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‘ a anska Crna’ and ‘Tisel’. The latest leaf unfolding was observed in ‘Titania’ and ‘Tsema’, whereas the latest occurrence of the other phenological events was recorded in ‘Ben Lomond’. As for treatments, the earliest onset dates of all phenological stages were in black currant cultivars grown under foil mulch, and the latest in those under bare fallow treatment. Climatic factors, particularly air temperature, have a large effect on the occurrence of phenological stages.

Key words: black currant, phenological traits, bare fallow, sawdust mulch, black plastic mulch

(*Ribes nigrum* L.)

(Miši , 2002).

(Sonstebey

et al., 2012).

(Robinson, 1991; Dale, 2000; Kivijarvi et al., 2005).

INTRODUCTION

Black currant (*Ribes nigrum* L.) is a small fruit crop that enters the growing season earlier than other continental fruit crops (Miši , 2002). The onset and length of phenological stages are genotype-specific and affected not only by heritable traits but also by agroenvironmental conditions. Day length and temperature have a direct effect on phenological stages in general and flowering in particular, due to which black currant cultivars respond differently to climatic conditions (Sonstebey et al., 2012).

Black currant fares well in a variety of soils and under diverse soil management systems. Under the agroenvironmental conditions of Serbia, the most common soil management system is bare fallow i.e. continuous tillage. More recently, mulching with sawdust or foil has been increasingly used in orchard floor management for black currants. Numerous studies have shown a positive effect of sawdust or foil mulches on phenological stages, vegetative and generative potential, and fruit chemical properties (Robinson, 1991; Dale, 2000; Kivijarvi et al., 2005).

Mulching orchard soils with foil directly promotes early fruit ripening (Larsson, 1997), whereas in peanut plants it increases

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(A) (VG)
Table 2. Average monthly rainfall total (mm m⁻²), annual rainfall total (A) and rainfall total for the vegetative growth period (VG)

Year/ month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	A	VG
2012	60	70	10	47	68	38	22	0	7.2	30	23.7	87.6	463.5	212.2
2013	51	68	65.7	37	78.5	61.5	10	62.5	87	17.2	40.5	4	582.9	353.7
2014	21.5	6	52.5	104.5	125	103.5	163	56	101	50	19	90	892	703

UPOV, 2009).

(CPVO-TP/040/2

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Phenological traits were determined by phenological observation using international descriptors for black currants (CPVO-TP/040/2 – UPOV, 2009). The following phenological parameters were examined: 1. Time of leaf unfolding – recorded as the date of first leaf emergence from the winter bud. Based on leaf unfolding date, the tested cultivars were classified into early, medium and late leaf unfolding; 2. Time of inflorescence emergence i.e. the date of first inflorescence emergence from the generative bud; 3. Time of beginning of flowering – i.e. the moment when 10% of the total number of flowers were open; full flowering – the moment when 90 % of flowers were open. According to their flowering times, the cultivars were classified into early, medium and late flowering. Length of flowering period i.e. the period between the beginning of flowering and first berry set, expressed as the number of days. According to this parameter, the cultivars were grouped into cultivars with either a short, medium or long flowering period; 4. Time of first berry set – recorded as the date of first berry set, and 5. Time of berry ripening i.e. date of full or harvest maturity of the fruit (harvest date), according to which the cultivars were classified into very early, early, medium, late and very late ripening. The results are presented in tabular form.

RESULTS AND DISCUSSION

Under the agroenvironmental conditions of Serbia, the average first leaf unfolding date for all cultivars, treatments and

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years was 13 March, whereas full leaf unfolding occurred on average 5 days after the first leaf unfolding day. The shortest period between first leaf unfolding and full leaf unfolding was 5 days in 2013, and the longest 7 days in 2012 and 2014. Data on leaf unfolding dates in the tested black currant cultivars are presented in Table 3.

The studied cultivars differed in the time of first leaf unfolding. The earliest leaf unfolding occurred in ' a anska Crna' and 'Tisel' in all treatments and years, and the latest in 'Titania' and 'Tsema'. The difference in the average date of first leaf unfolding between the cultivars with the earliest and latest entry into this phenological stage was 13 days, whereas the difference in the average date of full leaf unfolding was 12 days. In all treatments and years, the period between beginning of leaf unfolding and full leaf unfolding was the shortest in 'Ben Sarek' (4 days on average) and the longest in ' a anska Crna', 'Tisel' and 'Tiben' (6 days on average). The lowest variation in the time of leaf unfolding across the experimental years was exhibited by 'Titania' and 'Tsema' (1 day on average) and the highest by 'Ben Sarek' (6 days on average).

In all experimental years, the beginning of leaf unfolding and full leaf unfolding were the earliest in cultivars under black plastic mulch treatment, and the latest in those under bare fallow. The difference in the beginning of leaf unfolding in 2012 between bare fallow and sawdust treatments and between sawdust and black plastic mulch treatments, depending on cultivar, was 1 to 2 days, whereas the difference between bare fallow and black plastic mulch treatments ranged from 2 to 4 days. In 2013, the difference was smaller i.e. 1 day between bare fallow and sawdust mulch treatments, 1 day between sawdust and black plastic mulch, and 2 days between bare fallow and black plastic mulch treatments.

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Table 3. Dates of beginning of leaf unfolding and full leaf unfolding

Cultivars	Treatments	Beginning of leaf unfolding			Average across cultivars	Full leaf unfolding			Average across cultivars
		2012	2013	2014		2012	2013	2014	
'Ben Lomond'	bare fallow	19.03.	12.03.	17.03.	16.03.	24.03.	15.03.	25.03.	21.03.
	sawdust	18.03.	11.03.	15.03.	15.03.	23.03.	14.03.	24.03.	20.03.
	black plastic	17.03.	10.03.	13.03.	13.03.	22.03.	13.03.	23.03.	19.03.
'Ben Sarek'	bare fallow	18.03.	09.03.	16.03.	14.03.	22.03.	12.03.	22.03.	19.03.
	sawdust	16.03.	08.03.	13.03.	12.03.	21.03.	11.03.	21.03.	18.03.
	black plastic	14.03.	07.03.	10.03.	10.03.	20.03.	10.03.	20.03.	17.03.
'Tsema'	bare fallow	20.03.	22.03.	21.03.	21.03.	25.03.	26.03.	26.03.	26.03.
	sawdust	19.03.	21.03.	20.03.	20.03.	24.03.	25.03.	25.03.	25.03.
	black plastic	18.03.	20.03.	19.03.	19.03.	23.03.	24.03.	24.03.	24.03.
'Titania'	bare fallow	20.03.	22.03.	21.03.	21.03.	25.03.	27.03.	27.03.	26.03.
	sawdust	19.03.	21.03.	20.03.	20.03.	24.03.	26.03.	26.03.	25.03.
	black plastic	18.03.	20.03.	19.03.	19.03.	23.03.	25.03.	25.03.	24.03.
'a anaska Crna'	bare fallow	11.03.	07.03.	08.03.	09.03.	21.03.	11.03.	12.03.	15.03.
	sawdust	09.03.	06.03.	05.03.	07.03.	20.03.	10.03.	09.03.	13.03.
	black plastic	07.03.	05.03.	02.03.	05.03.	19.03.	09.03.	06.03.	11.03.
'Tisel'	bare fallow	11.03.	07.03.	08.03.	09.03.	21.03.	11.03.	12.03.	15.03.
	sawdust	09.03.	06.03.	05.03.	07.03.	20.03.	10.03.	09.03.	13.03.
	black plastic	07.03.	05.03.	02.03.	05.03.	19.03.	09.03.	06.03.	11.03.
'Tiben'	bare fallow	20.03.	14.03.	14.03.	16.03.	25.03.	19.03.	21.03.	22.03.
	sawdust	19.03.	13.03.	12.03.	15.03.	24.03.	18.03.	19.03.	20.03.
	black plastic	18.03.	12.03.	10.03.	13.03.	23.03.	17.03.	17.03.	19.03.
Average across treatments	bare fallow	17.03.	12.03.	14.03.	14.03.	23.03.	18.03.	21.03.	20.03.
	sawdust	15.03.	11.03.	12.03.	13.03.	22.03.	16.03.	19.03.	19.03.
	black plastic	13.03.	10.03.	09.03.	11.03.	21.03.	15.03.	17.03.	18.03.
Overall average across cultivars and treatments		15.03.	11.03.	12.03.	13.03.	22.03.	16.03.	19.03.	19.03.

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The differences between the treatments in 2014 were larger compared to the other experimental years, and ranged from 1 to 3 days between bare fallow and sawdust mulch, and between sawdust and black plastic mulch treatments, and 2-6 days between bare fallow and black plastic mulch treatments. In terms of full leaf unfolding, in 2012 and 2013, the difference between bare fallow and sawdust mulch, and between sawdust and black plastic mulch treatments was 1 day, and that between bare fallow and black plastic mulch treatments was 2 days. In 2014, the difference between the treatments was the same as in the beginning of leaf unfolding.

Leaf unfolding was the earliest in 2013 and the latest in 2012. The date of first leaf unfolding between the earliest and the latest year showed a difference of 3 days, and the difference in full leaf unfolding date was 6 days.

Based on the time of first leaf unfolding, the cultivars were classified into two groups: 1. medium early (6-10 March): ' a anaska Crna', 'Tisel' and 2. late (after 11 March): 'Ben Lomond', 'Ben Sarek', 'Tiben', 'Titania', 'Tsema'.

Inflorescence emergence occurred 15 days on average after full leaf unfolding. For all cultivars, treatments and years, the average date of inflorescence emergence was 3 April. The shortest period between full leaf unfolding and inflorescence emergence was 8 days in 2013 and 2014, and the longest 17 days in 2012. Data on inflorescence emergence dates in the tested cultivars are presented in Table 4.

Inflorescence emergence in all experimental years and treatments was the earliest in ' a anaska Crna' and 'Tisel' and the latest in 'Ben Lomond'. The difference in the average date of inflorescence emergence between the cultivars with the earliest and latest entry into this phenological stage was 6 days. In all treatments and years, the shortest period between full leaf unfolding and inflorescence emergence (13 days on average) was observed in 'Titania' and

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'Tsema', and the longest in ' a anaska Crna' and 'Tisel' (17 days on average). The lowest variation in the time of inflorescence emergence across the experimental years was exhibited by 'Titania' and 'Tsema' (5 days on average), and the highest by 'Tiben', 'Tisel' and ' a anaska Crna' (9 days on average).

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Table 4. Inflorescence emergence dates

Cultivars	Treatments	Inflorescence emergence			Average across cultivars
		2012	2013	2014	
'Ben Lomond'	/ bare fallow	08.04.	12.04.	31.03.	07.04.
	/ sawdust	07.04.	11.04.	30.03.	06.04.
	/ black plastic	06.04.	10.04.	29.03.	05.04.
'Ben Sarek'	/ bare fallow	05.04.	09.04.	29.03.	04.04.
	/ sawdust	04.04.	08.04.	28.03.	03.04.
	/ black plastic	03.04.	07.04.	27.03.	02.04.
'Tsema'	/ bare fallow	08.04.	12.04.	05.04.	06.04.
	/ sawdust	07.04.	11.04.	04.04.	05.04.
	/ black plastic	06.04.	10.04.	03.04.	04.04.
'Titania'	/ bare fallow	08.04.	12.04.	05.04.	06.04.
	/ sawdust	07.04.	11.04.	04.04.	05.04.
	black plastic	06.04.	10.04.	03.04.	04.04.
' a anaska Crna'	/ bare fallow	04.04.	06.04.	24.03.	01.04.
	/ sawdust	03.04.	05.04.	23.03.	31.03.
	black plastic	02.04.	04.04.	22.03.	30.03.
'Tisel'	/ bare fallow	04.04.	06.04.	24.03.	01.04.
	/ sawdust	03.04.	05.04.	23.03.	31.03.
	/ black plastic	02.04.	04.04.	22.03.	30.03.
'Tiben'	/ bare fallow	08.04.	09.04.	27.03.	04.04.
	/ sawdust	07.04.	08.04.	26.03.	03.04.
	/ black plastic	06.04.	07.04.	25.03.	02.04.
Average across treatments	/ bare fallow	06.04.	09.04.	28.03.	04.04.
	/ sawdust	05.04.	08.04.	27.03.	03.04.
	/ black plastic	04.04.	07.04.	26.03.	02.04.
Overall average across cultivars and treatments		05.04.	08.04.	27.03.	03.04.

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The earliest beginning of inflorescence emergence was recorded in cultivars grown under black plastic mulch treatment, and the latest in those under bare fallow. The difference in the time of inflorescence emergence in all experimental years was 1 day between bare fallow and sawdust mulch, and between sawdust and black plastic mulch treatments, as opposed to the 2-day difference between bare fallow and black plastic mulch treatments.

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Inflorescence emergence was earliest in 2014 and latest in 2013. The difference in inflorescence emergence date between the earliest and the latest year was 12 days.

The beginning of flowering was 8 days on average after inflorescence emergence, and full flowering was 7 days after the beginning of flowering. Data on flowering dates in the tested cultivars are given in Table 5.

Flowering in all treatments and experimental years was the earliest in ' a anaska Crna' and 'Tisel', and the latest in 'Ben Lomond' and 'Tsema', with the difference of 9 days in the average date of the beginning of flowering between the cultivars that entered this phenological stage at the earliest and latest dates. The shortest period between inflorescence emergence and beginning of flowering in all treatments and experimental years was observed in 'Titania' and 'Tsema' (5 days on average), and the longest in 'Ben Sarek' (10 days on average). The period between the beginning of flowering and full flowering was shortest in 'Ben Sarek' (6 days on average), and longest in 'Tiben' (8 days on average). Variations in flowering date across experimental years were smallest in 'Titania' (3 days on average), and largest in 'Tisel' and ' a anaska Crna' (10 days on average).

In all experimental years, foil mulching resulted in the earliest flowering, whereas bare fallow treatment led to the latest flowering event. The difference in the time of beginning of flowering and full flowering in all experimental years was 1 day between bare fallow and sawdust mulch treatments, and sawdust and black plastic mulch treatments, and 2 days between black plastic mulch and bare fallow treatments.

The beginning of flowering and full flowering were earliest in 2014 and latest in 2013. The difference in the time of the beginning of flowering between the earliest and the latest year was 14 days, whereas the difference in the time of full flowering was 10 days.

