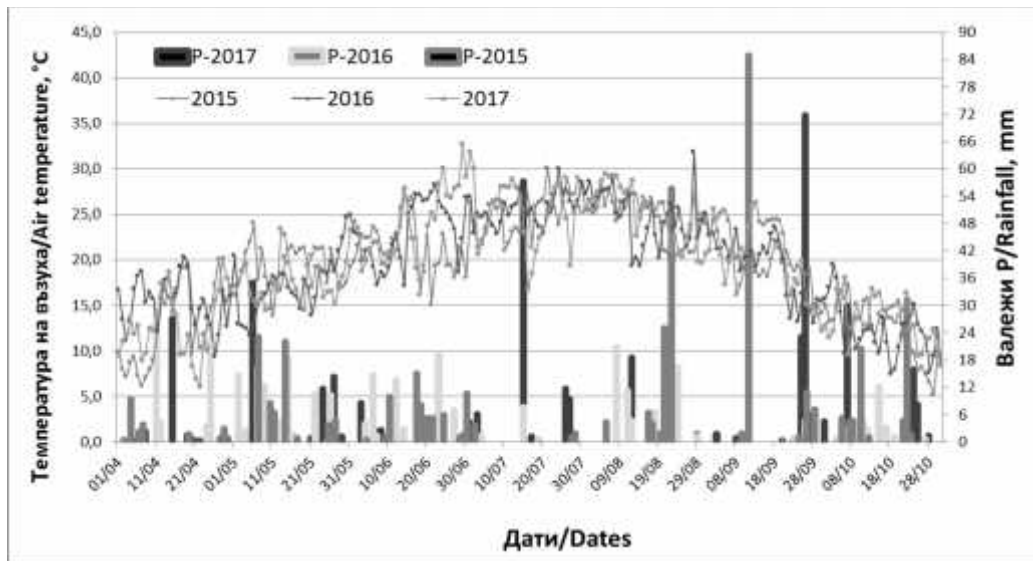
1.
 Brix, %
 100% -
 2017 .
 1.

1.

2015-2017 .

Table 1. Biochemical composition of peach fruits of 'Glohaven' cultivar at different fertilization levels of organic products for the period 2015-2017

Варианти Variants / Rate	Bio-hummus			Agrifull		Humustim			Control 0
	0.6 kg/tree	1.2 kg/tree	1.8 kg/tree	0.5 L/da	1.0 L/da	100 ml/da	120 ml/da	150 ml/da	
2015									
Brix, %	10	10,2	10,6	10	9,8	10,2	11	11,5	10,1
Общи захари Total sugars, %	8	8,56	8,56	8	7,64	8,34	7,78	8,34	5
Инвертна захар Inverted sugar, %	2,6	2,14	2,28	2,4	2,4	1,88	1,8	2,6	2,44
Захароза Sucrose, %	5,53	6,1	5,97	5,32	4,98	6,14	5,68	5,46	2,97
Киселини Total acids, %	0,65	0,62	0,65	0,65	0,65	0,65	0,6	0,64	0,62
pH	3,62	3,65	3,5	3,71	3,57	3,65	3,55	3,59	3,57
Acidimetric coefficient	12,31	13,81	13,17	12,31	11,75	12,83	12,97	13,03	8,06
Витамин С Vitamin C	11,11	10,98	10,86	11,50	11,10	11,76	10,72	10,86	10,13
2016									
Brix, %	12,3	12,6	13	12,2	13,8	13,7	13,1	14	11,3
Общи захари Total sugars, %	7,3	8,56	8,98	9,26	9,4	5,12	6,74	7,3	5,28
Инвертна захар Inverted sugar, %	0,96	1,8	2,88	1,74	1,94	1,68	1,42	2,88	1,48
Захароза Sucrose, %	7,22	5,225	5,795	7,144	7,087	3,268	5,054	4,199	6,46
Киселини Total acids, %	0,571	0,654	0,621	0,522	0,489	0,604	0,621	0,621	0,610
pH	3,7	3,64	3,68	3,79	3,81	3,79	3,62	3,69	3,68
Acidimetric coefficient	14,98	11,17	14,47	17,73	19,21	8,47	10,86	11,76	8,66
2017									
Дози Rate	1.2 kg/tree	2.4 kg/tree	3.6 kg/tree	1.0 L/da	2.0 L/da	200 ml/da	240 ml/da	300 ml/da	0
Brix, %	12,5	10,8	11,4	10,6	10,7	11,6	10,1	11,9	11,2
Общи захари Total sugars, %	6,82	7,22	7,92	5,86	7,36	7,08	5,6	5,12	5,03
Инвертна захар Inverted sugar, %	1,48	1,48	1,68	1,48	1,48	2	1,14	1,48	1,47
Захароза Sucrose, %	5,073	5,453	5,928	4,161	5,586	4,826	4,237	3,458	3,63
Киселини Total acids, %	0,517	0,435	0,550	0,599	0,468	0,649	0,419	0,517	0,534
pH	3,55	3,5	3,41	3,36	3,47	3,57	3,66	3,57	3,52
Acidimetric coefficient	13,18	16,59	14,40	9,78	15,73	10,91	13,37	9,90	9,42



1. 2015, 2016 2017 .
Fig. 1. Rainfall and course of the average daily temperature during the vegetation period of 2015, 2016 and 2017

Brix
 10,0% 14,0%.
 (100, 120 150 ml/da),
 13,1% 14,0%,
 1L/da – 13,8%.
 2016 ..
 296 mm,
 34 % (1).
 5%
 9,4 %
 1L/da
 0,6kg 1,8kg/tree

The soluble dry matter content (Brix) varied from 10,0% to 14,00%. The best results were established in the variants treated with Humustim at the three rates (100, 120 and 150 ml/da), the values being 13,7%; 13,1% and 14,0%, respectively, as well as those with Agrifull at a rate of 1 L/da – 13,8%. Applying increasing levels of Biohumus also resulted in an increase in the values of the soluble dry matter. The highest values were established in 2016, when the most favourable for peach climatic characteristics were reported. The rainfall during the vegetation period was 296 mm and the year was characterized as medium-wet with a probability level of 34% (Figure 1).
 The total sugar content in fruit flesh of the treated trees was higher than the control. The sugar content varied from 5% in the control to 9,4% in the variant fertilized with 1 L/da Agrifull.
 The increase of the Biohumus rate from 0,6 kg to 1,8 kg per tree resulted in an increase in total sugars by 1.68%. Total

	1,68%.		
	0,5	1,0L/da,	-
			0,17%.
			-
		2017 .	-
			-
	5,12%	7,92%.	-
	-		,
			-
		(2).	-
			.
	2,7	-	,
			.
2016 .			.
			.
	: 7,14%	7, 08%	-
			.
		7%,	
		3,2%	5,9 %.
			,
			,
	11,76% vit. C		-
2015 .	1,63%	100 ml/da	
		vit. C	.
			.
		vit. C. (1)	-
	0,43%,	0,65%	

sugars increased by 0,17% with the increase of Agrifull rate from 0,5 to 1,0 L/da. When Humustim was applied at increasing levels, there was no such pronounced tendency to an increase in total sugars.

In 2017 there was a decrease in the total sugar content in all the treated variants. The values were about one unit lower than the previous two years and ranged within 5,12% to 7,92%.

Increasing fertilization levels led to a higher average yield, but fruit quality was worse. The fruits obtained had a lower mean weight (Table 2).

Sucrose is the dominant sugar in peach fruits. They are characterized by high values of that component, which is about 2,7 times higher than inverted sugars. The highest results were obtained in 2016 when applying Biohumus and Agrifull at the studied rates. The variants treated with Agrifull at the two experimental rates had the highest sucrose content: 7,14% and 7,08%, respectively. The sucrose content was over 7%, while in the other variants treated with Biohumus and Humustim, the sucrose content ranged from 3,2% to 5,9%.

Vitamin C content was higher in the treated trees compared to the control. The highest value of vitamin C content (11,76%) was established in the variant with the application of Humustim at the rate of 100 ml/da in 2015, which was by 1,63% more than the content of vitamin C in the fruits of the control variant. No significant increase in the vitamin C content was observed after increasing the fertilization levels (Table 1).

