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Original Article

Impact of Raspberry (*Rubus idaeus* L.) Primocane Tipping on Fruit Yield and Quality

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Abstract

High temperature stress, which has been occurring more often in recent years, usually coincides with the flowering of primocane raspberries and causes a negative effect on fruit quality parameters. One of the methods of delaying raspberry flowering and fruit development to avoid high summer temperatures is tipping the young primocanes. The aim of the study was to investigate how this practice affects the fruit characteristics and primary and secondary metabolites of two primocane raspberry cultivars ('Amira' and 'Polka'). For this purpose, we performed primocane tipping on two different dates in late spring and analyzed the berries from three subsequent sampling dates. High performance liquid chromatography-mass spectrometry (HPLC-MS) analyses were used for the identification and quantification of individual phenolic compounds and HPLC analyses for individual sugars and organic acids. Primocane tipping had great influence on the beginning of the harvest season of both cultivars. The impact on fruit yield was insignificant. Sampling date had a greater influence on fruit metabolite contents than did different treatments, with cultivar 'Polka' showing a greater response to primocane tipping than cultivar 'Amira'. Based on primary and secondary metabolites, it is difficult to say which treatment provided the best results, since dissimilar patterns were shown at different sampling dates and between cultivars. With negligible differences in fruit quality, primocane tipping was shown to be a good cultivation practice for delaying the production season of raspberries.

Keywords: anthocyanins, fruit weight, cane removal, seasonal variation, sugar/organic acid ratio, phenolic compounds

Introduction

Raspberry (*Rubus idaeus* L.) is an important berry crop for both the fresh and the processing market. In addition to vitamins and minerals, a diverse range of polyphenols are present in the berries, including flavonoids such as anthocyanins, flavonols and flavanols, ellagitannins and ellagic acid derivatives (Wang *et al*., 2009; Veberic *et al*., 2015; Kula *et al*., 2016; Sójka *et al*., 2016).

Although genetic predisposition is the major factors influencing fruit quality (Milivojevic *et al*., 2011; Kula *et al*., 2016), different agricultural practices (Qiu *et al*., 2016; Palonen *et al*., 2017), fruit maturity (Stavang *et al*., 2015) and environmental conditions can also affect berry metabolite amounts and composition (Anttonen and Karjalainen, 2005; Remberg *et al*., 2010; Mazur *et al*., 2014a,b). Raspberries, like some other berries, ripen successively and are harvested over a period of a few weeks. As the harvest season progresses, climatic conditions can significantly impact on fruit quality (Miletić *et al*., 2015; Zorenc *et al*., 2016). Climatic factors, which can change a great deal in the natural environment and can significantly affect growth and fruit quality, are light conditions (intensity, quality, photoperiod), temperature and precipitation (Sønsteby and Heide, 2012; Mazur *et al*., 2014b; Woznicki *et al*., 2016), although there are also other abiotic and biotic stress factors. The highpoint of raspberry harvesting in the Slovene climate is usually in late July or early August, which coincides with high temperatures, frequently causing thermal shock for plants. Stress due to high temperatures can affect raspberry flower development and therefore berry size, fruit yield and other quality parameters (Gotame *et al*., 2013). To avoid such stress conditions, young first-year canes (primocanes) can be removed when they are approximately 1 m or less tall, to delay or extend the harvest season. Tipping the primocanes in the late spring/early summer stimulates the growth of lateral branches and delays fruiting by 3 or more weeks (Oliveira *et al*., 1998; Drake and Clark, 2003; Thompson *et*

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al., 2009; Strik, 2012). On the other hand, late season production can influence fruit quality parameters, since lower temperatures, light intensity and shorter day length are common in September/October (Ali *et al*., 2011).

Summer tipping of raspberry primocanes for off-season production was previously tested by Oliveira *et al*. (1996, 1998, 2004) in Portugal. The results showed that cutting the canes at ten nodes promoted better growth, increased yield and greatly advanced the harvest in various raspberry cultivars. Similar results were also shown by Strik and Buller (2012) for primocane-fruiting blackberry, while Drake and Clark (2003) reported no difference in yield and berry weight between canes tipped at 1 m height and untipped control. Young cane removal with a combination of plant growth retardant application was also previously researched on the raspberry cultivar 'Willamette' in Serbia (Poledica *et al*., 2012). Additionally, various cultivation techniques, including young cane removal, were also applied to various raspberry cultivars in Poland (Lewandowski *et al*., 2015). However, the two studies showed different influences on some vegetative and generative characteristics. Although no effect of cane removal on total anthocyanins, total phenolics and total antioxidant capacity has previously been reported (Poledica *et al*., 2012), the influence of primocane tipping on metabolites has not been researched in detail.