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Table 5. Dates of beginning of flowering and full flowering

Cultivars	Treatments	Beginning of flowering			Average across cultivars	Full flowering			Average across cultivars
		2012	2013	2014.		2012	2013	2014	
'Ben Lomond'	bare fallow	17.04.	21.04.	10.04.	16.04.	25.04.	27.04.	18.04.	23.04.
	sawdust	16.04.	20.04.	09.04.	15.04.	24.04.	26.04.	17.04.	22.04.
	black plastic	15.04.	19.04.	08.04.	14.04.	23.04.	25.04.	16.04.	21.04.
'Ben Sarek'	bare fallow	13.04.	20.04.	09.04.	14.04.	21.04.	25.04.	15.04.	20.04.
	sawdust	12.04.	19.04.	08.04.	13.04.	20.04.	24.04.	14.04.	19.04.
	black plastic	11.04.	18.04.	07.04.	12.04.	19.04.	23.04.	13.04.	18.04.
'Tsema'	bare fallow	17.04.	20.04.	10.04.	16.04.	25.04.	26.04.	18.04.	23.04.
	sawdust	16.04.	19.04.	09.04.	15.04.	24.04.	25.04.	17.04.	22.04.
	black plastic	15.04.	18.04.	08.04.	14.04.	23.04.	24.04.	16.04.	21.04.
'Titania'	bare fallow	11.04.	17.04.	12.04.	13.04.	20.04.	22.04.	18.04.	20.04.
	sawdust	10.04.	16.04.	11.04.	12.04.	19.04.	21.04.	17.04.	19.04.
	black plastic	09.04.	15.04.	10.04.	11.04.	18.04.	20.04.	16.04.	18.04.
' a anska Crna'	bare fallow	09.04.	14.04.	29.03.	07.04.	17.04.	20.04.	05.04.	14.04.
	sawdust	08.04.	13.04.	28.03.	06.04.	16.04.	19.04.	04.04.	13.04.
	black plastic	07.04.	12.04.	27.03.	05.04.	15.04.	18.04.	03.04.	12.04.
'Tisel'	bare fallow	09.04.	14.04.	29.03.	07.04.	17.04.	20.04.	05.04.	14.04.
	sawdust	08.04.	13.04.	28.03.	06.04.	16.04.	19.04.	04.04.	13.04.
	black plastic	07.04.	12.04.	27.03.	05.04.	15.04.	18.04.	03.04.	12.04.
'Tiben'	bare fallow	13.04.	17.04.	03.04.	11.04.	22.04.	24.04.	11.04.	19.04.
	sawdust	12.04.	16.04.	02.04.	10.04.	21.04.	23.04.	10.04.	18.04.
	black plastic	11.04.	15.04.	01.04.	09.04.	20.04.	22.04.	09.04.	17.04.
Average across treatments	bare fallow	13.04.	18.04.	04.04.	12.04.	21.04.	23.04.	13.04.	19.04.
	sawdust	12.04.	17.04.	03.04.	11.04.	20.04.	22.04.	12.04.	18.04.
	black plastic	11.04.	16.04.	02.04.	10.04.	19.04.	21.04.	11.04.	17.04.
Overall average across cultivars and treatments		12.04	17.04.	03.04.	11.04.	20.04.	22.04.	12.04.	18.04.

According to the time of the beginning of flowering, all cultivars were classified into late flowering cultivars (after 2 April). However, since ' a anska Crna' and 'Tisel' entered full flowering earlier in 2014, they were grouped into medium flowering cultivars (27 March – 1 April) in this year. Based on the length of the flowering period, the cultivars were grouped into: 1. cultivars with a short flowering period (up to 15 days): 'Ben Sarek', 'Tsema', 'Tiben' and 2. cultivars with a medium flowering period (16-18 days): 'Ben Lomond', 'Titania', ' a anska Crna', 'Tisel'.

Berry set occurred 15 days on average after full flowering, and berry ripening started 50 days on average after berry set. The period between full flowering and berry set was the longest in 2014 (18 days on average), and the shortest in 2013 (12 days on average), whereas the longest period between berry set and berry ripening was in 2014 (55 days on average), and the shortest in 2012 (46 days on average). Data on the onset dates of berry set and berry ripening in the tested cultivars are presented in Table 6.

' a anska Crna' and 'Tisel' had the earliest berry set date, and 'Ben Lomond' had the latest. The difference in the average berry set date between the cultivars with the earliest and those with the latest entry into this phenological stage was 9 days. The period between full flowering and berry set in all treatments and experimental years was the shortest in 'Tiben' and 'Ben Sarek' (14 days on average), and the longest in ' a anska Crna' and 'Tisel' (18 days on average). The variation in the onset date of berry set across experimental years was 3 days on average in 'Ben Lomond', 'Ben Sarek', 'Tsema' and 'Titania', and 4 days on average in ' a anska Crna', 'Tisel' and 'Tiben'. As for berry ripening date, the earliest date was recorded in 'Tisel', and the latest in 'Ben Lomond'.

According to the time of the beginning of flowering, all cultivars were classified into late flowering cultivars (after 2 April). However, since ' a anska Crna' and 'Tisel' entered full flowering earlier in 2014, they were grouped into medium flowering cultivars (27 March – 1 April) in this year. Based on the length of the flowering period, the cultivars were grouped into: 1. cultivars with a short flowering period (up to 15 days): 'Ben Sarek', 'Tsema', 'Tiben' and 2. cultivars with a medium flowering period (16-18 days): 'Ben Lomond', 'Titania', ' a anska Crna', 'Tisel'.

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' a anska Crna' and 'Tisel' had the earliest berry set date, and 'Ben Lomond' had the latest. The difference in the average berry set date between the cultivars with the earliest and those with the latest entry into this phenological stage was 9 days. The period between full flowering and berry set in all treatments and experimental years was the shortest in 'Tiben' and 'Ben Sarek' (14 days on average), and the longest in ' a anska Crna' and 'Tisel' (18 days on average). The variation in the onset date of berry set across experimental years was 3 days on average in 'Ben Lomond', 'Ben Sarek', 'Tsema' and 'Titania', and 4 days on average in ' a anska Crna', 'Tisel' and 'Tiben'. As for berry ripening date, the earliest date was recorded in 'Tisel', and the latest in 'Ben Lomond'.

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Table 6. Dates of beginning of berry set and berry ripening

Cultivars	Treatments	Beginning of berry set			Average across cultivars	Berry ripening			Average across cultivars
		2012	2013	2014		2012	2013	2014	
'Ben Lomond'	bare fallow	12.05	10.05	08.05	10.05.	28.06	25.06	30.06	28.06.
	sawdust	11.05	09.05	06.05	09.05.	27.06	24.06	29.06	27.06.
	black plastic	10.05	08.05	05.05	08.05.	26.06	23.06	28.06	26.06.
'Ben Sarek'	bare fallow	07.05	04.05	03.05	05.05.	18.06	21.06	23.06	21.06.
	sawdust	06.05	03.05	01.05	03.05.	17.06	19.06	22.06	19.06.
	black plastic	05.05	02.05	30.04	02.05.	15.06	17.06	20.06	17.06.
'Tsema'	bare fallow	09.05	06.05	04.05	06.05.	23.06	23.06	25.06	24.06.
	sawdust	08.05	05.05	02.05	05.05.	22.06	22.06	24.06	23.06.
	black plastic	07.05	04.05	01.05	04.05.	20.06	21.06	23.06	21.06.
'Titania'	bare fallow	09.05	06.05	04.05	06.05.	23.06	23.06	25.06	24.06.
	sawdust	08.05	05.05	02.05	05.05.	22.06	22.06	24.06	23.06.
	black plastic	07.05	04.05	01.05	04.05.	20.06	21.06	23.06	21.06.
'a anska Crna'	bare fallow	04.05	03.05	28.04	02.05.	14.06	21.06	22.06	19.06.
	sawdust	03.05	02.05	26.04	30.04.	13.06	19.06	21.06	18.06.
	black plastic	02.05	01.05	25.04	29.04.	11.06	17.06	19.06	16.06.
'Tisel'	bare fallow	04.05	03.05	28.04	02.05.	14.06	19.06	21.06	18.06.
	sawdust	03.05	02.05	26.04	30.04.	13.06	17.06	20.06	17.06.
	black plastic	02.05	01.05	25.04	29.04.	11.06	15.06	18.06	15.06.
'Tiben'	bare fallow	05.05	04.05	29.04	03.05.	30.06	24.06	25.06	26.06.
	sawdust	04.05	03.05	27.04	01.05.	29.06	23.06	24.06	25.06.
	black plastic	03.05	02.05	26.04	30.04.	28.06	22.06	23.06	24.06.
Average across treatments	bare fallow	07.05	05.05	02.05	05.05.	21.06	22.06	24.06	22.06.
	sawdust	06.05	04.05	30.04	03.05.	20.06	21.06	23.06	21.06.
	black plastic	05.05	03.05	29.04	02.05.	18.06	19.06	22.06	20.06.
/ Overall average across cultivars and treatments		06.05	04.05	30.04.	03.05.	20.06	21.06	23.06.	21.06.

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The difference in berry ripening date between 'Tisel' and 'Ben Lomond' was 10 days. The period between berry set and berry ripening in all treatments and experimental years was the shortest in 'Tisel' and 'Ben Sarek' (48 days on average), and the longest in 'Tiben' (56 days on average). The smallest variation across experimental years was exhibited by 'Titania' and 'Tsema' (1 day on average), and the largest by 'Tisel' and ' a anska Crna' (5 days on average).

Fruit set and fruit ripening occurred earliest in black plastic mulch treatment, and latest under bare fallow. The difference in berry set date in 2012 and 2013 was 1 day between bare fallow and sawdust mulch treatments, and between sawdust and black plastic mulch treatments, and 2 days between bare fallow and black plastic mulch treatments.

In 2014, the difference was higher i.e. 2 days between bare fallow and sawdust mulch, 1 day between sawdust mulch and black plastic mulch, and 3 days between bare fallow and black plastic mulch treatments. As regards berry ripening, a 1-day difference was observed between bare fallow and sawdust mulch treatments in all experimental years. Sawdust and black plastic mulch treatments showed a difference of 2 days in 2012 and 2013, and 1 day in 2014, whereas the difference between bare fallow and black plastic mulch was 3 days in 2012 and 2013, and 2 days in 2014.

In terms of experimental years, berry set occurred earliest in 2014 and latest in 2012. The difference in berry set between the earliest and the latest year was 6 days. Conversely, berry ripening was the earliest in 2012 and the latest in 2014, with the difference of 3 days between the earliest and the latest year.

According to berry ripening time, the tested cultivars were designated to three groups: 1. early (15-20 June): 'Tisel', ' a anska Crna'; 2. medium (21-25

Denisow (2004)

Laugale (2007),
Miši (2002),
Stanisavljevi et al. (2002) Nikoli
Milivojevi (2010),

(1997) Devi et al. (1991) Larsson

2013 2012.
2.6 °C
44.7 mm m⁻²
2013, 2012.

flowering cultivars, 'Tiben', 'Tisel' and 'Titania' into medium flowering cultivars, and 'Ben Sarek' into early flowering cultivars, whereas Denisow (2004) grouped 'Ben Lomond' and 'Titania' into medium early flowering cultivars. These findings are not in agreement with the present data. The classification of the tested cultivars according to the time of fruit ripening generally complies with the classification made by Laugale (2007), but not with that provided by Miši (2002), Stanisavljevi et al. (2002) and Nikoli and Milivojevi (2010) who classified ' a anska Crna' into medium early ripening cultivars, and 'Ben Lomond' and 'Ben Sarek' into early ripening.

Black plastic mulching in the experiment had a large effect on the phenological events in the tested cultivars. In all experimental years, the black currant cultivars grown under black plastic mulch showed the earliest entry into the phenological stages, whereas the phenological events in the cultivars under bare fallow occurred at the latest dates.

The earlier entry into the phenological stages in cultivars under black plastic mulch treatment is due to faster soil heating and higher soil moisture under this soil management system.

Black plastic mulch has the capacity to absorb higher amounts of sunlight, which directly leads to an increase in temperature compared to bare fallow. Black plastic mulching in the orchard, as reported by Devi et al. (1991) and Larsson (1997), results in earlier dates of flowering and fruit ripening, which is in agreement with the present results.

Significant differences were observed in the timing of the phenological stages across years. Leaf unfolding was the earliest in 2013, and latest in 2012. During the leaf unfolding stage, air temperature was 2.6°C higher and rainfall amount was 44.7 mm m⁻² higher in 2013 than in 2012. As compared to leaf unfolding, inflorescence emergence and

2014 . - 2013 . -
 2014 . 1.6 °C - ,
 - 37.7 mm m⁻²
 2013 .
 et al. (2012), Sonsteby
 Nikoli Milivojevi (2010) -
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 - 2014 -
 2012. 2.8 °C -
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 4 °C -
 mm m⁻² 227
 2014 . -
 2012 .

flowering were the earliest in 2014 and the latest in 2013. Air temperature in the first quarter of 2014 was 1.6°C higher and rainfall amount was 37.7 mm m⁻² lower than in 2013. The data are in agreement with the reports of Sonsteby et al. (2012) who found that the flowering rate is increased several times with increasing temperature.

Moreover, Nikoli and Milivojevi (2010) observed that flowering is dependent on cultivar and weather conditions (altitude, exposition and temperature), and that the warmer the weather during the flowering period, the shorter the length of flowering, and vice versa. Berry set occurred earliest in 2014 and latest in 2012.

Air temperature in 2014 during the period between flowering and berry set was 2.8°C higher and rainfall amount was 54.5 mm m⁻² higher than in 2012. This gave rise to an early berry set. Berry ripening was the earliest in 2012 and the latest in 2014.

During the period between berry set and berry ripening in 2012, an increase of 4°C in air temperature and a decrease of 227 mm m⁻² in rainfall amount were observed relative to 2014. The higher air temperature and the significantly lower rainfall amount in 2012 led to early fruit ripening in the tested black currant cultivars.

CONCLUSIONS

Knowledge of the phenology of black currant as a fruit species that demonstrates an early entry into the growing season and flowering is of particular importance when choosing an appropriate location, exposition and soil management system for a black currant planting and, accordingly, when making a proper choice of cultivars.

In lowland areas, when choosing a soil management system for black currant plantings, priority should be given to bare fallow since it postpones the entry of black

currant into the growing season, particularly the stage of flowering, thus preventing harmful effects of late spring frosts.

Climatic factors, particularly air temperature, have a large effect on the occurrence of phenological stages. The tested cultivars are suitable for growing under the agroclimatic conditions of a ak, Western Serbia, given their late entry into the flowering stage as their important characteristic in terms of preventing damage due to late spring frosts.