The content of acids is not significantly affected by the fertilizers applied. Acids were in the range of 0,43% to 0,65% in the different variants of fertilization. The acid content was slightly

2017 .
 -
 ,
 ,
 ,
 .
 -
 9,78 19,21.
 -
 ,
 .
 -
 -
 ,
 .
 (2).
 -
 2016 ., 2016 .
 ,
 ,
 ,
 ,
 200g.
 ,
 .
 2017 .
 .
 :
 20.9° 15%,
 41.2° 6%.
 330.5 mm,
 35-72 mm,
 ,
 .

lower in 2017.

The sugar-acid ratio determines fruit taste and it gives information about the balance between total sugars and acids in the fruit.

In the variants studied, the sugar-acid ratio ranged from 9,78 to 19,21. The results varied throughout the years of the experiment, but the values were optimal in all the variants. Over the three years of the study, the control plants had a lower sugar-acid ratio in comparison to the variants treated with organic products.

Fertilization with bio-products did not significantly affect fruit weight (Table 2). The highest average fruit weight was obtained in 2016. The medium-wet 2016 along with the frequent rainfalls in May, which in the first half of the month were even daily, were favourable for obtaining fruits with an average weight of over 200 g.

Typical of that characteristic is the fact that the control variant and the other variants in which different fertilization rates were applied, did not differ significantly between each other.

The different meteorological conditions exerted a substantial effect. In 2017 a decrease in the average fruit diameter was observed in all the variants. It should be noted that the year was hot: the average day and night temperature was 20,9 °C with 15% probability level and the maximum temperature reached 41,2 ° with 6% probability level. The rainfall sum in the vegetation period from April till September was 330,5 mm, which characterized the year as medium wet, but the precipitation sum was mainly formed by four rainfalls of 35-72 mm, distributed in April, May, August and late September, respectively.

2. g 2015, 2016 2017 .
Table 2. Average fruit weight in g, in 2015, 2016 and 2017

Variants	Doses	a Average fruit weight, g		
		2015	2016	2017
Bio-hummus	0.6kg/tree(1,2kg/tree)	156	222	163
	1.2kg/tree(2.4kg/tree)	144	177	166
	1.8kg/tree(3.6kg/tree)	173	187	150
Agrifull	0.5L/da (1.0L/da)	204	244	179
	1.0L/da (2.0L/da)	185	234	177
Humustim	100ml/da (200ml/da)	169	220	146
	120ml/da (240ml/da)	166	180	147
	150ml/da (300ml/da)	178	200	170
Control	untreated	207	223	128

2015 .
 372 mm,
 16 %.
 22.08 11.09
 63 %,
 :
 13.5%.
 20.6°
 35°
 (1).

In 2015 the rainfall sum during the vegetation period was 372 mm, the year was characterized as wet with 16% probability level. However, if the two major rainfalls on 22 August and 11 September are excluded due to the low efficiency of absorption, the precipitation was 63%, which characterized the year as medium wet. The year was hot: the average day and night temperature was 20,6 °C with 13.5% probability level. Maximum temperatures were over 35 °C, mainly during the second and third decades of August (Figure 1).

CONCLUSIONS

Peach fruits are characterized by a rich chemical composition. Sucrose is the dominant sugar in the fruit. Sucrose values are about 2,7 times higher on average compared to inverted sugars. With the application of Biohumus, Agrifull and Humustim at increasing fertilization levels, the content of dry matter and total sugars in the fruits of 'Glohaven' cultivar increased. Applied fertilization with organic products resulted in higher values of the sugar-acid coefficient, which determines the better flavour qualities of the peach trees, compared to the untreated control. The applied organic products did not significantly affect the acid content and pH of the fruit flesh.

pH

–

The average fruit weight was mainly influenced by the meteorological conditions – air temperatures and rainfalls, and less by the different fertilization levels of the applied organic products.

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a

(*Pyrus communis* L.)

*,
,
,
12, 4004 ,

Improvement of Fire Blight Resistance of Pear (*Pyrus communis* L.) by Treatment with the Regoplant Biostimulator

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SUMMARY

Burrill) (Erwinia amylovora,

(Rosaceae).

(Agrobiotech, Ukraina).

Fire blight (*Erwinia amylovora*, Burrill) is among the most destructive diseases registered in Bulgaria and worldwide and affects a wide range of hosts of the *Rosaceae* family. At the favorable conditions there is a rapid spread, affecting all plant parts.

The chemical control against the bacteria is particularly difficult and includes only preventive action. The aim of the study was to investigate the possibility to improve the resistance of pear plants to the fire blight by increasing the natural plant immunity.

Regoplant (Agrobiotech, Ukraina) is a new generation plant biostimulator whose action is based on synergic effect of products of biotechnological cultivation of fungi-micromycetes from root system of ginseng and Aversectine - biological product with antiparasitic action. Pear

“ ” (*Pyrus communis* L. ‘Packham’s Triumph’),
 (100µl l⁻¹)
Erwinia amylovora,
 Burrill.
 3,
 5
 (18,46%),
 2 (32.85%) (34.66%).
 : *Erwinia amylovora*,

plants (*Pyrus communis* L. ‘Packham’s Triumph’) treated with Regoplant (100µl l⁻¹) before and after inoculation with the bacterial suspension were used in the investigation.

As a control the same plants without Regoplant treatment were used. Best results were obtained in variant 3, in which the plants were treated with Regoplant, and 5 days later the artificial inoculation was done. In this variant, the lowest leaf attack index (18.46%) was recorded, followed by variant 2 (32.85%) and the control (34.66%).

Key words: *Erwinia amylovora*, bacterial diseases, artificial inoculation, biostimulators

Erwinia amylovora Burrill,
 (*Malus domestica communis*)
 1989
 (Bobev et al., 1999).
 2003-2007 .
 (Bobev et al., 2007).
 (*Pyrus communis* L. ‘Packham’s Triumph’) 1963 ,
 (*Venturia pyrina* (Bref.) Aderhold),
 (Iliev et al., 1984).

INTRODUCTION

Erwinia amylovora Burrill is the causative agent of fire blight, a bacterial disease existing as an unsolved problem in most countries where pome fruits like apple (*Malus domestica*) and pear (*Pyrus communis*) or ornamental plants of *Rosaceae* are grown.

The disease was established for the first time in Bulgaria in 1989 on a pear and still no chemical control (Bobev et al., 1999). During the period 2003-2007 the disease is enlarged area all over the country and causes significant damages to many pear, quince and apple orchards (Bobev et al., 2007).

The pear variety ‘Packham’s Triumph’ (*Pyrus communis* L.) was introduced in Bulgaria in 1963, until now the variety is a basic and standard grown in the country. It is cold resistant, scab sensitive (*Venturia pyrina* (Bref.) Aderhold) and medium sensitive to fire blight (Iliev et al., 1984).

In our previous studies, the reaction of the variety after artificial inoculation with

Erwinia amylovora (Alexandrova and Dzhuvinov, 2017). The plants grafted on a Ba29 quince rootstock have shown a susceptible to fire blight 10th days after the artificial inoculation a class 3 of infection (23.53%) was reported where necrosis reached to the leaf handle. The plants grafted on OHF333 pear rootstock, a degree of attack class 2 (25%) have reported by symptoms of infection only on the cut of site and nerve of a leaf.

Regoplant (Agrobiotech, Ukraine, <http://www.agrobiotech.com.ua>) is a new generation plant biostimulator whose action is based on synergic effect of products of biotechnological cultivation of fungi-micromycetes from root system of ginseng and Aversectine - biological product with antiparasitic action.

According to the results of n m renk et al. (2012), Regoplant increases the resistance of corn and soybean to *Fusarium*. Koshevskij et al. (2015) reported a positive effect on the soybean yield and reduced the attack of fungal diseases after treatment with Regoplant.

Applied at a concentration of 50 µl l⁻¹, it stimulated growth and improved the acclimatization of micropropagated OHF 333 pear plants (Dimitrova et al., 2017).

The aim of the study was to investigate the possibility to improve the resistance of pear plants to the fire blight by natural biostimulator Regoplant.

MATERIAL AND METHODS

The research was conducted during the period April and May 2016 in the Laboratory of Phytopathology in Department of Agrotechnics and Plant Protection at the Fruit Growing Institute in Plovdiv.

As a plant material was used the micropropagated pear plants (*Pyrus*

(*Pyrus communis* L. 'Packham's Triumph').

15-20 cm

➤ 3325 - 16.05.2013 ;
➤ 3345 - 27.06.2013 .