With primocane tipping, we wanted to delay fruit ripening and discover how this culture practice influences fruit weight, yield and primary and secondary metabolites, since previous studies have shown various effects on fruit quality. We therefore selected two primocane fruiting cultivars ('Amira' and 'Polka') and performed primocane tipping on two different dates (in late spring) and analyzed berries from three sampling dates. We also had an untipped control to test the fruit quality among different treatments.

Materials and Methods

Plant material

The study was conducted in 2016 at the experimental station of the Agricultural Institute of Slovenia, at Brdo pri Lukovici (46°10′N, 14°41′E). Plants of primocane fruiting cultivars 'Amira' and 'Polka' were planted in June 2014 on beds with polypropylene foil, with a planting distance of 3 × 0.4 m. The cultivation system was narrow, supported by two wires (0.7 and 1.5 m). Canes were cut back to soil level every December, while in spring they were reduced to 5 canes per plant. The orchard was covered with polyethylene foil (tunnel) from April to the end of the harvest season. Both cultivars were hand-harvested in a single harvesting period (from summer to late autumn) on first-year canes (primocanes). Data on temperature, precipitation and solar radiation during the span of the raspberry growing season are presented in Fig. 1. Drip irrigation, fertilization and pest control were applied according to integrated practice.

The experiment consisted of three treatments, in relation to the cultivation technique. The first treatment was untipped control, the second was primocane tipping performed on 18 May and the third was primocane tipping on 2 June 2016. In both treatments, tipping was performed on the 10th node of the primocane (around 0.6 m height). In order to evaluate the fruit quality, fruits from three harvest dates (when all treatments were ripe) were chosen: sampling dates of 27 July (S1), 1 September (S2) and 29 September (S3) 2016. Only ripe and undamaged fruits were used for the analysis. Immediately after harvest, fruit characteristics (fruit length, width and weight) were measured on thirty raspberry fruits for each treatment (8 plants for each treatment) and sampling date, and fruits were frozen in liquid nitrogen and stored for up to 1 month at -20 °C until chemical analysis.

Extraction and determination of sugars and organic acids

Primary metabolites (sugars and organic acids) were analyzed in whole berry fruits. For each raspberry cultivar and individual sampling date and treatment, five replications were carried out; each replication included several fruits. For the extraction of primary metabolites, 2 g of fruit was ground to a fine paste in a mortar, homogenized with 10 mL of double-distilled water, and left for 30 min at room temperature with continuous stirring. After the extraction, the homogenate was centrifuged and the supernatant was filtered into a vial and used for further analysis on the high performance liquid chromatography (HPLC) system (Thermo Scientific, Waltham, MA, USA). Further analysis of primary metabolites was performed as reported by Mikulic-Petkovsek *et al*. (2012b). Sugars and organic acids content levels were expressed in mg g^{-1} fresh weight (FW) of raspberries.

Extraction and determination of phenolic compounds using HPLC–DAD–MS^n analysis

As for sugars and organic acids, five replications (each included several fruits) were carried for phenolic