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31093,

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**E-mail: jtomic@institut-cacak.org*

Effect of cultivar and cultivation system on production characteristics and fruit quality of early ripening strawberry cultivars

Jelena Tomić*, Marijana Pešaković, Žaklina Karaklajić-Stajić, Rade Miletić, Svetlana M. Paunović, Mira Milinković

Fruit Research Institute, Cacak, Republic of Serbia

SUMMARY

The paper presents the results of the research into the impact made by cultivar and cultivation system on the vegetative and generative potential, phenological properties and fruit quality of the early ripening strawberry cultivars 'Clery' and 'Garda' in the first year of fruiting. Two types of cultivation system were applied – the open field and low tunnels.

Rosette height was stimulated by interaction effect cultivar/cultivation system, in particular 'Clery' grown in open field. Significantly higher value for number of leaves per plant and number of fruits per fruiting stalks were determined in 'Clery' strawberries. The examination of phenological properties show that the onset of flowering and ripening in both cultivars was earlier in low tunnels compared to open field system. The analysis of the fruit quality showed that the effect of cultivar and interaction was

- more stressed than effect of cultivation system. When interacting with open field cultivation system, 'Garda' gave the highest values in terms of fruit width and firmness.

- The analysis of the production characteristics, phenological properties and quality of strawberry 'Clery' and 'Garda' in two cultivation system suggests that the best performance in terms of fruit quality was exhibited by 'Garda', which can be recommended for further promotion and expansion in strawberry growing regions. Low tunnel cultivation system exhibited the highest effectiveness in terms of the time of flowering and ripening. Therefore, low tunnel cultivation system can be considered an appropriate practice to protect strawberry from late spring frost and ensure early strawberry fruit production.

Key words: strawberry, cultivation system, vegetative potential, generative potential, fruit quality

INTRODUCTION

One of significant factors of highly intensive strawberry production is the innovation of varieties, i.e., introduction of new, promising varieties with different ripening times with the aim of better and more balanced market supply. Strawberry assortment in Serbian plantations is heterogeneous, whereby in particular, over the last decade, certain dynamics has been observed in the group of table varieties.

Due to its earliness, good adaptability to different growing technology, a good fruit quality and high tolerance to root and leaf diseases (Martinelli and Leis, 2012), cultivar 'Clery' is very dominant in Serbian plant production.

In the last few years in Italy newer

2012),

(Martinelli and Leis,

CRA-FRF -

5-10%.

(43° 53' N, 20° 20' E, 225 m
2016

2015

30 cm.

30 x

20
3

strawberry cultivars, have been introduced, including early strawberry cultivar 'Garda', created under the CRA-FRF - Agricultural Research Council - Fruit Crop Research Unit Forli, Italy. When defining the production and use value of introduced varieties, apart from the genotype influence, the system of breeding, agricultural and pomotechnical measures play the important role too.

In planting strawberry seedlings in Serbia, there are different systems of growing, among which, in recent years the growing technology on joists covered with a polyethylene foil (PE) is dominant, with obligatory installation of the irrigation system. However, apart from the plants based on the principles of modern growing technology in the open field, strawberries planted in greenhouses accounts for 5–10% only. One of the newer ways of intensive strawberry production in greenhouses is setting low tunnels. The main objective of this research was to examine the effect of genotype and cultivation system on the vegetative and generative potential, phenological properties and fruit quality of the early ripening strawberry cultivars.

MATERIAL AND METHODS

The study was performed at the experimental strawberry plantation at the Fruit Research Institute in a ak (43° 53' N, 20° 20' E, 225 m altitude) in 2016. The planting of frigo plants was performed in August 2015, in the form of the banks, covered in black polyethylene foil with a planting distance 30 x 30 cm. The layout of the experiment was a completely randomized design, with the effect of two factors, viz. genotype and cultivation system, analyzed. The experiment was conducted on 2 early ripening strawberry cultivars 'Clery' and 'Garda', in 2 cultivations system (open field and low tunnels) with 20 plants in each treatment in 3 replications. During the experiment,

the plantation was subjected to standard cultural practices, including a drip-irrigation system.

Determination of the vegetative potential parameters was performed by standard morphometric methods and counting. The following parameters were observed: rosette height (cm), the number of crowns per rosette and the number of leaves per rosette.

Within generative potential of strawberry, the following parameters were monitored: number of fruiting stalks per plant, number of fruits per fruiting stalks, number of fruits per plant and yield per plant (g). Measuring was performed and monitored by counting and yield per plant determined by weight measuring of harvested fruit in every harvest and yield summing of all vintages.

Within fruit quality, the following parameters were monitored: fruit weight (g), fruit length (mm), fruit width (mm), index of fruit shapes, firmness and soluble solids content. The mentioned parameters are determined by standard morphometric methods on the sample of 20 fruit per replication (a total of 60 fruits per a harvest, 3 replications, 20 fruits each) in the full maturity phase. Fruit weight is determined by measuring on analytical scale *Mettler*, with accuracy of ± 0.01 g. The value of fruit shape index is obtained by calculation, establishing relationship between fruit length and fruit width, measured by digital calliper (*Carl Roth, Germany*) accuracy of ± 0.05 mm. Fruit firmness is determined by using a penetrometer.

Within phenological characteristics, phenology phase of flowering time (start, end, duration) and ripening (beginning, end, duration) was tested. Flowering time of strawberry was determined by recording the start date (when open 10% of flowers), and the end of flowering (when 90% of flowers decline coronal leaflets). Growth phenology phase was determined by the recording start date (when ripen 10%) and the end of maturity

(g),

(mm),

(mm),

20

60

20

3

Mettler

± 0.01 g.

± 0.05 mm

(*Carl Roth, Germany*).

10%

(

90%

the plantation was subjected to standard cultural practices, including a drip-irrigation system.

Determination of the vegetative potential parameters was performed by standard morphometric methods and counting. The following parameters were observed: rosette height (cm), the number of crowns per rosette and the number of leaves per rosette.

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(10%)
 ()
 (ANOVA, F test)
 STATISTICA
 Inc., Tulsa, OK,). 8.0 (StatSoft,
 LSD 0.05.

(the last day of the harvest). Duration of the mentioned phases is expressed in days.

The data obtained in the research was processed applying the Fisher model of variance analysis (ANOVA, F test) and the statistics software package STATISTICA version 8.0 (StatSoft, Inc., Tulsa, OK, USA). The analyses were performed in three replications and the obtained values were expressed as the mean value. Testing the significance of differences between the means of the treatments and their interaction effects was performed using the LSD test and the significance level of 0.05.

RESULTS AND DISCUSSION

(Pešakovi et al., 2015; Tomi , 2016). Chercuitte et al. (1991)
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 (Shaw, 1993).
 (Chercuitte et al., 1991).

Bearing in mind that strawberry yield positively correlates with the development of a plant, it is very important that by modern technology of growing the best possible development of each of the crown in a plant and of the entire plant is obtained (Pešakovi et al., 2015; Tomi , 2016). Chercuitte et al. (1991) point out that the most vigorous plants give highest yields. Table 1. shows the impact made by the genotype and cultivation system on the parameters of vegetative potential in strawberry. Significantly higher values for number of leaves per rosette was determined in 'Clery' compared to 'Garda'. Also, the highest rosette height was obtained through the interaction between 'Clery' and low tunnels production system (Figure 1). With cultivar 'Garda' there was no significant difference in plant height in both cultivation system examined. However, great vigour does not always result in high yields, particularly if the vigorous genotype is combined with agro-technical practices that further favour vigorous tillers (Shaw, 1993). For this reason, at maximizing the growth potential of strawberries, a particular attention should be paid to a balanced ratio between vegetative growth and productivity. (Chercuitte et al., 1991). A

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high value of the number of fruits per fruiting stalks in cultivar 'Clery' did not result in highest yields of the cultivar. 'Clery' had moderately high productivity, which was close to the productivity of 'Garda'. Cultivation system and interaction of study factors (genotype and production system) have not caused any changes in parameter values of the generative potential of the tested fruit varieties (Table 2).

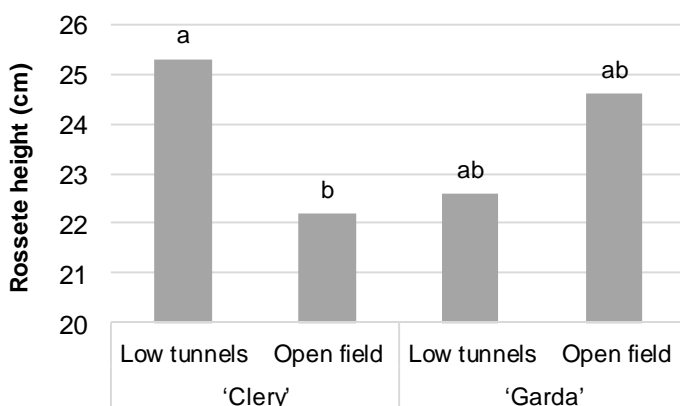
Table 1. Impact made by the genotype and cultivation system on the parameters of vegetative potential in strawberry

		/Rosette height (cm)	/Number of crowns per rosette	/Number of leaves per rosette
/Cultivar (A)	'Clery'	23.8 ± 0.9 a	2.5 ± 0.1 a	16.8 ± 1,3 a
	'Garda'	23.6 ± 0.7 a	2.3 ± 0.1 a	8.6 ± 0.4 b
/Cultivation system (B)	/Open field	23.9 ± 0.9 a	2.5 ± 0.1 a	13.7 ± 2.4 a
	/Low tunnels	23.4 ± 0.8 a	2.3 ± 0.1 a	11.7 ± 1.5 a
ANOVA				
A		ns	ns	*
B		ns	ns	ns
AxB		*	ns	ns

P 0.05 LSD ()

LSD

The different small letter(s) in column indicate significant differences among means at P 0.05 by LSD test



. 1.

Fig. 1. Influence of genotype and production system on rosette height

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Table 2. The influence of genotype and production system on the generative potential and productivity of strawberry

		/Number of fruiting stalks per plant	/Number of fruits per fruiting stalks	/Number of fruits per plant	/Yield per plant (g)
/Cultivar (A)	'Clery'	2.8 ± 0.1 a	8.2 ± 0.4 a	22.4 ± 1.4 a	467.6 ± 38.1 a
	'Garda'	2.8 ± 0.2 a	6.1 ± 0.4 b	24.1 ± 3.0 a	512.5 ± 50.1 a
/Cultivation system (B)	/Open field	2.8 ± 0.1 a	7.1 ± 0.8 a	21.6 ± 1.8 a	486.6 ± 51.2 a
	/Low tunnels	2.8 ± 0.2 a	7.2 ± 0.4 a	24.9 ± 2.6 a	493.4 ± 39.2 a
ANOVA					
A		ns	*	ns	ns
B		ns	ns	ns	ns
AxB		ns	ns	ns	ns

P 0.05 LSD

The different small letter(s) in column indicate significant differences among means at P 0.05 by LSD test.

3. Dates for the beginning, end and duration of flowering and ripening are shown in Table 3.

3

Table 3. The influence of genotype and production system on the phenological traits of the strawberry

Flowering	/Open field			/Low tunnels		
	Beginning	End	Duration	Beginning	End	Duration
'Clery'	10.04.	05.05.	25	04.04.	28.04.	24
'Garda'	11.04.	06.05.	25	02.04.	28.04.	26
/Average	11.04.	06.05.	25	03.04.	28.04.	25
Ripening	Open field			Low tunnels		
	Beginning	End	Duration	Beginning	End	Duration
'Clery'	11.05.	02.06.	22	01.05.	29.05.	28
'Garda'	10.05.	01.06.	22	02.05.	01.06.	30
/Average	11.05.	02.06.	22	02.05.	31.05.	29

The average values show that the onset of flowering in low tunnels system for both cultivars was at the beginning of April (on 4 April in 'Clery' and on 2 April in 'Garda'). Flowering in the open field started at an average of 8 days later (on 10 April in 'Clery' and on 11 April in 'Garda') than in the low tunnels, while the average duration of flowering was the same in both systems of cultivation (25

(25 days). The ripening stage began in the first decade of May in low tunnels system (on 1 May in 'Clery' and on 2 May in 'Garda'), and in the second decade of May in open field (on 11 May in 'Clery' and on 10 May in 'Garda').

The cultivars of garden strawberry have a fruit with various size, which depending on the genotype, ecological factors, the cultivation system, the age and the time of ripening. The results of physical properties of strawberries depending on the genotype and cultivation system are shown in Table 4.

4.

days). The ripening stage began in the first decade of May in low tunnels system (on 1 May in 'Clery' and on 2 May in 'Garda'), and in the second decade of May in open field (on 11 May in 'Clery' and on 10 May in 'Garda').

The cultivars of garden strawberry have a fruit with various size, which depending on the genotype, ecological factors, the cultivation system, the age and the time of ripening. The results of physical properties of strawberries depending on the genotype and cultivation system are shown in Table 4.

4.

Table 4. The influence of genotype and production system on the physical traits of the strawberry fruit

		Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Shape index	Fruit firmness (N)
Cultivar (A)	'Clery'	20.7 ± 1.4 a	39.5 ± 1.2 a	33.1 ± 0.3 b	1.2 ± 0.0 b	10.6 ± 0.6 b
	'Garda'	21.7 ± 1.4 a	39.5 ± 0.8 a	35.3 ± 0.7 a	1.1 ± 0.0 a	15.1 ± 0.9 a
Cultivation system (B)	Open field	22.2 ± 1.2 a	39.8 ± 0.3 a	34.7 ± 0.9 a	1.2 ± 0.0 a	13.6 ± 1.6 a
	Low tunnels	20.3 ± 0.6 a	39.1 ± 0.7 a	33.7 ± 0.3 b	1.2 ± 0.0 a	12.1 ± 0.6 a
ANOVA						
A		ns	ns	*	*	*
B		ns	ns	*	ns	ns
AxB		ns	ns	*	ns	*

P 0.05 (LSD test).

Values within each column followed by the same letter are not significantly different at the P 0.05 (LSD test).

Based on the analysis of the data obtained, it can be seen that the width, shape index and fruit firmness varied under the influence of the genotype, and were the highest average values in respect of aforementioned parameters registered in cultivar 'Garda'. The fruit width varied under the influence of cultivation systems and had significantly higher value recorded in the system of cultivation strawberries in the open field as compared to the low tunnels. In accordance with a significantly greater width of strawberries cultivated in an open field, a significant higher value of

(Tulipani et al., 2008).
 5,
 11.0%.
 Milivojevi et al. (2015)
 5.