26°
King's B (King et al., 1954),

$3 \cdot 10^8$ /ml.

1/3

(100µl l⁻¹)

1 _____ 3 - ;
2 _____ - ;
3 _____ 5 - .

25° 80-90%

5 .
10

5 Zeller
and Wolf (1996),

0 - ;

communis L. 'Packham's Triumph'). The plants were grown in a greenhouse with actively growing shoots of 15-20 cm in length.

Artificial inoculation of two isolates suspension was used:

➤ Ea 3325 - isolated from apple on 16.05.2013 in Petrich (Bulgaria);
➤ Ea 3345 - isolated from pear on 27.06.2013 in Botevgrad (Bulgaria).

The bacteria were cultured for 48 hours at 26°C on King's B media (King et al., 1954), which they were washed off the agar surface and the suspension was adjusted to a density of 3×10^8 cells/ml.

The artificial inoculation was made by cutting 1/3 of the top three leaves with scissors dipped in the bacterial suspension.

Regoplant treatment (100µl l⁻¹) was done in 3 ways:

1st variant - inoculation with the bacterial suspension, after 20 minutes treatment with Regoplant;

2nd variant - treatment with Regoplant, followed by inoculation with the bacterial suspension;

3th variant - treatment with Regoplant, after 5 days inoculation with the bacterial suspension.

As a control, artificially inoculated plants without Regoplant treatment were used. All plants were placed in controlled-environment chamber at optimal conditions for the development of the bacteria - temperature 25°C and 80-90% relative humidity. Five plants were tested for each variant and control. The results were recorded 10 days after artificial inoculation.

Reaction of plants were classified by the grading scale of disease severity of Zeller and Wolf (1996):

Class 0 - infected plants are no visible symptoms of infection;

- 1 - ;
- 2 - ;
- 3 - ;
- 4 - e ;
- 5 - ;

Class 1 - blackening only in place of the cut;
 Class 2 - visible symptoms of the place of cut and stem;
 Class 3 - browning of leaf and petiole;
 Class 4 - from symptoms necrosis on apices of the plant;
 Class 5 - blackening the whole plant.

To determine the disease attack, the results were transformed by the Mc Kiney formula as follows:

$$I = \frac{E(n.k).100}{N.K}$$

I - (%)
 E(n.k) - (n) ()
 N - ()
 K - ()

I - percentage Index of attack (%)
 E (n.k) - sum of the works of the number of attacked plants or organs (n) in the corresponding attack ball (k)
 N - the total number of plants examined (organs)
 K - the highest grade in the corresponding scale

RESULTS AND DISCUSSION

The first symptoms of infection were noticed on the 5th day in most inoculated plants.

In control plants not treated with Regoplant, the highest number of inoculated leaves (53%) showed class 1 infection (necrosis only in place of the cut). No leaves with the lowest and highest response degree rates from the scale used were counted.

Progressive infection with symptoms of necrosis at the shoot apex (class 4) was only observed in control plants (7%).

In the first variant of treatment (artificial inoculation followed by treatment with Regoplant), the effect of the existing contamination was monitored. The results

5
 k
 (53%)
 1
 e
 (4)
 (7%).
 ()

44%
 -
 3 (37%).
 0
 4

obtained showed that 44% of the treated leaves reacted with class 1 infection, the highest response rate was Grade 3 of infection (37%). No leaves without visible symptoms of Infection (0 class) was counted. In this variant, there was no class 4 infection, in contrast to the control.

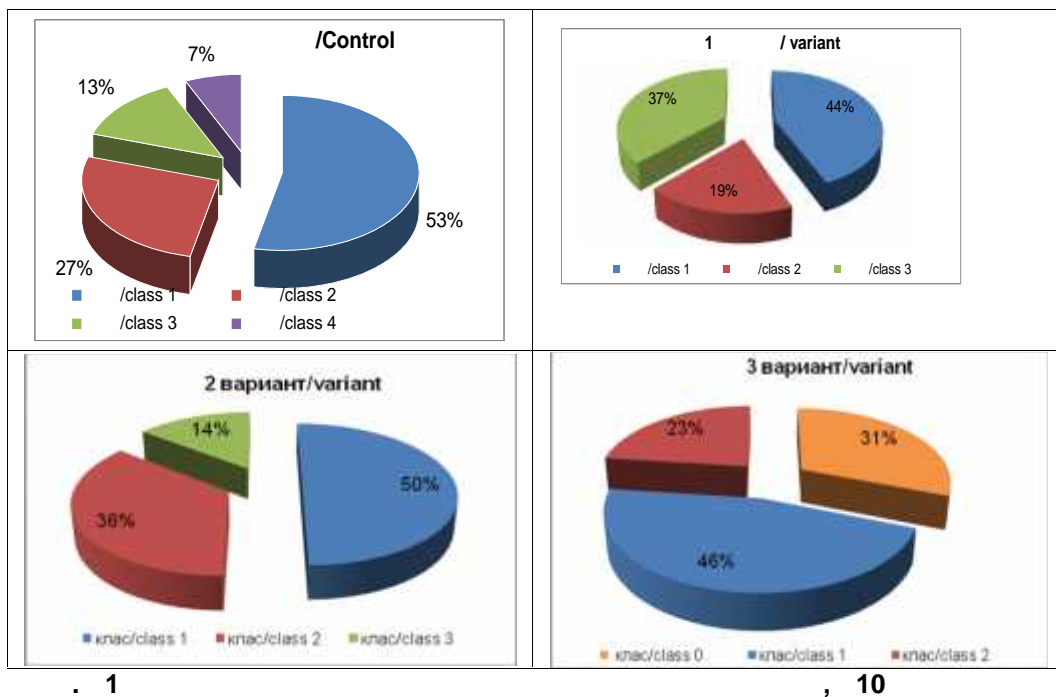


Fig. 1 Reaction of the pear variety Packham's Triumph, 10 days after artificial inoculation in three variants study and control

(/)
 -
 1 (50%) (1).
 3 ()
 14%

In a second variation of the study (Ragoplant treatment after that artificial inoculation), the inoculated plants were distributed into three reaction classes, with the highest number of leaves (50%) responding to Class 1 of infection (Figure 1). Class 3 of the reaction (necrosis reached the leaf handle) was reported in 14% of the artificially inoculated leaves.



Fig. 2. Packham's Triumph, reacted with 3 class of susceptibility, 10 days after infection



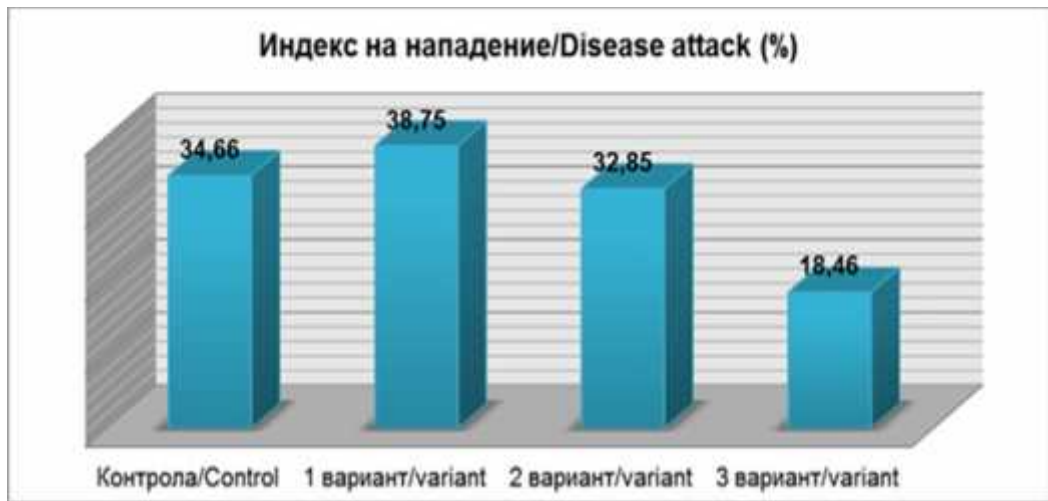
Fig. 3. Packham's Triumph, reacted with 2 class of susceptibility, 10 days after infection



Fig. 4. Packham's Triumph, reacted with 0 class of susceptibility, 10 days after infection

3 (/) 31% (0), - (46%) 1 (1). , 2 (23%).