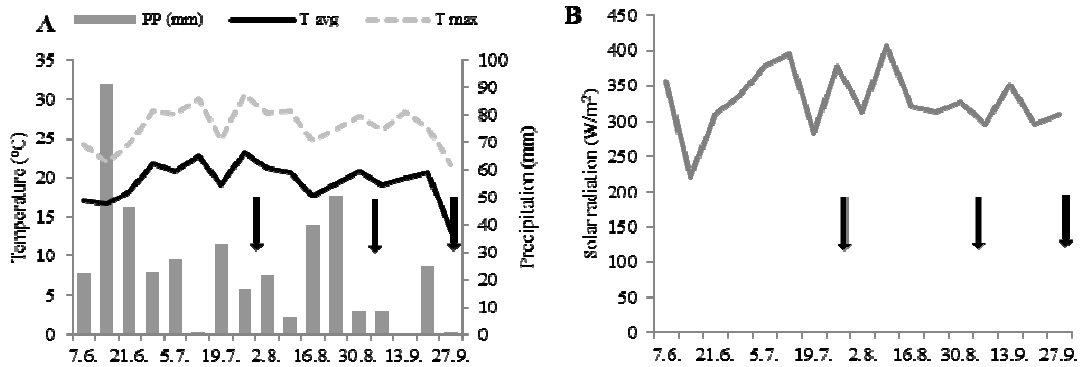

Fig. 1. Daily average and maximum temperatures (°C), precipitations (mm) (A) and solar radiation (W/m^2) (B) from the beginning of June till the end of September 2016. The sampling dates are represented by black arrows

compounds determination. Berries were ground to a fine paste in a mortar chilled with liquid nitrogen and 2.5 g was extracted with 8 mL of methanol containing 3% (v/v) formic acid in a cooled ultrasonic bath for 1 h. After extraction, the fruit extracts were centrifuged at 9700 × *g* for 7 min at 4 °C, and the supernatant was filtered into a vial. Phenolic compounds were analyzed on a Thermo Finnigan Accela HPLC system (Thermo Scientific). The procedures were described previously by Mikulic-Petkovsek *et al*. (2012a). Concentrations of phenolic compounds were calculated from peak areas of the sample and the corresponding standards and expressed in mg 100 g^{-1} FW of raspberries. For compounds lacking standards, quantification was carried out using similar compounds as standards.

Chemicals

The following standards were used for the determination of sugars and organic acids: fructose, glucose and sucrose; citric and malic acid from Fluka Chemie (Buchs, Switzerland) and shikimic and fumaric acid from Sigma-Aldrich Chemie (Steinheim, Germany). The following standards were used for the quantification of phenolic compounds: cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside, quercetin-3-*O*-glucuronide and ellagic acid from Sigma-Aldrich Chemie; caffeic and (+)-catechin from Roth (Karlsruhe, Germany), (−)-epicatechin, quercetin-3-rhamnoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, *p*-coumaric acid, procyanidin B1 and kaempferol-3-*O*-glucoside from Fluka Chemie; isorhamnetin-3-*O*-glucoside, and peonidin-3-glucoside from Extrasynthese (Genay, France). Methanol for the extraction of phenolics was acquired from Sigma-Aldrich Chemie. The chemicals for the mobile phases were HPLC-MS grade acetonitrile and formic acid from Fluka Chemie. Water for the mobile phase was double distilled and purified with the Milli-Q system (Millipore, Bedford, USA).

Statistical analysis

Results were evaluated with the Centurion XV.II program (Statpoint Technologies Inc., Warrenton, VA, USA). Differences in the content levels of the analyzed primary and secondary metabolites were tested among sampling dates (S), treatments (T) and their interaction (S × T) using two way analysis of variance (ANOVA). The differences among treatments of each sampling date were tested with the LSD test at a significance level of 0.05. Multivariate statistical analysis (hierarchical cluster analysis) was conducted in order to interpret the differences in secondary metabolites among different treatments.

Results and Discussion

The different cultivation techniques affected the beginning of the harvest season of both raspberry cultivars, since berries from the control treatment were collected first on 27 June, the berries from the May tipping were collected on 8 July (11 days after the control), while berries from the June tipping treatment were collected on 14 July ('Amira') and 18 July ('Polka') (17 and 21 days after the control) 2016.

Fruit characteristics

Since fruit color, in addition to berry size, is the most important parameter, especially for fresh consumption, we measured the CIE *L*a*b** color parameters of the berry skin, to test differences in the color values. Since the harvest season significantly affects raspberry fruit color (Mazur *et al*., 2014a,b) and high light intensity usually enhances red color development (Wang *et al*., 2009), we expected differences due to changes in climatic conditions during the summer (Fig. 1). However, we did not find any significant differences among the sampling dates and treatments of each cultivar, because of which the data are not presented in the results. Fruit length, width and weight, on the other hand, differed somewhat among the different treatments, while fruit shape (length/width ratio) and yield were not significantly affected (Table 1). The yield potential of raspberries is mainly determined by cultivar predisposition, although environmental conditions during floral initiation in the previous season also play an important role (Sønsteby *et al*., 2009; Palonen *et al*., 2017), which may be why fruit yield was not affected by treatment in our study, since all plants yielded approx. 1.1 kg fruit per plant. The fruit weights of both cultivars were highest and acceptable for the fresh market on the first sampling date, while fruit weights decreased on the second date, most prominently in the control treatments, while in both tipping treatments, weights diminished more constantly from the first to the last sampling date. On the first sampling date, the highest fruit length, width and weight of cv. 'Amira' were measured in the untipped control, in contrast to the second date, in which the highest values were measured in the June tipping treatment. On the third sampling date, differences were only noticed in fruit length. On the other hand, cv. 'Polka' showed differences in fruit length and width on the second