Consumer acceptance of strawberry fruits depends to a great extent on fruit flavour, closely associated with soluble solids content (Tulipani et al., 2008).

Based on the data presented in Table 5 we can concluded that genotype and production system had not significant effect on soluble solids in strawberry fuit. Average values of soluble solids content ranged from 10.7% to 11.0%.

By studying cultivars/new selections of different ripening time, Milivojevi et al. (2015) founded significant variation in respect of soluble solids content among tested cultivars in all ripening stages, except in varieties of early ripening time, whit no significant difference noted.

Table 5. The influence of genotype and production system on the soluble solids of the strawberry fruit

		Soluble solids (%)
/Cultivar (A)	'Clery'	10.9 ± 0.1 a
	'Garda'	10.8 ± 0.2 a
./Cultivation system (B)	/Open field	10.7 ± 0.2 a
	/Low tunnels	11.0 ± 0.1 a
A		ns
B		ns
AxB		ns

P 0.05 (LSD).
 Values within each column followed by the same letter are not significantly different at the P 0.05 (LSD test).

CONCLUSIONS

Taking into consideration that strawberry fruits grown in low tunnels ripen 10 days earlier in relation to open field, growing strawberry in low tunnels can be considered justified from the aspect of obtaining fruits for early strawberry fruit production.

Apart from its moderate productivity and earliness, changes occurred in

" " physical characteristics of the fruit variety
 - Garda (width, shape index and firmness)
 - indicate that it can be recommended for
 more intense expansion in the production.

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(23.61 g)

(0.12)

(11.46%)

pH

(15.50%),

(5.58%)

(4.33)

(7.25%)

locality Bresnica .

The highest yield per tree (23.61 kg) and unit area and yield efficiency (0.12) were observed in this cultivar on locality Stapar . The largest soluble solids content (15.50%), total sugars (11.46%) and sucrose (5.85%) contents and juice pH value (4.33) were recorded in cultivar Nada grown on locality Bresnica , while the highest invert sugars content (7.25%) and amount of total acids were found in this cultivar grown on locality Ljubi .

Key words: productive traits, Nada , three localities, yield, chemical composition

INTRODUCTION

Plum is a traditional fruit crop in Serbia, and the most important one in terms of the volume of production. Serbia was ranked first in Europe and second worldwide after China with an annual plum production of 507,987 t (average 2010-2014) on over 120,000 ha land (FAOSTAT, 2016). The Serbian plum production is characterized by extensive growing technology, low unstable yields, low-quality fruit, problems induced by Šarka virus and a multitude of cultivars (Nenadovi -Mratini et al., 2007; Milošević et al., 2012; Milošević et al., 2013). The most common cultivars include ' a anaska Lepotica', ' a anaska Rodna', 'Stanley', ' a anaska Najbolja' and ' a anaska Rana' (Milošević and Milošević , 2012). Paunovi et al. (2011) specified that myrobalan (*Prunus cerasifera* Ehrh.) seedling is the dominant and practically the only rootstock used for plum grafting. Development of new plum cultivars of high quality, high yielding potential and tolerance or resistance to diseases, particularly to Šarka virus is very important way to overcome disadvantages in plum production. Since 1979 to 2012 fifteen plum cultivars were named and released at Fruit Research Institute, a ak. Some of these cultivars,

(

2010-2014 .)
(FAOSTAT, 2016).

507.987
120000

(Nenadovi -Mratini et al., 2007; Milošević et al., 2012; Milošević et al., 2013).

" " " " " "
" " " " " "
" " " " " "
(Milošević and Milošević , 2012). Paunovi et al. (2011)

Prunus cerasifera Ehrh.).

(*Prunus*

2012 .

1979

Ohaus Adventurer (Parsippany, NJ, USA). (kg) (kg ha⁻¹)

Electronic Scale) (Zhejiang, China). (cm) 20 cm

(TCSA, cm²). (kg cm⁻²)

(TCSA).

(%)

(Athol, NE, USA) cm. Starrett, 727 0,01

(ATC, Rocky Mount, NC, USA) (Brix). (Brix). (%)

0.1 N NaOH. pH

Cyber Scan 510 pH- (Nijkerk, The Netherlands).

Luff-Schoorl, Schneider (1979).

: $SU = (TS - RS) \times 0,95$. %

(ANOVA)

(LSD test)

P 0.05, Microsoft Office Excel (Microsoft Corporation, Redmond, WA,).

recorded by Ohaus Adventurer technical scale (Parsippany, NJ, USA). Yield per tree (kg) and hectare (kg ha⁻¹) were measured in period 2014–2016 using an ACS System Electronic Scale (Zhejiang, China). Trunk circumferences (cm) were measured at the end of growing season 20 cm above the graft union and used to calculate the trunk cross-sectional area (TCSA, cm²). The yield efficiency (kg cm⁻²) was calculated as the ratio between yield per tree and trunk cross-sectional area (TCSA). For determining flesh/stone ratio fruits were cut in half horizontally with a stainless-steel knife and the stones were removed and weighed.

The flesh percentage (%) was calculated by subtracting the stone weight from the whole plum fruit weight. For each plum fruit, three linear dimensions, length, width and thickness were determined by using a digital caliper Starrett, 727 Series (Athol, NE, USA) with a sensitivity of 0.01 cm. Soluble solids content was defined by Milwaukee MR 200 hand refractometer (ATC, Rocky Mount, NC, USA) at 20°C (Brix). Titratable acidity, as malic acid (%), were determined by titration with 0.1 N solution of NaOH. The juice pH was assessed by a Cyber Scan 510 pH meter (Nijkerk, The Netherlands). The total sugars and invert sugars content were defined on triplicate samples by the Luff-Schoorl method previously described by Schneider (1979). The sucrose content was calculated according to the relationship: $SU = (TS - RS) \times 0.95$. The results were expressed in % of fresh weight. All data in the present study were subjected by analysis of variance (ANOVA) and means were separated by LSD test at P 0.05 using Microsoft Office Excel software (Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

Phenological characteristics of cultivar Nada grown on three localities are given in Table 1. Flowering onset was recorded in mid-April. The earliest flowering onset was observed at locality 'Ljubi' (April 14), and the latest at locality 'Stapar' (April 17). Full flowering was assessed between April 17 ('Ljubi') and April 21 ('Stapar'), and end of flowering between April 23 ('Ljubi') and April 27 ('Stapar'). Flowering time of cultivar Nada on all three localities and years was within the long-term average for examined regions (Ogašanovi et al., 2005; Gliši et al., 2013; Gliši et al., 2016). According the classification of flowering time of European plum cultivars, stated by Neumüller (2010), it can be said that cultivar Nada belongs to group of cultivars with very late flowering time. Latter blooming period could be important to avoid late spring frosts in some years. Ripening time was similar on all three localities and years. The earliest averaged ripening time was found at locality 'Ljubi' (August 16), and the latest at locality 'Stapar' (August 20). The earliest ripening time was observed in 2014, and the latest in 2015. Similar ripening time of cultivar Nada was found in study of Gliši et al. (2015) and Gliši et al. (2016). This trait has been established as characteristic of each genotype, and quantitatively inherited (Dirlewanger et al., 1999).

1. (Ogašanovi et al., 2005; Gliši et al., 2013; Gliši et al., 2016).
Neumüller (2010),
" (16),
" (20).
2014., 2015.
Gliši et al. (2015) Gliši et al. (2016).
(Dirlewanger et al., 1999).

1. **2014-2016**
Table 1. Averaged flowering time of cultivar Nada grown on three localities in period 2014-2016

Locality	Flowering time			Harvesting time
	Onset	Full	End	
/ Ljubi	14.04.	17.04.	23.04.	16.08.
/ Bresnica	16.04.	20.04.	26.04.	18.08.
/ Stapar	17.04.	21.04.	27.04.	20.08.

The average values of fruit and stone weight and the flesh percentage differed significantly depending on the examined localities and years (Table 2).

The largest fruit and stone weight as well as flesh percentage of cultivar Nada were observed at locality Ljubi, while the smallest these values were found at locality Bresnica.

These parameters had the highest values in 2016. These results were in accordance with findings of Gliši et al. (2015) and Gliši et al. (2016) in similar conditions. Differences among localities can be explained with different climatic and soil conditions. Considering that fruits of this cultivar had averaged fruit weight on all localities higher than 40 g, Nada could be classified as plum with large-size fruits according to similar data obtained by Blažek and Pišt ková (2009) for some cultivars.

According to Depypere et al. (2007) the stone weight is considered to be stable cultivar-specific characteristic. In our study, the positive correlation between the fruit and the stone weight was found, which was confirmed by results of Okut and Akca (1995). Previous works in plum showed that flesh percentage is a very important characteristic of plum and depends on cultivar (Nenadovi -Mratini et al., 2007).

Regarded to this trait, our results are in accordance with results obtained by Gliši et al. (2015) in similar conditions. Cultivar Nada grown on locality Ljubi showed the largest values of fruit height, width and thickness. On the other hand, these parameters were the smallest when this cultivar was grown at locality Bresnica (Table 2). The fruit dimensions can be useful in determining size of apertures and components of machines especially for mechanical harvesting (Jannatizadeh et al., 2008).

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2. " ",
2014-2016 .

Table 2. Fruit characteristics of cultivar Nada grown on three localities in period 2014-2016

Locality	Year	Fruit weight (g)	Stone weight (g)	Flesh percentage (%)	Fruit height (mm)	Fruit Width (mm)	Fruit thicknes (mm)
Ljubi	2014	45.76±0.27 c	1.74±0.05 a	96.51±0.09 b	50.28±0.22 b	41.65±0.25 b	40.01±0.48 a
	2015	50.36±0.29 b	1.71±0.06 b	96.33±0.12 b	53.38±0.32 a	40.86±0.28 b	39.97±0.46 a
	2016	54.45±0.25 a	1.74±0.05 a	96.99±0.12 a	53.84±0.30 a	44.48±0.22 a	39.18±0.44 a
	/ Average	51.19±0.27 A	1.73±0.06 A	96.61±0.11 A	52.50±0.28 A	42.33±0.25 A	39.72±0.46 A
Bresnica	2014	36.26±1.00 c	1.52±0.05 b	96.02±0.12 b	45.10±0.49 c	31.28±0.37 c	34.99±0.53 b
	2015	40.12±1.01 b	1.54±0.06 a	95.90±0.12 b	47.68±0.41 b	39.84±0.41 b	34.97±0.50 b
	2016	42.54±0.99 a	1.56±0.05 a	96.74±0.12 a	50.11±0.48 a	43.54±0.45 a	39.48±0.53 a
	/ Average	39.64±1.00 C	1.54±0.06 B	96.22±0.12 B	47.63±0.46 B	38.22±0.41 B	36.48±0.52 B
Stapar	2014	40.76±0.54 a	1.62±0.02 b	96.12±0.07 a	47.21±0.2 b	39.18±0.30 b	37.45±0.23 c
	2015	42.65±0.52 a	1.64±0.01 b	95.87±0.07 b	45.14±0.22 b	37.92±0.28 c	38.54±0.21 b
	2016	44.45±0.62 a	1.69±0.04 a	96.34±0.10 a	51.38±0.22 a	41.49±0.32 a	39.18±0.22 a
	/ Average	42.62±0.56 B	1.65±0.02 A	96.11±0.08 B	47.91±0.22 B	39.53±0.30 B	38.39±0.22 A

LSD

P 0.05

The different letters in columns showed significant differences among means by LSD test at P 0.05

2016 . 2014 . Tree growth from 2014 to 2016 slowly increased with significant differences among years except locality Ljubi . Differences among all localities were significant, and the highest trunk cross sectional area (TCSA) was determined at locality Stapar and the smallest at locality Ljubi (Table 3). Similar results for different plum cultivars in Serbian conditions were obtained by Nenadovi -Mratini et al. (2007). Data in Table 3 showed that significant differences among years and localities are presented in cultivar Nada regarding to yield per tree. The largest yield was found at locality Stapar and the smallest at locality Bresnica . Regarding examined years, the largest yield was assessed in 2016. Considering the yield efficiency, there were no significant differences among localities and years. Generally, yield efficiency was smaller than obtained by Miloševi et al. (2009) and Miloševi et al. (2011) for different cultivars in Serbian conditions. This is probably due to high vigour and high values of TCSA in our study likely caused by Myrobalan seedling as a rootstock.

3. " ",
2014-2016 .

Table 3. Yield parameters of cultivar Nada grown on three localities in period 2014-2016

Locality	Year	Yield per three (kg)	TCSA (cm ²)	Yield efficiency (kg cm ⁻²)
Ljubi	2014	21.72±0.27 b	201.48±7.55 b	0.11±0.00 a
	2015	21.44±0.29 b	203.42±6.88 a	0.10±0.00 a
	2016	23.11±0.25 a	204.88±5.82 a	0.11±0.01 a
	/ Average	22.09±0.28 B	203.26±6.75 A	0.11±0.00 A
Bresnica	2014	18.88±0.30 c	187.77±4.73 b	0.10±0.00 b
	2015	20.12±0.31 b	189.14±5.41 b	0.11±0.00 b
	2016	22.08±0.32 a	191.53±6.45 a	0.12±0.01 a
	/ Average	20.36±0.31 C	189.48±5.53 A	0.11±0.00 A
Stapar	2014	24.44±0.26 a	196.57±8.70 c	0.12±0.00 c
	2015	20.94±0.30 b	197.52±8.38 b	0.11±0.01 b
	2016	25.45±0.31 a	199.01±9.32 a	0.13±0.01 a
	/ Average	23.61±0.29 A	197.70±8.80 A	0.12±0.01 A

LSD

P 0.05

The different letters in columns showed significant differences among means by LSD test at P 0.05

4

" "

Data in Table 4 showed the existence of significant variations among fruit chemical properties of cultivar Nada in examined localities and years.

4. " ",
2014-2016 .