In variant 3 (treatment with Regoplant/artificial inoculation after 5 days), 31% of the inoculated leaves were free of visible symptoms of infection (class 0), which was lacking in the control. This has confirmed our hypothesis about the stimulating effect of Regoplant on plants to overcome bacterial infection. Again, the highest number of inoculated leaves (46%) responded to class 1 infection (Figure 1). The highest infection rate reported in this variant was Grade 2 of infection (23% of the leaves).



. 5.

Erwinia amylovora

Fig. 5. Disease attack of the pear variety Packham's Triumph to the causative agent of *Erwinia amylovora* in the three studied variants

(5). - -
 1, -
 - 38,75%,
 (34,66%) -
 2 (32,85%). -
 (18,46%),
Erwinia
amylovora 5
 .
 .
 (Brisset et al., 2000;
 Baysal Zeller, 2004).

The disease attack depended on Regoplant treatment method (Figure 5). The most pronounced symptoms of the disease showed variant 1, in which reported a maximum diseases attack of 38.75%, followed by the control (34.66%) and the variant 2 (32.85%). The best results were obtained in the third variant (18.66%), in which the inoculation with *Erwinia* suspension was done 5 days after treatment with Regoplant.

We assume that during this period, Regoplant has stimulated the plant's resistance and thus the spread of the infection is limited.

Induction of resistance by extracts of a number of plant species against a wide spectrum of pathogens has already been demonstrated (Brisset et al., 2000; Baysal and Zeller, 2004). For the development of resistance, plants need some time before being challenged with a pathogen. Further studies are needed to determine optimal concentrations and modes of treatment with Regoplant, as

well as the phenophases in which it is best applied.

CONCLUSIONS

The highest class of infection was reported only in control plants (class 4) that had not been treated with Regoplant.

Only in variant 3, ten days after inoculation some of plants showed no symptoms of *Erwinia* infection. In this variant, the lowest diseases attack was also reported - 18.66%.

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(*Cydalima perspectalis* Walker) –

, 4000 ,

Box Tree Moth (*Cydalima perspectalis* Walker) – Potentially Dangerous Pest by Boxwood and in Our Country

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SUMMARY

- Climate change in the past years,
- free trade and permanent imports of fruits,
- vegetables and ornamental plants to meet
- the needs of the market, create a
- prerequisite for porting new insect pest.
- Exactly such species is box tree moth
- (*Cydalima perspectalis* Walker). He
- applied serious damage over boxwood,
- which is used as a result of landscaping in
- the parks and gardens in Bulgaria to
- which shrubs are deforested completely
- and its decoration is disturbed. Given on
- this arises the need of the search for
- means of control, that can be used to
- combat this species in practice.

With establishment damages from
the box tree moth during the period
2016-2017 in the region of Kozloduy,
boxwood observations were made using
standard entomological methods. In
parallel were tested the insecticides in
laboratory conditions: Fastac New 100 EC
(alpha - cypermethrin) and Nurelle D
(chlorpyrifos-ethyl + cypermethrin) against
caterpillars of the box tree moth
(*C.perspectalis*).

2016-2017 .

: 100

(-)
(- +)

(*C. perspectalis*).

(Beshkov et al., 2015).
(Pencheva and Yovkova, 2016).

(*C.perspectalis*)

Buxus microphylla, *B.microphylla* var. *Insularis*, *B.sempervirens*, *. olchica*, *B.sinica*, *B.balearica* (Todeschini, 2014).
Ruscus aculatus L. *Prunus leucocerasus* L.

Cylindrocladium bixicola enr.,
(*C. perspectalis*)

(*C. perspectalis*),
(Choo et al., 1991),
(Kawazu et al., 2007)
(Zhou et al., 2005).

(Nacambo et al., 2014)
(Leuthardt and Baur, 2013).

C. perspectalis

(Maruyama and Shinkaji, 1987; Zhou

in Balchik and Varna) (Beshkov et al., 2015). The species is expanding its range and reaches Dobrich, Golden Sands, Sozopol, Primorsko, Burgas, Kalofer, Plovdiv, Sofia, Dragalevtsi, Gravatikovo and others (Pencheva and Yovkova, 2016).

The caterpillars of the box tree moth (*C.perspectalis*) are fed the leaves of different varieties of boxwood: *Buxus microphylla*, *B.microphylla* var. *Insularis*, *B.sempervirens*, *. olchica*, *B.sinica*, *B.balearica* (Todeschini, 2014). In Russia attacking also *Ruscus aculatus* L. and *Prunus leucocerasus* L. Damaged by box tree moth plants they lag behind in development and are attacked by the fungus *Cylindrocladium bixicola* enr., which causes necrosis, causing the branches to dry.

To limit the spread of the box tree moth (*C.perspectalis*) is essential performing monitoring in nurseries, garden centers for flowers and ornamental plants, on the basis of which restrictive and sanitary measures are applied to localize the species.

Control in East Asia, where it was found box tree moth (*C.perspectalis*), includes biological control with nematodes (Choo et al., 1991), mating interruption between butterflies (Kawazu et al., 2007) and chemical control (Zhou et al., 2005).

Natural enemies box tree moth have multifaceted parasites (Nacambo et al., 2014) and birds, but they exhibit less predation, due to the high levels of toxic alkaloids contained in caterpillars (Leuthardt and Baur, 2013).

Control of box tree moth (*C.perspectalis*) in parks, green belts or nurseries in Japan and China is mainly achieved through the application of insecticides. Synthetic pyrethroids (cypermethrin, deltamethrin) exhibit good efficacy (Maruyama and Shinkaji, 1987;

et al., 2005; Ma et al., 2006; Xi et al., 2009).

perspectalis

(Zhou et al., 2005).

Chrysantemus,

Zhou et al., 2005; Ma et al., 2006; Xi et al., 2009).

Prolonged use of Chlorofluazuron in the province Shandong for control of *C. perspectalis* lead to development of sustainability and because of that recommends the use of spinosad and fipronil (Zhou et al., 2005).

Can be used and insecticides, extracted from the genus *Chrysantemus* mixed with rapeseed oil. Their toxicity is similar to that of synthetic pyrethroids, which is why it is recommended that the application be carried out in the evening to protect natural pollinators and beneficial species.

To increase the effectiveness of the pest control insecticides applied, an adhesive is recommended, as the boxwood leaves are smooth.

The chemical controls against contact insecticides have proven to be very effective, but it can harm the arthropods, that use boxwood plants for shelter.

MATERIAL AND METHODS

The studies were conducted in the period 2016-2017 in the region of Kozloduy. Individual plants were marked, which were reviewed periodically and damage was monitored, applied by the Box tree moth (*C. perspectalis*). In parallel under laboratory conditions in the Agricultural University of Plovdiv tested the insecticides: Fastac New 100 EC (alpha - cypermethrin) with a dose of 3 ml/10 l water and Nurelle D (chloropyrifos-ethyl + cypermethrin) - 7 ml/10 l water.

The experiment was based on three variants and three replicates: Variant I - control (water treatment), Variant II - treatment with Fastac New 100 EC and Variant III - treatment with Nurelle D. In each variant, 20 caterpillars were placed in plastic containers box tree moth (*C. perspectalis*) on fresh boxwood and treated with the above agents. Readings

2016-2017 .

(*C. erspectalis*).

: 100
(-) 3 ml/10 l

+ () - 7 ml/10 l

a : -
(), -
100

20 -
(*C.perspectalis*)

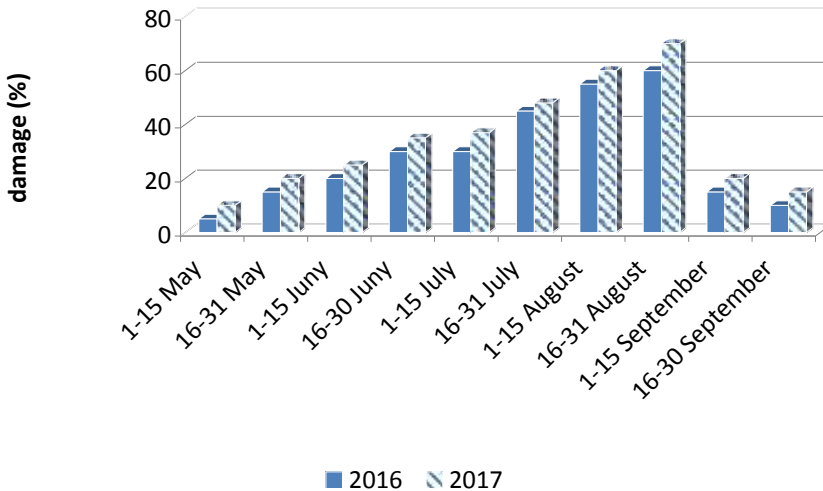
7-
t-
3
(*C. erspectalis*)
2016 .
5 %, (1).
2017 . - 10 %
60 % - 2016 . 70%
2017 .

were made on the 3rd, 5th and 7th day after treatment.