Table 1. Fruit characteristics of 'Amira' and 'Polka' raspberry cultivars

Cultivar	Treatment	Fruit length (mm)			Fruit width (mm)			Length/width ratio			Fruit weight (g)			Yield (g plant^{-1})
		S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	
'Amira'	Control	25.84 ± 0.43 a	17.72 ± 0.30 c	18.84 ± 0.31 a	19.51 ± 0.32 a	14.92 ± 0.21 c	16.55 ± 0.29 ns	1.32 ± 0.02 ns	1.19 ± 0.02 ns	1.14 ± 0.02 ns	5.03 ± 0.05 a	2.44 ± 0.06 c	2.78 ± 0.09 ns	1073.9 ± 28.4 ns
	May tipping	24.47 ± 0.34 b	18.83 ± 0.45 b	17.26 ± 1.26 ab	19.22 ± 0.35 ab	15.68 ± 0.23 b	16.36 ± 0.46 ns	1.27 ± 0.02 ns	1.20 ± 0.02 ns	1.06 ± 0.07 ns	4.40 ± 0.10 b	3.07 ± 0.05 b	3.12 ± 0.07 ns	1217.9 ± 54.9 ns
	June tipping	22.74 ± 0.62 c	20.18 ± 0.34 a	16.47 ± 0.67 b	18.33 ± 0.27 b	16.91 ± 0.22 a	15.87 ± 0.37 ns	1.24 ± 0.02 ns	1.19 ± 0.02 ns	1.04 ± 0.04 ns	4.00 ± 0.03 c	3.49 ± 0.12 a	3.17 ± 0.07 ns	1063.1 ± 31.8 ns
'Polka'	Control	23.77 ± 0.27 ns	15.39 ± 0.38 b	18.19 ± 0.49 a	18.94 ± 0.24 ns	15.84 ± 0.23 b	17.60 ± 0.27 a	1.26 ± 0.01 ns	0.97 ± 0.02 ns	1.03 ± 0.02 ns	4.65 ± 0.07 ns	2.34 ± 0.08 b	2.35 ± 0.11 ns	1084.1 ± 73.0 ns
	May tipping	22.81 ± 0.37 ns	18.71 ± 0.36 a	15.99 ± 0.59 b	19.64 ± 0.25 ns	17.31 ± 0.24 a	16.19 ± 0.50 b	1.16 ± 0.01 ns	1.08 ± 0.01 ns	0.99 ± 0.03 ns	4.62 ± 0.06 ns	3.13 ± 0.09 a	2.27 ± 0.13 ns	1050.8 ± 57.7 ns
	June tipping	23.27 ±0.39 ns	19.09 ± 0.22 a	16.07 ± 0.45 b	19.49 ± 0.33 ns	17.56 ± 0.23 a	16.08 ± 0.37 b	1.19 ± 0.02 ns	1.09 ± 0.01 ns	1.00 ± 0.03 ns	4.43 ± 0.11 ns	3.37 ± 0.07 a	2.69 ± 0.07 ns	1058.1 ± 54.0 ns

Different letters in columns denote significant differences among treatments within each sampling date (S1-S3) and cultivar (LSD test, $P < 0.05$).

and third sampling dates, while weights differed among treatments only on the second date. The untipped control had the lowest values on the second date, in contrast to the third sampling date (Table 1).