Table 4. Chemical properties of cultivar Nada grown on three localities in period 2014-2016

Locality	Year	Soluble solids (°Brix)	Total sugars (%)	Invert Sugars (%)	Sucrose (%)	Total acids (%)	pH
Ljubi	2014	13.74±0.06 b	9.86±0.05 c	6.92±0.05 b	3.28±0.22 b	0.33±0.01 b	4.17±0.00 a
	2015	14.36±0.08 b	11.71±0.04 a	6.94±0.05 b	3.38±0.32 a	0.36±0.01 b	3.97±0.01 a
	2016	15.61±0.07 a	10.74±0.05 b	7.89±0.05 a	3.84±0.30 a	0.48±0.01 a	3.38±0.01 b
	/ Average	14.57±0.07 B	10.77±0.04 B	7.25±0.05 A	3.68±0.03 C	0.39±0.01 A	3.84±0.01 C
Bresnica	2014	15.75±0.11 a	12.28±0.09 a	5.25±0.07 b	5.10±0.49 c	0.28±0.00 c	4.54±0.01 a
	2015	14.61±0.12 b	11.54±0.09 a	5.20±0.06 b	4.68±0.41 b	0.32±0.01 b	3.97±0.01 b
	2016	16.14±0.13 a	10.56±0.10 b	6.74±0.07 a	5.11±0.48 a	0.36±0.01 a	4.48±0.01 a
	/ Average	15.50±0.13 A	11.46±0.09 A	5.73±0.07 C	5.85±0.05 A	0.32±0.01 B	4.33±0.01 A
Stapar	2014	13.17±0.11 a	10.94±0.04 b	6.38±0.06 a	4.21±0.2 b	0.31±0.00 c	4.15±0.01 a
	2015	14.02±0.10 a	10.04±0.05 b	6.87±0.07 a	4.14±0.22 b	0.35±0.00 b	3.74±0.01 b
	2016	15.41±0.09 a	11.69±0.04 a	6.34±0.06 a	5.38±0.22 a	0.45±0.00 a	4.02±0.02 a
	/ Average	14.20±0.10 B	10.89±0.05 B	6.53±0.06 B	4.21±0.03 B	0.37±0.00 A	3.97±0.01 B

LSD

P 0.05

The different letters in columns showed significant differences among means by LSD test at P 0.05

ripening time was similar on all localities and years and was established in mid-August. The highest values of fruit parameters were found in cultivar Nada grown at locality Ljubi. The largest yield per tree and yield efficiency were obtained when this cultivar was grown at locality Stapar. The highest values of soluble solids, total sugars and sucrose content, as well as juice pH value were determined when cultivar Nada was grown at locality Bresnica, while the highest values of invert sugars and total acids were found when this cultivar was grown at locality Ljubi. Generally, it can be said that cultivar Nada showed very good results grown at all examined localities and years and could be very interesting as a cultivar in new commercial orchards in Serbia.

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TR 31064.

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*E-mail: rmiletic@institut-cacak.org

Biological and pomological characteristics of promising walnut genotypes

Rade Mileti *, Svetlana M. Paunovi , Žaklina Karaklaji -Staji ,
Jelena Tomi , Mira Milinkovi , Marijana Pešakovi

Fruit Research Institute Kralja Petra I/9, 32000 a ak, Republic of Serbia

SUMMARY

	2008	2016	
28			In the period between 2008 and 2016, research was conducted into major characteristics for 28 walnut genotypes, which had previously been selected in the region of East Serbia. By further grafting, grouping and cultivation under same conditions in the a ak region, five promising genotypes were selected. Genotype CV/3 is characterised by an average fruit weight of 13.6 g and kernel ratio of 55.0%. The fruit is oval in shape, with a smooth shell, which is coloured light brown and breaks easily.
13.6 g,		CV/3	
55.0%.			
CV/4			The kernel is medium-rough, light brown in colour and of an excellent taste. Genotype CV/4 is very large, with the fruit mass of 16.8 g and kernel ratio of 55.0%. It is round in shape, with a thin and light-coloured shell, light-yellow kernel and a very good taste. Genotype CV/11 is characterised by a large fruit, having a weight of 15.5g and kernel ratio of 52.2%. The fruit is elongated, mildly rough, light brown in colour. The kernel is medium-
16.8 g			
55.0%.			
CV/11			
		15.5 g	
		52.2%.	

18 g
52.0%.

2
16.5 22.0 g

50% 52%.

. CV12

) (Mitrovi et al., 2007).

(
Gnomonia leptostylai
Xanthomonas juglandis) (Germain, 1883;
Kora et al., 1990; Mitrovi et al., 2005).

, Miletic (1994)

, Mileti et al. (2003).

rough, with a good taste. CV/12 is characterised by an average fruit weight of 18 g and kernel ratio of 52.0%. The fruit is elongated, mildly rough and of thin, light-colour shell. The kernel is light yellow, easily separated from the shell and of a very good quality. Genotype 2 has a fruit weight in the range between 16.5 and 22.0 g and the usual kernel ratio between 50% and 52.0%. It is round in shape, with a thin, which is almost completely smooth, and of a darker colour. The kernel is light-brown, medium-rough and of a very good quality.

Key words: walnut, genotype, fruit, mass, kernel, kernel ratio

INTRODUCTION

Individual positive selection of the population is a worldwide method mainly used to obtain new genotypes of walnut. Exceptions are a small number of genotypes obtained through planned hybridization (USA, France) (Mitrovi et al., 2007). New walnut cultivars should have regular and vigorous birth rate, fruiting in clusters and on lateral buds. Significant objectives of walnut breeding are also high kernel ratio and kernel quality, resistance to late spring frosts and heavy snow, as well as tolerance to parasites (preferably tolerant/resistant to *Gnomonia leptostylai* and *Xanthomonas juglandis*) (Germain, 1883; Kora et al., 1990; Mitrovi et al., 2005).

In his long research, Miletic (1994) studied a population of walnuts in Eastern Serbia during which time he separated and described a larger number of selections, Mileti et al. (2003). After several years of testing, collection, under the same conditions in the region of Cacak, this paper presents the most important qualities of promising genotypes of walnut in line to join the procedure for the registration of varieties.

MATERIAL AND METHODS

2008
(28)

2010 2016

Gnomonia leptostyla.

(IPGRI).

0.01
(Mettler).

mm,

Examinations were carried out in a collection of walnut genotypes planted at the Fruit Research Institute in Cacak in 2008. All planted genotypes (28) originated from the region of Eastern Serbia. According to all indicators, five promising genotypes with the most favourable properties were singled out. For each walnut genotype in the period 2010 to 2016 phenological characteristics were monitored, as well as susceptibility to low winter and late spring frosts. Fruit coarseness, weight and kernel ratio were determined. All characteristics of fruit and kernel were assessed as well as susceptibility to pests *Gnomonia leptostyla*.

All the above features were evaluated according to the Descriptors for Walnut (IPGRI). The dimensions of the foetus were determined by measuring the digital floating criterion digital caliper with a resolution of 0.01 mm, and weight with a technical scale (Mettler).

RESULTS AND DISCUSSION

(28)

(4)

CV/11,

(1).

Selected walnut genotypes are characterized by early beginning of the growing season. The average time from the beginning of the season is the end of March (28th March) until the beginning of April (4th April). Pollen shedding, depending on the genotype happens in the second half of April or early May. Average time of flowering of female flowers is the third decade of April or early May. For these reasons, walnut genotypes are characterized by protrandry type of flowering. The exception is genotype CV/11, which is characterized by protogyny. The fruits ripen in the second half of September, while shedding or the end of the growing season is in the first week of November (Table 1).

1.

Table 1. Phenological characteristics of walnut genotypes

Genotype	Leafing onset	Pollen shedding	Flowers for fertilization	Flowering type	Ripening Onset	Shedding
CV/3	31.03.	15.04.	20.04.	/Protradry	25.09.	05.11.
CV/4	28.03.	25.04.	18.04.	/Protradry	22.09.	05.11.
CV/11	04.04.	17.04.	20.04.	/Protogyny	18.09.	10.11.
CV/12	04.04.	17.04.	01.05.	/Protradry	15.09.	08.11.
Genotype 2	28.03.	5.05.	01.05.	/Protradry	25.09.	05.11.

The fruits of the selected genotypes are large and very large, of weight 13.6 g (CV/3 to 18.0 g (CV/12). The kernel weight is from 7.5 g to 9.4 g. Therefore, the yield in all cases is over 50,0%, that is from 52.0 to 55.0% (Table 2).

2.

Table 2. Fruit weight

Genotype	Length (mm)	Width (mm)	Thickness (mm)	Fruit weight (g)	Kernel weight (g)	% Kernel content (%)
CV/3	38.2	32.4	33.1	13.6	7.5	55.0
CV/4	43.2	39.4	39.5	16.8	9.2	55.0
CV/11	47.3	36.6	34.0	15.5	8.1	52.2
CV/12	49.2	36.4	39.4	18.0	9.4	52.0
Genotype 2	53.0	36.1	37.4	16.5	8.6	52.0

According to shape factor, fruits of selected genotypes are rounded or elongated. The top seam is poorly expressed or expressed. The shell is smooth or slightly wrinkled, thin or medium. Shell colour is bright or light brown, while in genotype 2 dark. The shell of the fruit is generally easy to break (Table 3).

3.

Table 3. Important fruit characteristics

Genotype	Shape factor	Top seam	Seam	/Shell appearance	/Shell thickness	/Colour scales	/Breaking strength
CV/3	Oval	Poorly expressed	Strong	/Smooth	/Medium	Bright red	/Light
CV/4	Round	Poorly expressed	Medium	Slightly wrinkled	/Thin	/Bright	/Light
CV/11	Elliptic	/Expressed	Strong	/Smooth	/Medium	Bright red	/Light
CV/12	Elliptic	/Expressed	Strong	Slightly wrinkled	/Thin	/Bright	/Light
Genotype 2	-	-	Strong	/Smooth	/Thin	/Dark	/Medium

Kernel is in all cases bright, yellow or light brown, medium or coarse rough. It can be easily separated from the shell. Its quality is excellent, very good or good. It is characteristic that the primary membrane is thin which also affects the ease of removing kernel and a better kernel ratio (Table 4).

(4).

4.

Table 4. Main characteristics of the kernel

Genotype	/Primary membrane	Kernel colour	/Kernel roughness	Extraction of kernel	Kernel taste
CV/3	/Medium	Light brown	/Medium	/Easy	Excellent
CV/4	/Thin	/Yellow	/Medium	/Easy	Very good
CV/11	/Thin	Light brown	/Medium	/Easy	/Good
CV/12	/Thin	Light brown	/Medium	/Easy	Very good
Genotype 2	/Thin	/Yellow	/Medium	/Easy	Excellent

CV/3,
2017

2 CV/11,
CV/4 CV/12,
-20 -25 °C
2012

Gnomonia leptostyla.

CV/4, (5).

The highest birth rate was expressed by genotype 2 and CV/11, slightly lower by genotypes CV/4 and CV/12 while the lowest birth rate showed genotype CV/3, which later entered the fertility period. Extremely low temperatures between -20 and -25 °C were particularly expressed in January 2012 and 2017, but did not significantly affect bud damage and decrease in fertility.

Late spring frosts occurred in three years during the study period, but the effects did not significantly affect the decrease in fertility. It is obvious that the selected genotypes were characterized by more expressive resistance to low air temperatures. Depending on the beginning of growing season and rainfall, the genotypes without application of pesticide showed a low sensitivity to *Gnomonia leptostyla*. Sensitivity was very low and low, while in the genotype CV/4, medium (Table 5).

5.

Tab 15. Productive and biological properties

Genotype	Birth rate	Low winter temperatures (0-5)	Late spring frosts	Gnomonia	
				Susceptibility to Gnomonia (1-9)	Susceptibility to Gnomonia (1-9)
CV/3	++	5	++++	3/	/small/
CV/4	+++	4	++++	5/	/medium/
CV/11	++++	5	+++	3/	/small/
CV/12	+++	5	++++	3/	/small/
Genotype 2 ²	++++	4	+++	1/	/very small/

Aforementioned genotypes according to their size and kernel weight and kernel ratio were at the same level or slightly better than the standard domestic varieties Champion, a special selection of Rasna, which described Korac et al. (1998). According to the same properties, they were better than varieties of walnuts of the Eastern Serbia described by Miletic et al. (2002a; 2002b), as well as Mitrovic et al. (2007) from the territory of Central Serbia. By many characteristics, they surpass the varieties described by Džuvinov et al. (2013) in Bulgaria. However, according to the beginning of vegetation and the time of shedding and flowering, they were slightly earlier than all known and described varieties. An advantageous feature of the fruit and the kernel, yield and resistance to low winter temperatures and parasites were the advantages of these genotypes when it came to their commercial cultivation.

CONCLUSIONS

Genotype CV/3 is characterized by the fruit of average weight of 13.6 g and kernel ratio of 55.0%. The shape is oval, with a smooth shell, light brown colour, easily breakable. The kernel is medium rough, light brown colour, of an excellent flavour.

Genotype CV/4 is really large, with a weight of 16.8 g and kernel ratio of 55.0%. Round in shape, thin and light shell, kernel is bright yellow, of a very good taste.

Genotype CV/11 is characterized by a large fruit with the weight of 15.5 g

		52.2%	
	CV/12		
18 g		52.0%	
	2	16.5	16.5
22.0 g	50	52.0%	
	31093	46008,	

and kernel ratio of 52.2%. The fruit is elongated, slightly rough, light brown in colour. The kernel is medium rough, of a good taste.

Genotype CV/12 is characterized by the weight of 18 g and an average kernel ratio of 52.0%. The fruit is elongated, slightly rough, bright colours and thin shell. The kernel is light yellow, medium rough, easily singled out from shell, of a very good quality.

Genotype 2 weighs 16.5 and 16.5 to 22.0 g. Kernel ratio is mostly between 50 and 52.0%. The shape is rounded, almost smooth and thin shell, darker in colour. The kernel is light brown, medium rough, of a very good quality.

ACKNOWLEDGEMENTS

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(*Aronia melanocarpa*)

53,

1407

*E-mail: svetla.diankova@ikht.bg

Technological investigations for obtaining anthocyanins from black chokeberry (*Aronia melanocarpa*)

Svetla Dyankova*, Maria Doneva, Gabriela Marinova

*Institute of Cryobiology and Food Technologies, 53 Cherni Vrah Blvd.,
1407 Sofia, Bulgaria*

SUMMARY

The fruits of chokeberry are a valuable source of bioactive compounds that may be used in the production of new functional foods and beverages. It is believed that chokeberry juice has a beneficial effect in many diseases - hypertension, atherosclerosis, diabetes and the like. This effect is mainly due to anthocyanins, polyphenols and vitamins contained in it.