Averages of 3 independent samples were compared with the t-criterion.

RESULTS AND DISCUSSION

The first damage from the caterpillars of the box tree moth (*C. perspectalis*) in 2016 on boxwood shrubs have been revealed at the beginning of May and they reached 5% and in 2017 - 10% (Figure 1). Initially the caterpillars partially bite the leaves, and then completely destroy them, leaving only the handles intact. The attacked plants withered and covered with cobwebs. In mass multiplication, the caterpillars bite the bark of the younger twigs. With the weather warming and as the species multiplied, the damage gradually began to increase. The mass attack on boxwood shrubs for both years was found in the second half of August 60% in 2016 and 70% in 2017



1. (%) (*C. erspectalis*)

Fig. 1. Damaged plants (%) of box tree moth (*C. perspectalis*) in the region of Kozloduy

10 %
 (C. erspectalis)
 (Var.1)
 2).

In the beginning of September, the density of caterpillars began to decrease, with about 10-15% of damaged plants found at the end of the month.

The analysis of data from the treatments performed against the caterpillars of the box tree moth (*C. perspectalis*) show a clear differentiation of the control (Var.1) from the other variants. This is evident from the analysis of variance carried out and the comparison of the mean values (Figure 2).



2.

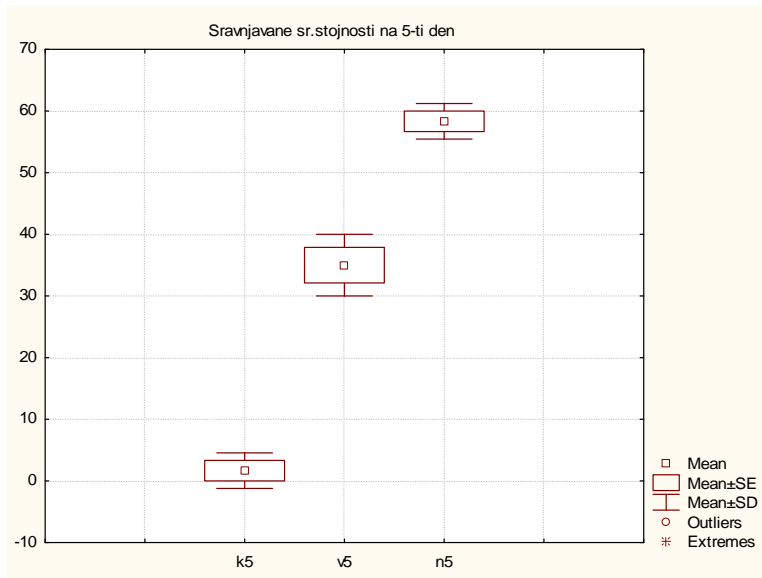
100
 (C. erspectalis)

Fig. 2. Mean values on the third day after treatment with Fastac New 100 EC and Nurelle D compared to the control in the box tree moth (*C.perspectalis*)

3-
 100 (Var. 2)
 10 %, - 20% (Var. 3)
 (2).
)
 -
 35 % (Var. 2),
 58% (3).
 100 (Var. 3) -

When conducted first readings on the 3rd day efficiency of the insecticide Fastac New 100 EC (Var. 2) is 10% and for Nurelle D - 20% (Var. 3) (Figure 2).

In subsequent reports (on five day) efficiency is almost three times higher than day three as at Fastac New 100 EC reached 35% (Var. 2), and at Nurelle D (Var. 3) - 58% (Figure 3).

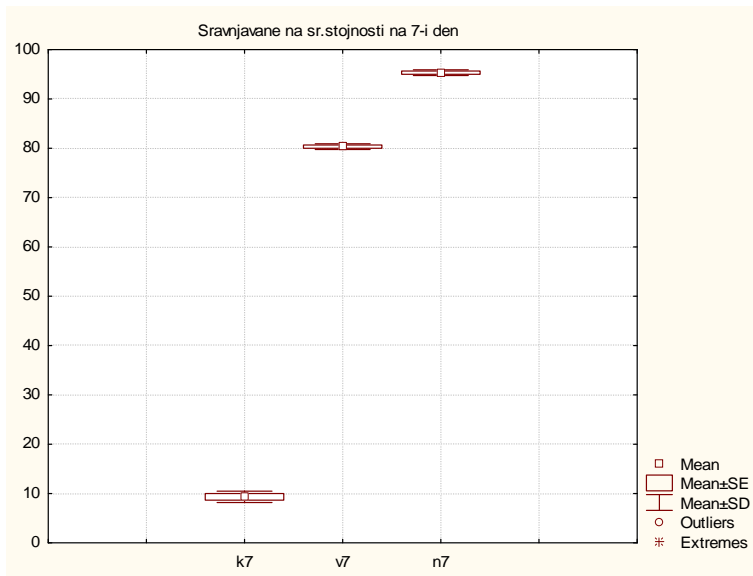


. 3.

100

(*C. erspectalis*)

Fig. 3. Mean values on the fifth day after treatment with Fastac New 100 EC and Nurelle D compared with the control in the box tree moth (*C.perspectalis*)



. 4.

100

(*C. erspectalis*)

Fig. 4. Mean values on the seventh day after treatment with Fastac New 100 EC and Nurelle D compared with the control of the box tree moth (*C.perspectalis*)

- . . 80%

- | On the seventh day and for both insecticides the efficacy is significantly higher 80% at Fastac New 100 EC (Var 100

(Var 2) 95% (Var. 3)
(4).

2) and 95% Nurelle D (Var. 3) (Figure 4).

What is more noticeable is the higher efficiency of Nurelle D, which shows that this insecticide has a good duration of action, which in its application reduces the number of sprays against the enemy. Fastac New 100 EC is a synthetic pyrethroid and short duration is normal.

CONCLUSIONS

As a result of conducted observations can be made the following conclusions:

- During the 2016-2017 observations in the region of Kozloduy, the first caterpillars of the box tree moth (*C.perspectalis*) were detected in May and their multiplication was recorded in August when the damage reached 60-70%.

- Fastac New 100 EC and Nurelle D insecticides have good efficacy against the caterpillars of the box tree moth (*C. perspectalis*) and can be used successfully to control this pest. The Nurelle D insecticide shows a longer duration of action, which ensures the protection of the boxwood by this pest for a long period of time.

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INTRODUCTION

Chemigation has been used to apply a wide variety of chemicals to many crops through different irrigation systems. Pesticides are added to the irrigation water and, depending on their type, the process is defined as herbigation, fungigation, nematogation and fertigation.

Thus, the specific conditions lead to the designation of the specialized applications of the chemigation. The introduction of pesticides through the irrigation waters could be applied to different crops, both in their field growing and for the production of seedlings under different conditions (Sun and Yuan, 2011).

The studies in this area cover almost the whole range of agricultural production, including the cultivation of decorative and ornamental plants. Both methods, the conventional spraying and herbigation, have also been compared for the cultivation of corn, cotton, soybeans, potatoes, etc. The results have revealed a good potential for increasing the herbicidal solutions efficiency (Eberlein et al., 1999; Eberlein et al., 2000; Abbasi et al., 2006; Keshtkar et al., 2010; Nalayini et al., 2013).

Micro-irrigation might be successfully used for the application of herbicides in orchards. Experimental work in cherry plantations had not reported a negative effect of herbigation on both the growth of the cherry trees, their fruitfulness and the activity of the soil microorganisms. The highest efficacy of the herbicide was recorded in the variant of herbigation with micro-spraying (Rankova et al., 2007; Kolev et al., 2007; Rankova et al., 2016). Successful application of a number of herbicides (Stomp, Agil, Betanal ultra, etc.) by herbigation had been found in the production of apple and citrus propagation material (Xin et al., 1997; Kviklys, 2009).

(Sun and Yuan, 2011).