Primary metabolites

Since the composition and content of sugars and organic acids, as well as their ratio, significantly impact on fruit flavor, the primary metabolites of the different treatments and sampling dates of 'Amira' and 'Polka' cultivars are presented in Table 2 and Fig. 2. Total sugars and organic acids were significantly impacted in both cultivars by tipping treatment, although sampling date had a greater effect. Glucose and fructose were the prevalent sugars in both cultivars, while sucrose was generally detected in somewhat lower amounts, as in previous studies (Wang *et al*., 2009; Mikulic-Petkovsek *et al*., 2012b). Cultivar 'Amira' showed differences in glucose and fructose contents among treatments only on the second sampling date, in which the total sugar content was almost 1.3-fold higher in the untipped control than in primocane tipping treatments. In cv. 'Polka', differences in individual and total sugars were measured among treatments in all three samplings. On the first and third sampling dates, the lowest glucose, fructose and total sugar contents were observed with the control and on the second date with June primocane tipping. Sucrose somewhat deviated from this pattern. Poledica *et al*. (2012) similarly found that the soluble solids content was lower in control berries than in berries from young cane removal treatment, while Lewandowski *et al*. (2015) in contrast showed no effect of different cultivation techniques on soluble solids.

Citric acid was the major organic acid in both raspberry cultivars (Table 2), while malic acid was present in a lesser amount, as found earlier (Mikulic-Petkovsek *et al*., 2012b). Cultivar 'Amira' showed differences among treatments for citric acid only on the first sampling date and for malic acid on the second and third sampling dates, while in cv. 'Polka' differences in citric acid were observed on the second and third dates and in malic acid on the first harvest date. Total organic acid contents differed among treatments in 'Amira' only on the last harvest date, in which the first (May) primocane tipping showed a 1.2-fold higher content, which is similar to higher titratable acidity measured in fruits of removed canes (Poledica *et al*., 2012). Conversely, in cultivar 'Polka', the highest total organic acids were measured in the untipped control on the second and third harvest dates.

The sugar/organic acid ratio increased from the first to the third sampling date in both cultivars, meaning the berries became sweeter (Fig. 2). This may be connected with the fact that later harvested berries were smaller (Table 2); i.e., the fruits contained a lower percentage of water than those harvested first. The results calculated on a dry weight basis showed similar trends (data not shown). Remberg *et al*. (2010) and Mazur *et al*. (2014a,b) similarly reported that dry matter, soluble solids, total acidity and their ratio increased in raspberry fruit with the progress of harvest season as the result of the complex responses to a range of environmental conditions (especially temperature). Increased sugar/acid ratio in our study may also be due to weather, with low precipitation in September (Fig. 1a), since conditions with a high amount of rain reduce the sugar content, and/or due to the shorter day, which can also change their levels (Mazur *et al*., 2014b). Among treatments, different patterns for sugar/organic acid ratios were noted for each cultivar. 'Amira' had the highest ratio in control treatments (S2, S3), in addition to the second (June) primocane tipping (S3) (Fig. 2). Conversely, 'Polka' had the highest ratio in the second (June) tipping (S1, S3) and in the first (May) primocane tipping (S2).

Secondary metabolites

It is widely known that secondary metabolites protect plants against various pathogens and kinds of environmental stress and thus contribute to the overall quality of fruits. Differences among the cultivation techniques on the three sampling dates in total anthocyanins, flavanols, flavonols, ellagitannins and ellagic acid derivatives and hydroxycinnamic acids of the two primocane raspberry cultivars are presented in Table 3. The phenolic profile of cv. 'Amira' was analyzed for the first time in this study. Total analyzed phenolics (TAP), representing the sum of total amounts of all polyphenol groups, are additionally presented in Fig. 3.

Total anthocyanin content, determined as the sum of 10 individual anthocyanins (data not shown), showed similar contents as in previous studies, in which the anthocyanin

Table 2. Individual and total sugars and organic acids (mg g^{-1} FW) of raspberry fruits and Two-Way ANOVA of sampling date (S1-S3), treatment (control, May and June tipping), and their interaction (S × T)