The aim of the study is to make a comparative analysis of the content of anthocyanins in the fruit juices and pulps from chokeberry. Technological researches for ultrasonic extraction were carried out with water-ethanol mixtures at a ratio of material:solvent – 1:1 and 1:2. The influence of two factors was studied: the concentration of ethanol and the duration of the process on the extraction of anthocyanins. It was found that the total quantity of extracted anthocyanins is greater in hydro modul 1:2. Under these conditions, the highest yield of anthocyanins was observed for the

1:1 1:2.

1:2.

75 %
 64 882,5 mmol/l
 Vit. C.

solvent – 75 % ethanol. The analysis of antioxidant activity (radical scavenging activity) of the produced extracts showed very high – up to 64 882,5 mmol/l equivalents of Vit. C.
Key words: chokeberry, extraction, anthocyanins, antioxidant activity

INTRODUCTION

Anthocyanins are among the most important plant pigments. They belong to a widespread class of phenolic compounds called flavonoids. Anthocyanins are glycosides of polyhydroxy or polymethoxy, derivatives of 2-phenylenebenzopyrylium (flavillium) salts (Figure 1).

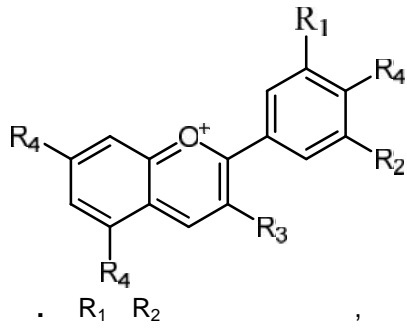


Fig. 1. The flavillium cation. R1 and R2 stand for H, OH or OCH3; R3 is glycosyl or H; R4 is OH or glycosyl

The differences between the individual anthocyanins are based on the number of hydroxyl groups, the type and number of sugars, attached to the molecule, the position of the bound of sugar residues and the type and number of aliphatic or aromatic acids, bound to the sugars in the molecule (Castaneda-Ovando et al., 2009; Scotter, 2011). To date, 17 types of anthocyanidins or aglycons and over 400 anthocyanidin glycosides have been detected. The most common anthocyanidins in higher plants are - pelargonidin (Pg), peonidin (Pn), cyanidin (Cy), malvidin (Mv), petunidin (Pt) and delphinidin (Dp). The glycosides of the three non-methylated

(Castaneda-Ovando et al., 2009; Scotter, 2011).
 17
 400
 (Pg),
 (Pn),
 (Mv),
 (Pt)
 (Cy),
 (Dp).

(Cy, Dp Pg)

80%

69% (Kong et al., 2003).

(Tsuda, 2012).

Rosaceae) Aronia (

Aronia melanocarpa (Michx.) Ell.,
Aronia arbutifolia (L.)
 Pers.– (Kokotkiewicz et al., 2010; Kulling and Rawel, 2008).
prunifolia ()
A. melanocarpa *A. arbutifolia*
 (Kokotkiewicz et al., 2010).
A. melanocarpa

(Bräunlich et al., 2013).

1790 mg/100 g

(- -) -3-
 -3-) (Oszmianski
 Wojdylo, 2005).

anthocyanidins (Cy, Dp and Pg) are the most common in nature and are present in 80% of the pigmented leaves, 69% of the fruit and 50% of the flowers (Kong et al., 2003).

It is believed that anthocyanins have a beneficial effect on obesity, diabetes, cardiovascular diseases and also on cognitive function (Tsuda, 2012). These effects are mainly due to their high antioxidant capacity. Consumption of foods rich in anthocyanins reduces blood pressure and insulin resistance and increases the resistance of capillaries.

The Aronia genus (Rosaceae family) includes two species of shrubs, originating from East North America and eastern Canada: *Aronia melanocarpa* (Michx.) Ell., known as black chokeberry and *Aronia arbutifolia* (L.) Pers. - red chokeberry (Kokotkiewicz et al., 2010; Kulling and Rawel, 2008). *Aronia prunifolia* (purple chokeberry) is a hybrid between *A. melanocarpa* and *A. arbutifolia* (Kokotkiewicz et al., 2010). *A. melanocarpa* fruits are used to produce juice, syrup, jam and wine. They are a rich source of anthocyanins, proanthocyanidins, chlorogenic and neochlorogenic acids and exhibit high antioxidant activity (Bräunlich et al., 2013). The total amount of anthocyanins in fresh fruit of aronia, range from 357 to 1790 mg/100 g of fresh weight (FW). Compared to other fruits, chokeberry has a more uniform anthocyanin composition – mainly cyanidin glycosides (cyanidin-3-arabinoside, cyanidin-3-galactoside, cyanidin-3-glucoside and cyanidin-3-xyloside) (Oszmianski and Wojdylo, 2005).

The aim of the study is to make a comparative analysis of the anthocyanin content in fruit juices and pomace from chokeberry and to establish the technological extraction parameters that result in the highest yield per unit of plant material.

MATERIAL AND METHODS

Materials:

The subject of current research was black chokeberry fruit - *Aronia melanocarpa* (Michx.) Ell. The fruits were collected from the Berkovitsa region of Bulgaria in August 2016. After washing, the fresh fruit was distributed in polyethylene bags, frozen at (-20 ° C) and kept in a freezer until the time of analysis.

Methods:

The chokeberry samples were unfrozen at room temperature and pressed to separate the juice from the pulp. The extraction was carried out with 10 variants of solvents: water-ethanol solutions at a concentration of 45 to 95% v/v, and a plant material: solvent ratio of 1:1 and 1:2. The samples were placed in an ultrasonic bath (Model 7652 Ultrasonic System) and were sonicated for 20 minutes, then left in the dark at room temperature for 24 to 96 hours. The resulting extracts were filtered through a glass filter with a pore size of 100-160 µm.

Dry matter content - The dry matter content of the plant raw material was measured with a Sartorius Thermo Control YTC 01L balances.

Quantitative content of anthocyanins in the extracts

The total content of monomeric anthocyanins in the extracts was determined spectrophotometrically by the pH-differential method (Lee et al., 2005).

Principle of the method: Monomeric anthocyanin pigments reversibly change their color when changing the pH. At pH 1.0 they exist in stained oxonium form, and at pH 4.5, a colorless hemiketal form prevails. The difference in absorption of pigments at 520 nm is proportional to their concentration. Polymeric anthocyanins are resistant to pH change and are not included in the measurement. The calculations are according to the following formula:

Aronia melanocarpa (Michx.) Ell.,
 2016
 (-20°)
 10
 45 95% /
 1:2. (Model 7652 Ultrasonic System) 20
 24 96
 100-160 µm.
 Sartorius Thermo Control YTC 01L.
 pH (Lee et al., 2005).
 1.0
 4,5
 520 nm

$$\text{Anthocyanin pigment (cyd eq mg/l)} = \frac{A \cdot Mw \cdot DF \cdot 10^3}{\epsilon \cdot l} \quad (1)$$

$A = (A_{520\text{nm}} - A_{700\text{nm}}) \text{ pH } 1.0 - (A_{520\text{nm}} - A_{700\text{nm}}) \text{ pH } 4.5$;
 $Mw = 449.2 \text{ g/mol}$ (cyd-3-glu);
 $DF = 26900$;
 $l = \text{cm}$;
 $10^3 =$ factor for conversion from g to mg.

(cyd eq mg/l).

(DPPH (2,2-diphenyl-1-picrylhydrazyl radical)

(mmol/l) (Marinova and Batchvarov, 2011).

Microsoft Excel 2013. (SD) ANOVA. 0.01.

g/100g; - 30,70±4,56 g/100g.

95 % - 1:1.

where $A = (A_{520\text{nm}} - A_{700\text{nm}}) \text{ pH } 1.0 - (A_{520\text{nm}} - A_{700\text{nm}}) \text{ pH } 4.5$;
 $Mw = 449,2 \text{ g/mol}$ for cyanidin-3-glucoside (cyd-3-glu);
 $DF = 26900$, molar extinction coefficient for cyd-3-glu, and $10^3 =$ factor for conversion from g to mg.

The results are presented as equivalents of cyanidin-3-glucoside (cyd eq mg/l).

Determination of total antioxidant capacity

Antioxidant ability (radical scavenging activity) is determined by a DPPH (2,2-diphenyl-1-picrylhydrazyl radical) spectrophotometric method and the results are presented as the equivalent of vitamin C (mmol / l) by calibration curve and the sample dilution factor (Marinova and Batchvarov, 2011).

Statistical analysis

The statistical processing of data was done with the help of Microsoft Excel 2013. Data represent mean ± standard deviation (SD) of three independent experiments. The data were analyzed by one-way ANOVA. Differences were considered statistically significant when the p level was less than 0.01.

RESULTS AND DISCUSSION

After unfreezing, the fruits were pressed to separate the pulp and juice, which were then treated separately, i. e. two options were examined – juice and pulp. The analysis of dry matter content in the chokeberry samples showed the following results: juice – 18,66 ± 1,50 g/100g; pulp – 30,70 ± 4,56 g/100g.

Due to the high moisture content of the raw materials, the initial extraction experiments were carried out with 95% ethanol, with a raw material: extract ratio of 1: 1. The observed amount of monomeric anthocyanins in fruit juice is

403,83±19,64 cyd eq mg/l. 403,83 ± 19,64 cyd eq mg/l. Fruit pulp extracts have a more than 5 times higher concentration of anthocyanins, respectively 2230.97 ± 60.70 cyd eq mg/l. Similar results have been cited by other authors (Oszmia ski and Lachowicz, 2016). Based on the results, fruit pulp was used in the experiments to optimize extraction. Tables 1 and 2 present the data for total monomer anthocyanin content in extract variants with a raw material: solvent ratio of 1:1 and 1:2, respectively. The statistical analysis of the results shows reliable differences in the amount of anthocyanins extracted depending on the concentration of the ethanol used.

1. : - 1:1.
± SD (n = 3)

Table 1. Total content of monomeric anthocyanins in chokeberry extracts at material:solvent ratio – 1:1. The results are presented as means ± SD (n = 3)

Ethanol concentration (%)	Anthocyanins content in extracts (cyd eq mg/l)			
	24 h	48 h	72 h	95 h
45	1665,1± 165,1	1723,3±83,5	1758,3±25,0	1619,8±28,2
50	1725,2± 205,2	1592,4±42,7	1756,5±100,0	1817,2±127,7
55	1863,7±273,7	1846,2±74,1	1847,1±26,5	1571,0±261,8
60	1852,0±252,0	1987,6±193,2	2140,9±15,0	2404,4±251,7
65	2097,2±197,2	2274,1±73,8	2182,5±38,5	2506,1±23,4
70	2177,3±177,3	2438,0±283,9	2339,4±50,1	1386,0±136,9
75	1853,2±296,8	2680,5±229,1	2124,2±38,5	2711,8±50,1
80	2267,4±177,4	2822,1±63,5	2785,4±20,3	2888,8±226,9
85	2185,5±85,5	2705,6±154,0	2775,3±20,9	2941,9±287,0
95	2316,0±416,0	2813,8±128,6	2798,7±11,5	2474,8±125,3

* ANOVA; (p < 0.01)
Statistical significance was determined by one-way ANOVA; significant differences between mean values (p < 0.01 for all variants)

2.

: - 1:2.
± SD (n = 3)**Table 2. Total content of monomeric anthocyanins in chokeberry extracts at material:solvent ratio – 1: 2. The results are presented as means ± SD (n = 3)**

Ethanol concentration (%)	Anthocyanins content in extract (cyd eq mg/l)			
	24 h	48 h	72 h	95 h
45	815,9±14,3	980,2±71,8	1245,7±66,8	1279,1±33,4
50	1266,9±36,5	1428,9±18,6	1885,2±76,9	1923,7±38,5
55	1438,8±13,2	1594,8±97,7	1842,2±60,8	1922,6±19,6
60	1068,3±141,8	1505,8±33,2	2255,2±275,5	2392,9±137,7
65	1777,5±50,0	2504,4±14,4	3029,0±53,3	3155,7±126,6
70	1444,9±153,2	2087,7±64,4	2620,0±56,0	2792,0±172,0
75	1450,9±201,2	2034,1±141,7	3091,5±281,0	3232,0±140,5
80	1228,8±100,0	1847,7±1,5	2129,6±213,0	2223,1±93,5
85	1387,9±90,2	1977,0±10,7	2571,6±204,6	2673,9±102,3
95	1078,7±38,2	1931,9±342,2	1895,3±128,6	1959,6±64,3

*

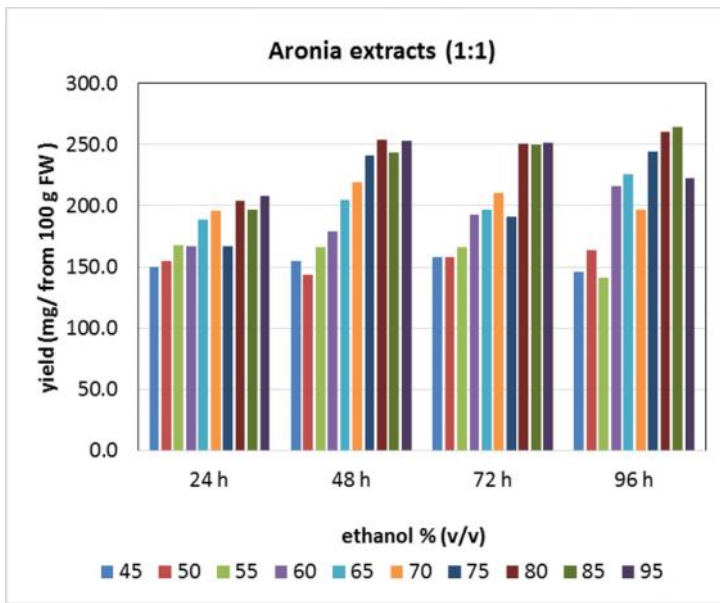
ANOVA;

(p < 0.01)

Statistical significance was determined by One-Way ANOVA; significant differences between mean values (p < 0.01 for all variants)

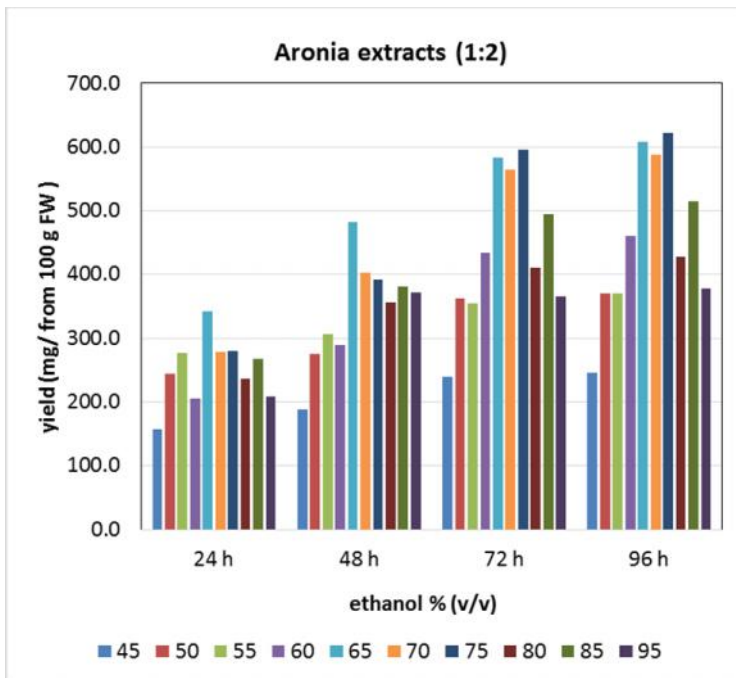
2 3
100 g
1:1 -
80 95%
48 h.
1:2 -
65 75%
- 72 h.
600 mg.
-

Figures 2 and 3 graphically show the results for the yield of anthocyanins of 100 g of fresh raw material in the different extraction variants. At a 1: 1 ratio, the highest values were observed at an ethanol concentration of 80 to 95% and a process duration of 48 hours. At a 1: 2 ratio, the best extraction of anthocyanins was obtained at an ethanol concentration of 65 to 75% and a duration of 72 hours. Under these conditions, the yield of anthocyanins reaches 600 mg. The content of anthocyanins in the extract remains unchanged for a longer duration of the process.