(Eberlein et al., 1999; Eberlein et al., 2000; Abbasi et al., 2006; Keshtkar et al., 2010; Nalayini et al., 2013).

(Rankova et al., 2007; Kolev et al., 2007; Rankova et al., 2016).

(Xin et al., 1997; Kviklys, 2009).

1992).

(Fourie,

Similar to fruit crops, grapevine also allows the use of chemigation. The herbicides simazine, oxadiazon, napropamide, orisalin, acetochlor were found to be more effective when applied through herbigation.

A study carried out in the Republic of South Africa showed that the criteria established for herbigation on sandy soil could be used as a guideline for other light soils (Fourie, 1992).

The objective of the present work was to investigate the opportunities for increasing the efficiency of the herbicide solutions by introducing them to the irrigating waters and optimizing the weed control in the vine nursery.

MATERIAL AND METHODS

Setting of the Trial

The trial was carried out during the period 2016-2018 in the vine nursery of the IVE, Pleven on a soil type leached chernozem. The grafted cuttings were grown without mulching the beds with polyethylene foil. They were supplied with water through a drip irrigation system with one irrigation wing located between the two rows. The water wings had built-in drippers at a distance of 15 cm with a flow rate of 1.0 L h⁻¹. The volume of the irrigation water was controlled by a water meter installed at the beginning of the system. Refreshing watering was performed two or three times a day, distributed before noon, at lunch and in the afternoon (in amount of 1-2 mm). The weed vegetation control was accomplished by treatment with herbicides. The dynamics of weed growing was reported on permanently marked parcels (4 per variant) of 1 m².

Herbicides

Herbicides for annual weeds found in the vine nursery (Gardoprim plus Gold) and perennial rhizome species (Gallant

2016-2018

cm

15

1.0 L h⁻¹.

(1-2 mm).

(4

1 m².

() super) were applied.
().

1. ,

Table 1. Herbicides, time of treatment and doses

Herbicides applied (formulated product)	Time of application	Active substance (g/l)	Dose (l/da)
Gardoprim plus Gold	preem.	312.5 g/l s-metolachlor + 187.5 g/l terbuthylazine	0,4
Gallant super	postem.	125 g/l haloxy-phetoxyethyl	0,25

„Water Bird VI Clasic”
 $q = 156 \text{ L h}^{-1}$ 0.2
 $r = 5.0$
 $m.$
 $= 1.42r = 7 \text{ m},$
 $F = 2r^2 = 50 \text{ m}^2,$
 $i = q/F = 3.12 \text{ mm/h.}$
 40 l/da (Pmax 300 kPa).

Manner of treatment

The herbicides were applied through the irrigation water by micro-sprinkling and using the conventional method with a backpack sprayer immediately after planting of the cuttings. The herbigation was carried out with „Water Bird VI Clasic” micro-sprinklers with flow rate $q = 156 \text{ L h}^{-1}$ at 0.2 pressure and radius of operation $r = 5.0 \text{ m}$. It was used a square layout with distance between the units $= 1.42r = 7 \text{ m}$, thus the irrigated area per unit was $F_i = 2r^2 = 50 \text{ m}^2$, while the intensity rate was $i = q/F_i = 3.12 \text{ mm/h}$. The spraying by backpack sprayer was realized at a rate of 40 l/da working solution (Pmax 300 kPa).

Variants

The trial variants were as follows:
1 (1) – **Technological control 1 (K1)** – introducing the herbicides with a backpack sprayer;
2 (2) – **Technological control 2 (K2)** – manually weeded out;
() – **Treated variant (H)** – introducing the herbicides by micro-sprinkling;

Methods of reporting and data analysis

(/m²) -
 60 , 90) (30 -
 The effect of Gardoprim plus Gold was estimated by the density of the weeds (pc./m²), total and per species, that was counted in dynamics (30th, 60th, 90th day of treatment) by the quantitative method. The efficiency of Gallant super was determined according to the scale of

al., 2017).
 Marinkov, 1999).

the International Weed Control Association (Zhelyazkov et al., 2017). Data were processed by analysis of variance (Dimova and Marinkov, 1999).

RESULTS AND DISCUSSION

17
 8
 : 2
 (Sorghum halepense
 (L.) Pers.) (Cynodon dactylon
 (L.) Scop.); 2
 (Aristolochia clematitis L.)
 (Rumex crispus L.); 4
 (Sonchus arvensis L.),
 (Cirsium arvense (L.) Scop),
 (Convolvulus arvensis L.)
 (Cardaria draba L.).
 9
 :1
 (Sinapis arvensis L.); 8
 (Chenopodium album
 L.), (Setaria viridis L.),
 (Amaranthus retroflexus
 L.), (Heliotropium europaeum
 L.), (Amaranthus blitoides
 L.), (Xanthium strumarium L.),
 (Portulaca oleracea L.)
 (Solanum nigrum L.).
 2016
 1
 (2).

Over the period of the study on the areas used for the vine nursery, the presence of 17 weed species was reported. The perennials were represented by 8 species, including: 2 rhizome – Johnson grass (*Sorghum halepense* (L.) Pers.) and Bermuda-grass (*Cynodon dactylon* (L.) Scop.); 2 with spindle root – birthwort (*Aristolochia clematitis* L.) and curly dock (*Rumex crispus* L.); 4 root-sprouting – field sow thistle (*Sonchus arvensis* L.), Canada thistle (*Cirsium arvense* (L.) Scop), field bindweed (*Convolvulus arvensis* L.) and whitetop (*Cardaria draba* L.). The annual weeds were 9 species, comprising: 1 early spring – field mustard (*Sinapis arvensis* L.); 8 late spring – lamb's quarters (*Chenopodium album* L.), green foxtail (*Setaria viridis* L.), amaranth (*Amaranthus retroflexus* L.), European heliotrope (*Heliotropium europaeum* L.), white amaranth (*Amaranthus blitoides* L.), common cocklebur (*Xanthium strumarium* L.), pigweed (*Portulaca oleracea* L.) and hound's berry (*Solanum nigrum* L.).

In 2016, the dynamics of weeding in the plots with herbigation and the technological control 1 developed in the same way – the total density of the weeds increased from the thirtieth to the sixtieth day (reported after treatment with Gardoprim plus Gold (Table 2). The lack of Canada thistle in the herbigated areas was more likely the result of its uneven dissemination due to the sector availability of the weed seeds.

The total density increase on the sixtieth day was due to the appearance of new stems of the rhizome species and field bindweed. The decrease on the nineteenth day was caused by the

ú

(92,5%) (85,71%)
 (1).
 (2)

reduction of the twitch grass and Johnson grass as a consequence of the action of Gallant super. Its efficiency, at the applied dose, was assessed as complete (according to the scale) for Johnson grass for both manners of treatment, almost satisfactory for twitch grass in case of herbigation (92.5%) and unsatisfactory (85.71%) for twitch grass in the technological control 1 (K1). Ninety days after treatment with Gardoprim plus Gold, pigweed plants were recorded in K1. The technological control 2 (K2) manually weeded out three times was characterized by a greater variety of species (especially annuals). As a result of the mechanical removal of weeds, their density decreased to the ninetieth day, but remained higher than that of the herbigated plot.

2. 2016 . (/m²)
Table 2. Dynamics of weed growing in 2016 (pc./m²)

	Species	Herbigation (H)			Technological control 1 (1)			Technological control 2 (2)		
		30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day
1	<i>A. blitoides</i>	-	-	-	-	-	-	1,8	0,5	-
2	<i>A. retroflexus</i>	-	-	-	-	-	-	0,5	-	-
3	<i>C. arvensis</i>	0,8	3,0	3,0	2,0	8,0	5,0	3,3	5,3	4,0
4	<i>C. arvensis</i>	-	-	-	0,5	0,8	1,5	0,8	-	0,3
5	<i>C. dactylon</i>	1,8	4,0	0,3	2,0	3,5	0,5	5,3	3,8	0,5
6	<i>Ch. album</i>	-	-	-	-	-	-	1,3	-	-
7	<i>H. europaeum</i>	-	-	-	-	-	-	0,3	-	-
8	<i>P. oleracea</i>	-	-	-	-	-	0,3	4,0	6,0	2,5
9	<i>R. crispus</i>	-	-	-	-	-	0,3	-	1,3	2,5
10	<i>Sonch. arvensis</i>	-	-	-	-	-	-	0,8	-	0,3
11	<i>S. halepense</i>	1,5	4,0	-	0,5	3,5	-	-	0,3	4,5
12	<i>X. strumarium</i>	0,8	1,0	1,5	1,5	3,0	3,5	3,0	-	-
		4,9	12,0	4,8	6,5	18,8	11,1	20,3	17,2	14,3

2017 .
 (1),
 (3).

In 2017, the efficacy of Gardoprim plus Gold introduced through herbigation did not differ significantly from that in the technological control 1 (K1), but the presence of white amaranth on the thirtieth day after treatment and European heliotrope on the sixteenth day was reported (Table 3). Despite the fact that both species exhibited susceptibility to

(Fetvadhieva et al., 1986; Prodanova-Marinova, 2015)
A. blitoides

1, - . -
 4,0 /m² (1,0 /m²)
 - 1). *S. nigrum*
 -
 2 (2)
 2017 . , ,

the active substances of the herbicide used (Fetvadhieva et al., 1986; Prodanova-Marinova, 2015), *A. blitoides* appeared also in the technological control 1, a month later. Ninety days after treatment, hound's berry plants (1.0 pc/m² in H and 4.0 pcs/m² in K1) were counted both in the control and the herbigated plot. *S. nigrum* was the species with the highest density throughout the entire vegetation period in the technological control 2 (K2), and that revealed significant availability of the area used for the nursery in 2017 with seeds of this weed.

3. 2017 . (/m²)
Table 3. Dynamics of weed growing in 2017 (pc./m²)

	Species	Herbigation (H)			Technological control 1 (1)			Technological control 2 (2)		
		30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day
		1	<i>A. blitoides</i>	0,3	1,3	1,3	-	1,0	3,0	2,8
2	<i>A. clematitis</i>	-	-	-	-	0,5	0,5	-	-	-
3	<i>C. arvensis</i>	6,8	8,8	9,8	5,0	7,8	10,0	2,3	1,5	1,5
4	<i>C. arvense</i>	3,0	3,5	-	1,0	1,0	1,0	-	-	-
5	<i>C. draba</i>	-	-	-	-	-	1,3	-	-	-
6	<i>H. europaeum</i>	-	0,3	1,3	-	-	-	0,8	-	0,3
7	<i>P. oleracea</i>	-	-	-	-	-	-	-	0,3	0,3
8	<i>S. arvensis</i>	-	-	-	-	-	-	2,3	4,8	-
9	<i>S. nigrum</i>	-	-	1,0	-	-	4,0	8,3	16,8	5,0
10	<i>S. viridis</i>	-	-	-	-	-	-	5,3	3,8	1,3
11	<i>X. strumarium</i>	0,5	0,8	3,8	1,0	1,8	2,8	2,8	3,8	2,0
		10,6	14,7	17,2	7,0	12,1	22,6	24,6	36,5	10,9

2018 .
 2016 2017 .
A. blitoides
 1
 (4).
 2 (2),
 2018
 (-
 0,25 l/da).
 ()

The structure of the weed association in the vine nursery in 2018 almost totally corresponded to that of 2016 and 2017. The presence of *A. blitoides* in the herbigated plots and in K1 on the thirtieth, sixtieth and ninetieth days after treatment with Gardoprim plus Gold was reported (Table 4). The total density of weeds on the thirtieth day was the highest in the technological control 2 (K2), the same as in the previous years. In 2018, Gallant super (at a dose of 0.25 l/da) was applied again. Its efficacy was complete (according to the scale) for both manners of treatment and unsatisfactory

70,59 % (87,97%

1).

for twitch grass (87.97% in the herbigated plot and 70.59% for the technological control 1).

4.

2018 . (/m²)

Table 4. Dynamics of weed growing in 2018 (pc./m²)

	Species	Herbigation (H)			Technological control 1 ¹ (1)			Technological control 2 ² (2)		
		30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day
1	<i>A. blitoides</i>	0,3	0,3	0,5	0,6	0,6	0,6	3,8	4,5	0,5
2	<i>A. clematitidis</i>	-	-	-	2,1	3,5	4,0	-	-	-
3	<i>C. arvensis</i>	11,0	11,0	11,91	14,1	15,0	16,0	12,3	5,5	1,5
4	<i>C. arvense</i>	1,0	1,0	1,0	-	-	-	-	-	-
5	<i>C. dactylon</i>	29,1	3,5	12,5	17,0	5,0	8,0	13,8	4,8	2,0
6	<i>H. europaeum</i>	-	-	0,1	-	-	-	0,8	0,3	0,3
7	<i>P. oleracea</i>	-	-	0,3	-	0,9	1,0	2,0	0,3	0,3
8	<i>Sonch. arvensis</i>	-	0,3	0,3	-	-	-	0,3	0,3	-
9	<i>S. halepense</i>	5,0	-	2,3	6,9	-	2,1	8,0	6,3	0,3
10	<i>S. nigrum</i>	0,5	0,9	1,0	0,3	0,5	0,5	18,3	10,0	6,0
11	<i>X. strumarium</i>	3,0	3,0	3,0	1,5	1,5	1,5	2,0	3,8	1,0
		49,9	20,0	39,9	42,5	27,0	33,7	61,3	35,8	11,9

(1, 2 3).

(2017 . (2).

(1). (< 5%),

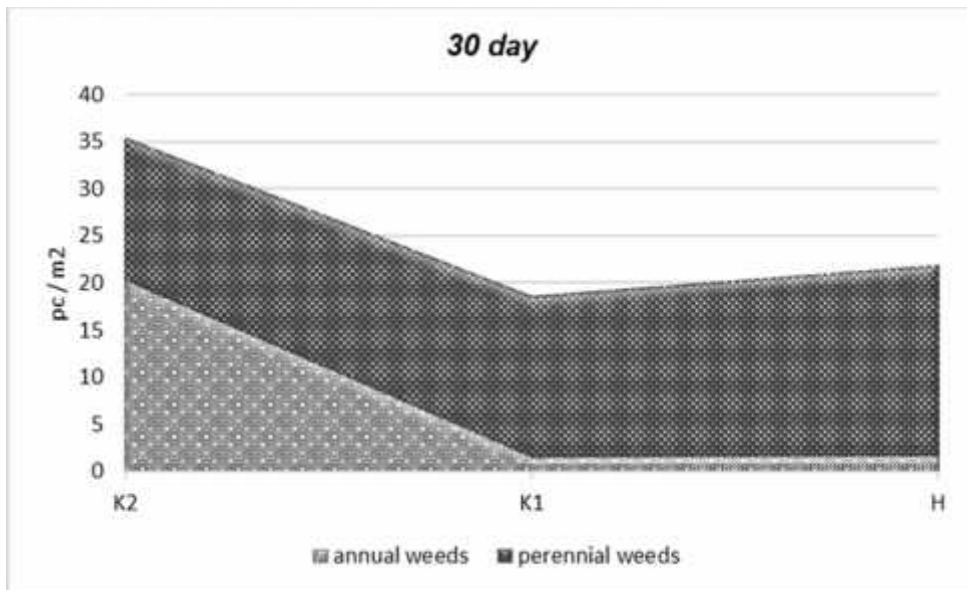
1

2 (= 1%).

Gardoprim plus Gold, applied to the soil, did not suppress the perennial species found in the nursery. Their density was determined by the natural availability of seeds and rhizomes in the areas and on the average for the study period it did not differ significantly in the trial plots (Figures 1, 2 and 3). The efficacy of Gardoprim plus Gold herbigation was directly related to its effect on the annual species. The treatment with foliar herbicides did not affect their density due to the fact that they were presented by only one species (green foxtail) disseminated in 2017 only in the manually weeded out control (K2).

Thirty days after the application, the density of the annuals in H variant was less than that in the technological control K1 by 0.2 pcs/m² (Figure 1). That difference was unproven (p <5%), but the analysis showed good assurance in the comparison of H and K1 with the technological control 2 (p = 1%). The data presented gave reason to believe that in the first month after the introduction of the

- herbicide, both manners of treatment were equally effective against the annual species.



. 1.

2016-2018 .

Fig. 1. Density of annual and perennial species thirty days after treatment with Gardoprim plus Gold, on the average for the period 2016-2018

(2).