. 2. 100 g : - 1:1

Fig. 2. Total yield of anthocyanins from 100 g of fresh raw material in the different extraction variants, ratio raw material:solvent – 1:1



. 3. 100 g : - 1:2

Fig. 3. Total yield of anthocyanins from 100 g of fresh raw material in the different extraction variants, ratio raw material:solvent – 1:2

-
-
DPPH.
DPPH
64882,5 mmol/l Vit. C
Barry Halliwell –
(Halliwell
et al., 1995).
(Denev et al., 2012; Hwang et al., 2014).

Extracts with the highest anthocyanin content were tested for antioxidant activity by the DPPH method. This analysis measures the ability of the extract to transfer electron and to scavenge the DPPH radical. The result obtained – 64882.5 mmol/l Vit. C equivalents are indicative of a strong antioxidant action. The classic definition of antioxidants is given by British scientist Barry Halliwell – substances, which in low concentrations inhibit or prevent oxidation of available oxidizable substrates (Halliwell et al., 1995).
Like vitamins C and E, many phytochemicals, especially polyphenols, have the ability to capture free radicals before they attack biologically important molecules. Thus, the antioxidant substances in the chokeberry extract act (Denev et al., 2012; Hwang et al., 2014).

CONCLUSIONS

–
1:2.
75
% – 600 mg/100 g

The influence of two factors – ethanol concentration and duration of the process on the yield of anthocyanins from black chokeberry fruit - has been investigated. It was found that the total amount of anthocyanins extracted was greater in the 1:2 ratio (raw material:solvent). Under these conditions, the highest yield of anthocyanins was observed for 75% ethanol – 600 mg/100 g of fresh raw material.
The radical scavenging capacity of the aqueous-ethanolic chokeberry extracts was analyzed and found to show high antioxidant activity due not only to the high content of anthocyanins but also to the accompanying phytochemicals. These results demonstrate the potential of the tested extracts to be used as a source of antioxidants.
The fruit pulp left after the production of aronia juice is an

inexpensive and affordable source of valuable biologically active substances.

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Chaenomeles sp.

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*E-mail: mistoyanova@abv.bg

Antibacterial activity of *Chaenomeles* fruits against plant pathogenic bacteria

Mariya Stoyanova^{1*}, Miroslava Valkova¹, Teodora Mihova²,
Nevena Bogatzevska¹

¹Institute of soil Science, Agrotechnologies and Plant Protection
"Nikola Pushkarov", 7 "Shose Bankya" Str., 1331 Sofia, Bulgaria

²Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str.,
5600 Troyan, Bulgaria

SUMMARY

Chaenomeles sp.
– *Xanthomonas vesicatoria*, *Xanthomonas euvesicatoria*, *Xanthomonas gardneri*, *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas syringae* pv. *tomato*.

The aim of this study was to test the effect of fruit extract of six genotypes of *Chaenomeles* sp. against phytopathogenic bacteria of tomato and pepper – *Xanthomonas vesicatoria*, *Xanthomonas euvesicatoria*, *Xanthomonas gardneri*, *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas syringae* pv. *tomato*. Freezed fruits from six genotypes were Soxhlet extracted with methanol and obtained extracts were concentrated in a vacuum vaporizer.

in vitro

Study was conducted in vitro by agar diffusion method in triplicate. Average diameter of inhibitory zones and standard deviation were calculated. All extracts possessed antibacterial activity against all tested bacteria. The diameter of sterile zones formed by 5% water solutions of the extracts on 24th hour was between 10

5%

24- 10 21 mm, C.
michiganensis subsp. *michiganensis* (15-21 mm),
P. syringae pv. *tomato* (12-18 mm),
X. euvesicatoria (11-14 mm),
C. michiganensis subsp. *michiganensis* (72-12-14 mm).
Chaenomeles sp.
 6'. 3 8h
 22 29,
 : *Chaenomeles*,
Xanthomonas, *Clavibacter michiganensis*,
Pseudomonas syringae pv. *tomato*

and 21 mm.
C. michiganensis subsp. *michiganensis* was most sensitive with zones between 15-21 mm followed by *P. syringae* pv. *tomato* (inhibitory zones 12-18 mm). Most resistant were the strains of *X. euvesicatoria* with zones between 11 and 14 mm. *C. michiganensis* relatively overcome the antibacterial effect of the extracts up to 72 hours after incubation (sterile zones 12-14 mm). The tested genotypes of *Chaenomeles* sp. showed different antibacterial activity against the pathogens. Lowest activity was observed for genotypes 3 8h and 6'. Genotypes 22 and 29, which were characterized by the largest and heaviest fruits, showed the highest activity. The concentration of tanning substances in these fruits was the lowest compared to the others which presumes that the established antibacterial properties were due to different substances.

Key words: *Chaenomeles*, antibacterial activity, extract, phytopathogens, tomato, pepper, *Xanthomonas*, *Clavibacter michiganensis*, *Pseudomonas syringae* pv. *tomato*

INTRODUCTION

(*Chaenomeles* sp. Lindl),
 . *Rosaceae*.
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 a
 (Mihova-

Japanese quince (*Chaenomeles* sp. Lindl) is a perennial decorative shrub from family *Rosaceae*. The plant originates from Eastern Asia. In Japan, Korea and China the plant has been grown from centuries for food, medicinal and ornamental purposes. As a crop *chaenomeles* is known also in many Northern countries like Canada, Sweden, Norway, Latvia, Lithuania, Belarus, Ukraine, and Russia but it is also grown in Australia. In Middle-Eastern Europe the plant was cultivated from the end of 18th century. Japanese quince was imported in Bulgaria as a fruit plant four decades ago. Since then, the plant has been widely distributed in the country as an ornamental because of its beautiful

Chavdarova, 2016).

Thunberg 1784. *Chaenomeles* sp.

Malus ()
(Rumpunen, 2002).

al. (1990)

Chaenomeles japonica
Chaenomeles superba.

50 g,
70-80 g.

(Mihova-Chavdarova, 2016).

Chavdarova, 2016).

20

(Mihova-Chavdarova, 2016).

flowers (Mihova-Chavdarova, 2016).

The plant was described for the first time from Thunberg in 1784. *Chaenomeles* sp. is from subfamily *Maloideae* and is most related to *Cydonia* (quince), *Malus* (apple) and *Pyrus* (pear) (Rumpunen, 2002). According to some authors the genus includes two subgenera with overall four species but Phipps et al. (1990) refers four species and four interspecies hybrids to genus *Chaenomeles*. There is no enough information about the morphological differences among the species and hybrids especially between *Chaenomeles japonica* and *Chaenomeles superba*.

Chaenomeles plants form golden in color aromatic fruits which due to their rich chemical composition have been used in medicine and gastronomy in Eastern Asia and Ancient Greece. The fruits are middle-sized with average weight of 20-50 g but some varieties can reach 70-80 g. The fruits contain organic acids, phenols, flavonoids, proanthocyanic oligomers, macro- and micro-elements, vitamins from B-group. Vitamin C is of notably high concentration but the fruits are not eatable due to the too acidic taste and hard texture.

Chaenomeles plants are characterized by resistance to drought and cold. They grow well on rich of organic matter, well structured, clay and clay-sandy soils. However, the plants can adapt to more rocky and gravel soils. These features describe *chaenomeles* as adaptive species in the changing soil-climatic conditions and with potential for widening of their distribution (Mihova-Chavdarova, 2016).

The chemical composition and pharmacological activity of the fruits have been studied in the past two decades, mainly in China. The potential for clinical applications was partly investigated (Mihova-Chavdarova, 2016). The antibacterial properties of essential oil

Chaenomeles speciosa - *Staphylococcus* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Proteus mirabilis* *Klebsiella pneumoniae* (Xie et al., 2007).

Chaenomeles sp.

Chaenomeles (Mihova-Chavdarova, 2016). -10° (Mihova et al. (2012). *Xanthomonas gardneri* (2), *Xanthomonas vesicatoria* (4), *Xanthomonas euvesicatoria* (3), *Pseudomonas syringae* pv. *tomato* (3) *Clavibacter michiganensis* subsp. *michiganensis* (1)

80 ° 5
55° , 300 mbar.

from dry fruits of *Chaenomeles speciosa* against *Staphylococcus* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Proteus mirabilis*, and *Klebsiella pneumoniae* has been established (Xie et al., 2007). However, the data of chaenomeles' antibacterial properties are only partially known as the plants have been grown mainly for the purposes of food industry.

The aim of this study was to test the effect of fruit extract of different genotypes of *Chaenomeles* sp. against phytopathogenic bacteria of tomato and pepper.

MATERIAL AND METHODS

Plant material: Fresh fruits were collected from four fields on the territory of Research Institute of Mountain Stockbreeding and Agriculture, Troyan, Bulgaria (Mihova-Chavdarova, 2016). Plant materials were used freeze-dried at -10 ° before extraction. Six genotypes of *Chaenomeles* were used in this study. Major characteristics were determined as described by Mihova et al. (2012): presence/lack of thorns, weight of fruits, dry matter, concentration of total carbohydrates, pectins, organic acids, vitamin C, and tanning substances.

Bacterial strains: Test bacteria were *Xanthomonas gardneri* (2 strains, isolated from tomato), *Xanthomonas vesicatoria* (4 strains, isolated from tomato), *Xanthomonas euvesicatoria* (3 strains from pepper), *Pseudomonas syringae* pv. *tomato* (3 strains), and *Clavibacter michiganensis* subsp. *michiganensis* (1 strain) from the collection of Prof. DSci N. Bogatzevska, ISSAPP "N. Pushkarov".

Extractions: Extractions were prepared with methanol in Soxhlet extractor at 80° for 5 hours and solvent was separated in vacuum vaporizer at 55° , 300 mbar. Extracts were further

72 mbar, 70° , 72 mbar. The extracts were stored at 18° in air tight brown bottles. Water solutions (% , w/v) were freshly prepared 1 h before the assay.

Antibacterial assay: The *in vitro* test for antibacterial activity was completed by the agar diffusion method on Nutrient agar with 0.2% glucose. Bacterial suspensions of 100 µl, 1.5x10⁷ cfu/ml from 24-hour bacterial cultures were used for inoculums. The wells were filled with 50 µl of each substance and left for 2 h prior to incubation. Incubation was held at 28°C for 48 h. The antibacterial activity was assessed by measuring the inhibition zones in millimetre (diameter) on the 24th, 72nd and 120th hour. The experiments were performed in triplicate and the standard deviation was calculated.

concentrated at decreasing pressure to 70° , 72 mbar. The extracts were stored at 18° in air tight brown bottles. Water solutions (% , w/v) were freshly prepared 1 h before the assay.

Antibacterial assay: The *in vitro* test for antibacterial activity was completed by the agar diffusion method on Nutrient agar with 0.2% glucose. Bacterial suspensions of 100 µl, 1.5x10⁷ cfu/ml from 24-hour bacterial cultures were used for inoculums. The wells were filled with 50 µl of each substance and left for 2 h prior to incubation. Incubation was held at 28°C for 48 h. The antibacterial activity was assessed by measuring the inhibition zones in millimetre (diameter) on the 24th, 72nd and 120th hour. The experiments were performed in triplicate and the standard deviation was calculated.

RESULTS AND DISCUSSION

Chaenomeles sp.

The genotypes of *Chaenomeles* sp. differ in presence or lack of thorns, fruit weight and concentration of total carbohydrates, organic acids, tanning substances, pectin, and vitamin C (Table 1).

1. ***Chaenomeles* sp.,**
Table 1. Tested genotypes of *Chaenomeles* sp.

Characteristics Genotype	Plantation	Thorns	Weight (g)	Dry matter (Re%)	Total carbohydrates (%)	Pectin (%)	Acids (%)	Vitamin C (mg)	Tanning substances (%)
3 8h	2	+	39,00	11,50	4,85	0,890	2,51	73,92	0,810
Tch	3	+	49,13	10,00	3,70	0,280	2,45	100,32	0,582
6'	1	-	37,90	11,50	2,40	0,430	2,27	149,60	0,763
22	4	-	62,60	13,00	2,25	1,580	2,54	96,80	0,580
27	2	+	58,08	15,00	6,85	1,360	2,36	136,40	1,073
29	2	+	60,23	12,00	2,10	0,990	2,74	140,80	0,392

All tested extracts have antibacterial activity against the pathogens of tomato and pepper. The

5%
24
10 21 mm.
-
24-
michiganensis subsp. *michiganensis* -
15 21 mm
P. syringae pv. *tomato* (-
12 18 mm) (2). -
X. euvesicatoria
11 14 mm

X. vesicatoria, *X. gardneri* *P.*
syringae pv. *tomato*
(2). 14 17 mm

diameter of sterile zones formed by 5% water solutions of the extracts on 24th hour was between 10 and 21 mm.