2 (2)

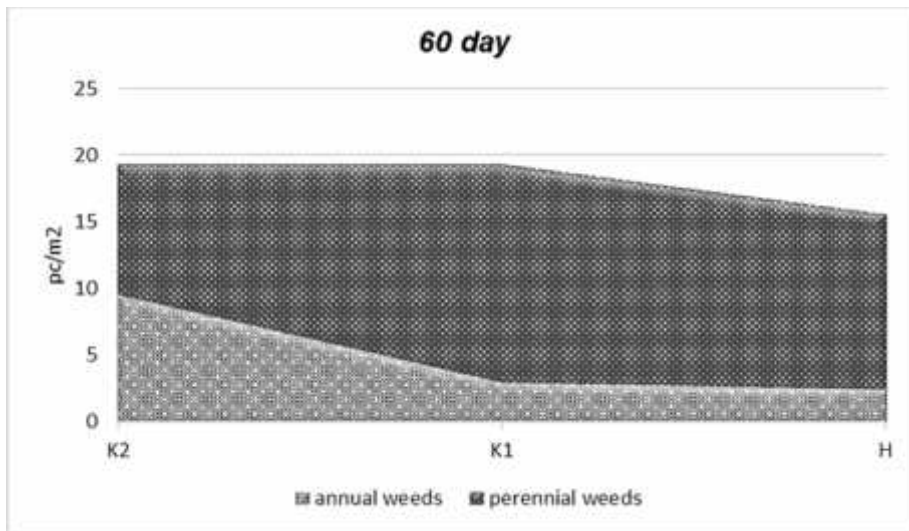
1 (1)

()

0,5 /m²
(< 5%)

1

The results for Gardoprim plus Gold effect sixty days after treatment were similar (Figure 2). The density of the annual and perennial species in the technological control 2 (K2) almost did not differ, whereas in the technological control 1 (K1) and the variant of herbigation (H), the annual weeds had a much smaller share in the overall weed association structure. The difference of 0.5 pc./m² between H and K1 was unproven (p <5%) and showed the efficient action of the herbicide irrespective of the mode of treatment.



. 2.

2016-2018 .

Fig. 2. Density of annual and perennial species sixty days after treatment with Gardoprim plus Gold, on the average for the period 2016-2018

(3).
 $2 \quad (6,6 \quad /m^2)$
 $5,7 \quad /m^2$).

16,03 $/m^2$ - 4,6 $/m^2$; 1 - 5,4
 $/m^2$ 16,5 $/m^2$)

- 0,8 $/m^2$

()
 (100,0% 100,0%
 1), . .

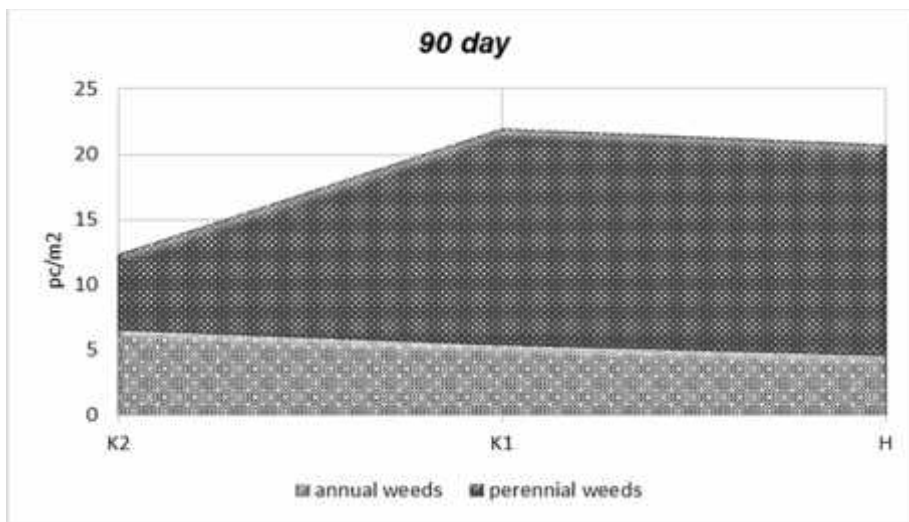
The tendency of the annual and perennial weeds ratio remained the same at the last recording (Figure 3). In the manually weeded out control K2, their density almost did not differ (6.6 - annual and 5.7 pc./m² - perennial). Ninety days after treatment with Gardoprim plus Gold in variant H and K1, the density of the annual species was significantly lower compared to the perennial ones (H - 4.6 pc./m² - annual and 16.03 pc./m² - perennial; K1 - 5.4 pc./m² - annual and 16.5 pc./m² - perennial).

The difference between the average density of the annuals in N and K1 continued to grow but remained almost insignificant - 0.8 pc./m² and unproven. Three months after the treatment, the herbicide efficiency did not differ significantly for both modes of application.

The efficiency of the foliar herbicide (Gallant super) against Johnson grass also did not change depending on the mode of treatment (100.0% for H and 100.0% for K1), i.e. in both cases it was complete. When recording its effect on

1. (90,23%),
(78,15%).

the other rhizome weed (twitch grass), differences in the results of H and K1 were observed. In the herbigated plots on the average it was almost satisfactory for the period (90.23%), while in the plots treated by a backpack sprayer the efficacy was evaluated on the scale as unsatisfactory (78.15%).

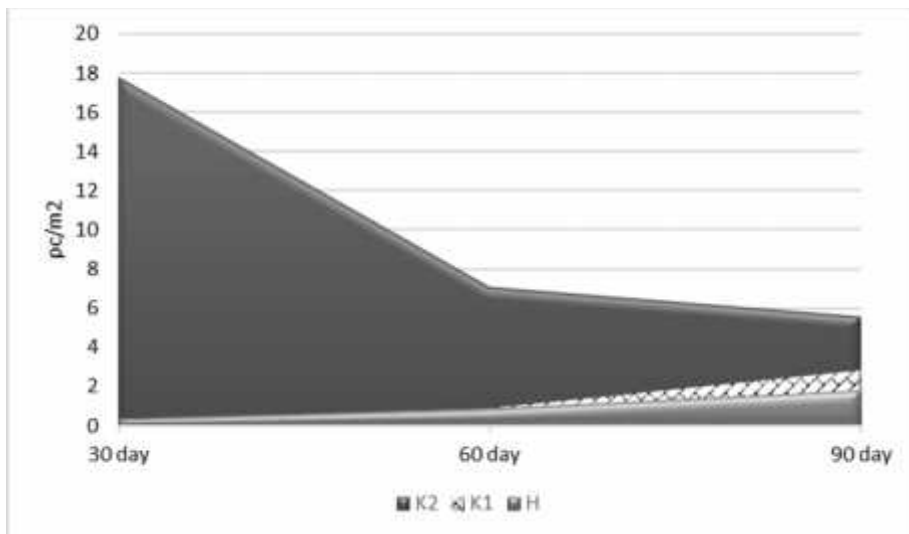


3. 2016-2018 .
Fig. 3. Density of annual and perennial species ninety days after treatment with Gardoprim plus Gold, on the average for the period 2016-2018

ú
-
() (1)
0,4 /m² () 0,3 /m²
(1), - 0,9 /m²
(4).
1,0 /m²

The common cocklebur is an annual weed not affected by Gardoprim plus Gold when applied before the emergence of its seeds and ignoring it as an element of the total density of the annual species allowed for a more specific assessment of the effect of the different treatment methods on susceptible species. In this case, the weed growing in the herbigated (H) and the control (K1) plots was practically the same - on the thirtieth day, respectively, the following rates were recorded - 0.4 pc./m² (H) and 0.3 pc./m² (K1), while on the sixtieth day - 0.9 pc./m² susceptible weeds irrespective of the mode of treatment (Figure 4). On the ninetieth day, a difference of 1.0 pc./m² was found in their density, with more efficient action

being achieved after the introducing of Gardoprim plus Gold with the irrigating waters.



4.

2016-2018 .

Fig. 4. Dynamics in the density of the susceptible species to Gardoprim plus Gold, on the average for the period 2016-2018

CONCLUSIONS

The efficiency of Gardoprim plus Gold to weed species susceptible to the active substances of the herbicide did not decrease when the plots were treated through the irrigation waters.

Micro-sprinkling with Gallant super solution (2.5 l/da dose) had total efficacy against Johnson grass and unsatisfactory to twitch grass.

Herbigation with Gardoprim plus Gold (soil) and Gallant super (foliar) could be successfully applied for weed vegetation control in the production of vine propagation material.

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