The *C. michiganensis* subsp. *michiganensis* was most sensitive with zones between 15-21 mm (Table 2) followed by *P. syringae* pv. *tomato* (inhibitory zones 12-18 mm). Most resistant were the strains of *X. euvesicatoria* with zones between 11 and 14 mm. *C. michiganensis* relatively overcome the antibacterial effect of the extracts up to 72 hours after incubation (sterile zones 12-14 mm). The inhibitory zones of *X. vesicatoria*, *X. gardneri*, and *P. syringae* pv. *tomato* are relatively close in size between 14 and 17 mm (Table 2).

2.

Chaenomeles* sp.** ***Xanthomonas , ***P.***
syringae* pv. *tomato ***C. michiganensis* subsp. *michiganensis***
(±)

Table 2. Antibacterial activity of extracts from *Chaenomeles* sp. against pathogens from genus *Xanthomonas* of tomato and pepper, *P. syringae* pv. *tomato* and *C. michiganensis* subsp. *michiganensis* of tomato (mean values of the inhibitory zones in mm ± standard deviation)

Genotype	Tch	3 8h	6'	22	27	29
Strain						
<i>X. gardneri</i> 66t	13.67 ± 0.58	12.33 ± 0.58	12.67 ± 1.15	14.33 ± 1.53	12.67 ± 0.58	16.67 ± 0.58
<i>X. gardneri</i> 73t	14.33 ± 0.58	12.67 ± 0.58	12.67 ± 1.15	15.00 ± 1.00	13.33 ± 1.53	16.33 ± 0.58
<i>X. vesicatoria</i> 31t	14.00 ± 0.00	13.00 ± 0.00	13.67 ± 1.15	15.00 ± 1.00	14.33 ± 0.58	17.67 ± 0.58
<i>X. vesicatoria</i> 39t	12.67 ± 0.58	12.00 ± 0.00	12.00 ± 0.00	13.67 ± 0.58	11.67 ± 0.58	14.33 ± 1.15
<i>X. vesicatoria</i> 53t	13.00 ± 1.00	11.33 ± 0.58	11.67 ± 0.58	14.33 ± 0.58	12.67 ± 1.15	16.67 ± 0.58
<i>X. vesicatoria</i> 58t	14.33 ± 1.53	12.33 ± 1.15	13.00 ± 1.00	14.33 ± 1.53	16.33 ± 1.53	17.67 ± 1.53
<i>X. vesicatoria</i> 1	16.00 ± 1.00	13.33 ± 1.15	15.67 ± 2.08	17.33 ± 0.58	14.00 ± 1.73	17.33 ± 1.53
<i>X. vesicatoria</i> 8	14.33 ± 1.53	12.00 ± 0.00	13.00 ± 1.00	15.67 ± 0.58	12.33 ± 0.58	16.67 ± 0.58
<i>X. vesicatoria</i> 14	16.00 ± 0.00	13.33 ± 0.58	13.67 ± 0.58	17.00 ± 1.00	15.00 ± 1.00	18.33 ± 0.58
<i>X. vesicatoria</i> 19	12.67 ± 1.53	11.33 ± 1.15	12.33 ± 1.15	14.33 ± 0.58	13.00 ± 0.00	16.00 ± 1.00
<i>X. vesicatoria</i> 53	14.33 ± 0.58	13.00 ± 0.00	13.33 ± 0.58	15.33 ± 1.15	13.33 ± 0.58	17.00 ± 1.00
<i>X. vesicatoria</i> 44M	14.67 ± 1.15	13.00 ± 0.00	14.00 ± 0.00	15.67 ± 1.53	13.33 ± 0.58	17.33 ± 0.58
<i>X. euvesicatoria</i> 5	11.00 ± 0.00	10.33 ± 1.53	12.67 ± 1.53	14.33 ± 1.15	12.00 ± 0.00	13.00 ± 0.00
<i>X. euvesicatoria</i> 10	11.33 ± 1.15	11.00 ± 1.00	11.33 ± 0.58	13.33 ± 1.53	11.33 ± 2.52	14.33 ± 0.58
<i>X. euvesicatoria</i> 61	12.67 ± 0.58	12.00 ± 1.00	11.67 ± 2.08	15.00 ± 1.00	12.00 ± 1.00	14.33 ± 0.58
<i>P. syringae</i> pv. <i>tomato</i> R0 - 1	15.33±0.58	12.00±0.00	13.57±0.58	16.33±0.58	14.00±1.00	17.00±1.00
<i>P. syringae</i> pv. <i>tomato</i> R0 - 2	15.33±0.58	12.33±0.58	13.33±0.58	16.67±1.15	14.33±0.58	17.33±0.58
<i>P. syringae</i> pv. <i>tomato</i> R1	15.67±0.58	13.00±1.00	14.33±0.58	16.33±1.15	15.00±0.00	17.67±0.58
<i>C. michiganensis</i> subsp. <i>michiganensis</i> (24 th hour/24)	18.67±0.58	15.00±1.00	15.33±2.08	21.33±1.15	17.33±0.58	19.67±1.53
<i>C. michiganensis</i> subsp. <i>michiganensis</i> (72 nd hour/72)	14.00±1.00	13.00±0.00	12.33±0.58	14.00±0.00	13.00±0.00	14.00±0.00

72

X. vesicatoria, *X. euvesicatoria*, *X. gardneri* *P. syringae* pv. *tomato*.
C. michiganensis subsp. *michiganensis*

mm (2).

P. syringae pv. *tomato* > *X. vesicatoria* > *X. gardneri* > *C. michiganensis* subsp. *michiganensis* > *X. euvesicatoria*,

P. syringae pv. *tomato*,
- *X. euvesicatoria*.

Chaenomeles sp.

(1 2).

Up to 72nd hour changes in the sizes of inhibitory zones of *X. vesicatoria*, *X. euvesicatoria*, *X. gardneri*, and *P. syringae* pv. *tomato* were not observed. *C. michiganensis* subsp. *michiganensis* relatively overcame the antibacterial activity of the extracts. The sterile zones at full growth of the pathogen varied between 12 and 19 mm (Table 2).

The antibacterial activity of methanol extracts of chaenomeles fruits against the pathogens of tomato and pepper was as follows:

P. syringae pv. *tomato* > *X. vesicatoria* > *X. gardneri* > *C. michiganensis* subsp. *michiganensis* > *X. euvesicatoria* where the best activity was observed against *P. syringae* pv. *tomato* and lowest against *X. euvesicatoria*.

The tested genotypes of *Chaenomeles* sp. showed different antibacterial activity against the pathogens (Figure 1 and 2).

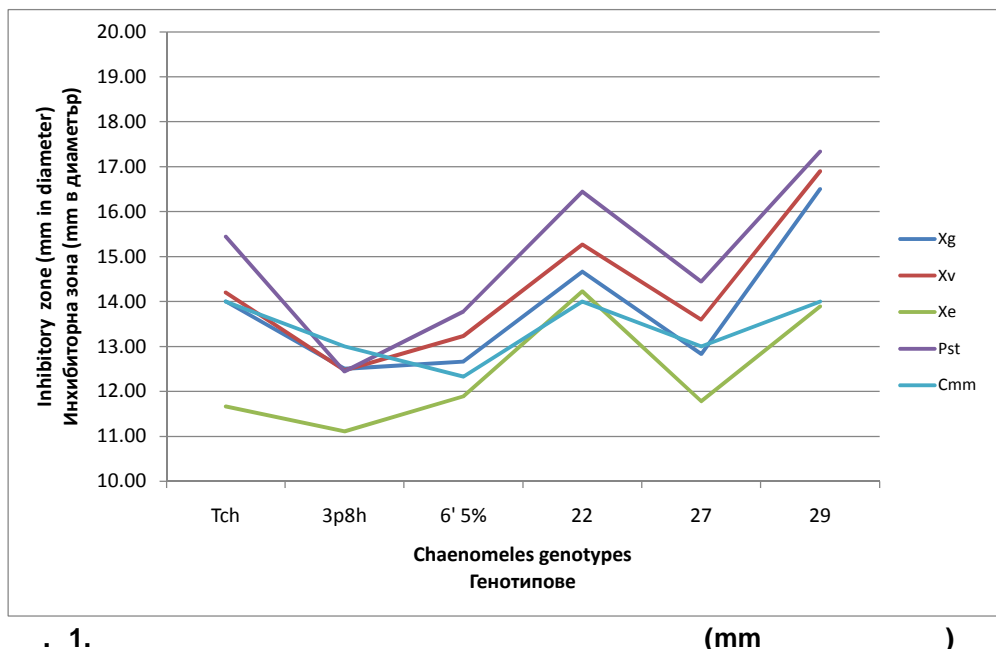
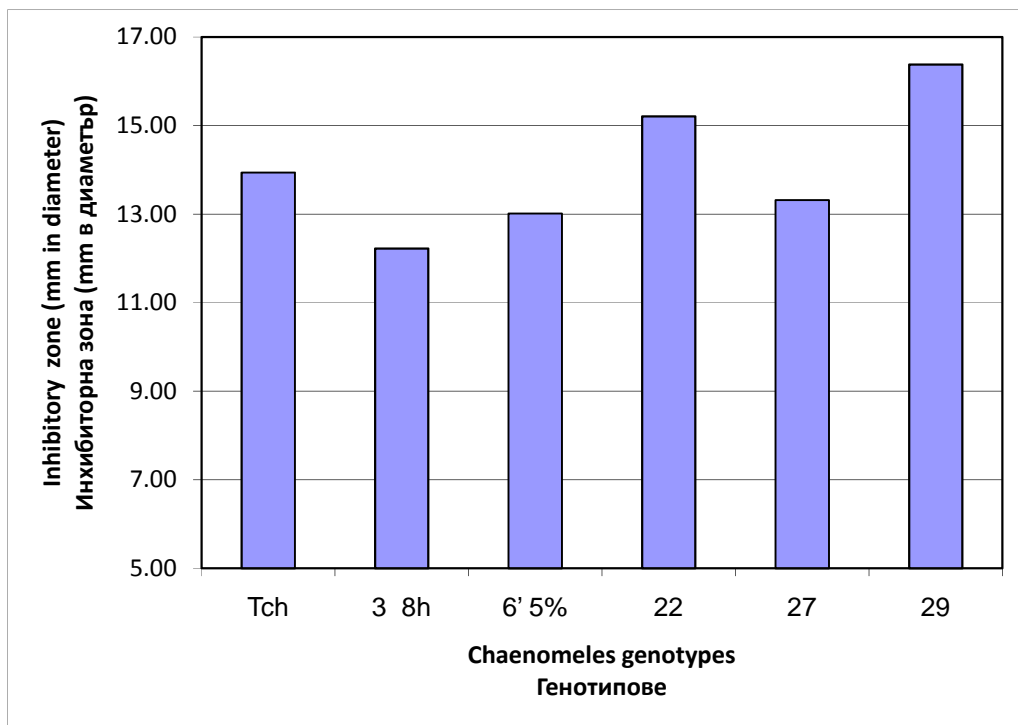


Fig. 1. Average values of inhibitory zones (mm in diameter) of different pathogenic species formed by the extracts from different genotypes *Chaenomeles* sp.



2. (mm)
Chaenomeles sp.

Fig. 2. Average values of inhibitory zones (mm in diameter) of the extracts from different genotypes *Chaenomeles sp.*

3 8h 6' 22 29,
Escherichia coli, Salmonella

Lowest activity was observed for genotypes 3 8h and 6'. Genotypes 22 and 29, which were characterized by the largest and heaviest fruits, showed the highest activity. The concentration of tanning substances in these fruits was the lowest compared to the others which presumes that the established antibacterial properties were due to different substances. The rest fruit features like dry matter, concentration of total carbohydrates, pectin, and vitamin C do not have relation to the antibacterial activity as these can be themselves nutrition substrates for the pathogenic bacteria.

In the past years antimicrobial effects were established for organic acids and small molecules against *Escherichia coli, Salmonella sp, Clostridium*

sp, *Clostridium perfringens*, *Listeria monocytogenes*, *Campylobacter* sp. (Brul and Coote, 1999) –

Chaenomeles speciosa -
Bacillus subtilis,

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Escherichia coli* *Salmonella typhi* (Gan et al., 2015).

10

(Xie et al., 2007).

(CN 1261085 A Google

Patents).

Chaenomeles

(Rumpunen, 2002; Mezhenskyj, 2004).

Xanthomonas P. syringae pv. *tomato*

(Vancheva et al., 2014).

C. michiganensis subsp. *michiganensis*

perfringens, *Listeria monocytogenes*, *Campylobacter* sp. (Brul and Coote, 1999) – all of which are present in the

fruits of *chaenomeles*. Alkaloids from *Chaenomeles speciosa* showed activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi* (Gan et al., 2015). The essential oil of fruits had activity against 10 micro-organisms, the Gram-negative bacteria being more sensitive (Xie et al., 2007). These characteristics of *chaenomeles* fruits made possible the construction of antimicrobial mold putty powder used in building (CN 1261085 A Google Patents).

Chaenomeles sp. are characterized with high adaptability and ability to be grown at different soil-climatic conditions. Some varieties and hybrids show high fruit yield (Rumpunen, 2002; Mezhenskyj, 2004). The plants do not require special cares which makes them suitable for industrial cultivation.

At the same time, the means of control of the plant pathogenic bacteria of tomato and pepper from genus *Xanthomonas* and *P. syringae* pv. *tomato* that are used now are copper-based products in combination with agrotechnical measures. Unfortunately, because of their extensive use many of the pathogenic strains have developed resistance to copper ions (Vancheva et al., 2014). Up to now, there are no known means of control of *C. michiganensis* subsp. *michiganensis* of tomato.

The morphological and biological characteristics, the high ecological plasticity of the *Chaenomeles* sp. and the established in this study antibacterial properties of the fruits against the economically important phytopathogens of tomatoes and pepper reveal good perspectives for the development of means of control based on plant fruit extracts.

CONCLUSIONS

– *Xanthomonas vesicatoria*, *Xanthomonas euvesicatoria*, *Xanthomonas gardneri*, *Pseudomonas syringae* pv. *tomato* *Clavibacter michiganensis* subsp. *michiganensis*.

22 29.

The methanol extracts from frozen fruits of six genotypes *Chaenomeles* possess antibacterial activity against the pathogens of tomato and pepper – *Xanthomonas vesicatoria*, *Xanthomonas euvesicatoria*, *Xanthomonas gardneri*, *Pseudomonas syringae* pv. *tomato*, and *Clavibacter michiganensis* subsp. *michiganensis*. Best activity possess the extracts from genotypes 22 and 29.

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”, . 02/4.”

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