Sampling date	S1			S2			S3			Factors		
Treatment	Control	May tipping	June tipping	Control	May tipping	June tipping	Control	May tipping	June tipping	S	T	S × T
'Amira'												
sucrose	1.68 ± 0.29 ns	1.47 ± 0.29 ns	1.02 ± 0.16 ns	0.90 ± 0.07 ns	0.88 ± 0.19 ns	1.28 ± 0.19 ns	1.02 ± 0.37 ns	0.72 ± 0.03 ns	1.05 ± 0.18 ns	*	NS	NS
glucose	15.2 ± 0.20 ns	13.5 ± 0.24 ns	14.5 ± 0.96 ns	27.1 ± 0.63 a	20.6 ± 0.84 b	20.9 ± 0.63 b	30.1 ± 0.70 ns	29.7 ±0.63 ns	30.8 ± 1.00 ns	***	**	***
fructose	17.4 ± 0.17 ns	16.0 ± 0.22 ns	17.0 ± 1.00 ns	31.6 ± 0.79 a	24.3 ± 0.96 b	24.2 ± 0.66 b	33.9 ± 0.85 ns	33.1 ± 0.71 ns	35.0 ± 0.92 ns	***	**	***
Total sugars	34.3 ± 0.44 ns	31.0 ± 0.69 ns	32.5 ± 2.13 ns	59.6 ± 1.44 a	45.8 ± 1.81 b	46.4 ± 1.22 b	65.0 ± 1.29 ns	63.5 ± 1.32 ns	66.9 ± 1.97 ns	***	*	**
citric acid	17.5 ± 0.38 c	19.4 ± 0.15 b	20.5 ± 0.24 a	18.5 ± 0.41 ns	19.3 ± 0.27 ns	18.4 ± 0.23 ns	14.8 ± 0.45 ns	16.9 ± 0.17 ns	14.8 ± 0.60 ns	***	**	***
malic acid	2.66 ± 0.07 ns	2.66 ± 0.07 ns	2.58 ± 0.06 ns	2.46 ± 0.05 a	2.44 ± 0.05 a	2.06 ± 0.04 b	1.70 ± 0.07 b	2.22 ± 0.14 a	1.94 ± 0.07 ab	***	**	***
Total acids	20.2 ± 0.25 ns	22.1 ± 0.11 ns	23.1 ± 0.28 ns	21.0 ± 0.43 ns	21.7 ± 0.30 ns	20.5 ± 0.28 ns	16.5 ± 0.48 b	19.1 ± 0.26 a	16.7 ± 0.73 b	***	*	**
'Polka'												
sucrose	14.1 ± 0.64 a	7.98 ± 0.38 c	11.8 ± 1.08 b	8.96 ± 1.55 ns	12.4 ± 0.74 ns	10.5 ± 0.91 ns	7.54 ± 2.12 b	15.3 ± 1.98 a	17.1 ± 1.56 a	*	*	*
glucose	7.84 ± 0.21 c	12.3 ± 0.19 b	15.6 ± 0.52 a	22.0 ± 0.70 a	22.0 ± 0.47 a	17.1 ± 0.49 b	22.4 ± 0.88 c	30.2 ± 0.87 a	26.1 ± 0.98 b	***	***	***
fructose	9.92 ± 0.23 c	14.6 ± 0.21 b	18.6 ± 0.66 a	26.8 ± 0.86 a	26.2 ± 0.57 a	20.5 ± 0.50 b	25.2 ± 0.97 c	34.0 ± 0.85 a	29.6 ± 1.08 b	***	***	***
Total sugars	31.9 ± 0.87 c	34.8 ± 0.40 b	46.0 ± 0.76 a	57.8 ± 2.77 a	60.6 ± 1.70 a	48.1 ± 1.66 b	55.1 ± 2.07 c	79.5 ± 1.54 a	72.8 ± 1.77 b	***	**	***
citric acid	15.9 ± 0.13 ns	16.5 ± 0.23 ns	17.6 ± 0.23 ns	18.6 ± 0.43 a	16.1 ± 0.31 b	15.6 ± 0.52 b	17.3 ± 0.91 a	15.9 ± 0.72 ab	14.0 ± 0.48 b	*	**	***
malic acid	3.97 ± 0.10 a	3.23 ± 0.06 b	2.78 ± 0.06 c	2.61 ± 0.03 ns	2.43 ± 0.11 ns	2.44 ± 0.10 ns	2.31 ± 0.06 ns	2.15 ± 0.05 ns	2.13 ± 0.08 ns	***	*	***
Total acids	19.9 ± 0.18 ns	19.7 ± 0.28 ns	20.4 ± 0.26 ns	21.2 ± 0.41 a	18.5 ± 0.38 b	18.0 ± 0.58 b	19.6 ± 0.92 a	18.1 ± 0.47 ab	16.1 ± 0.56 b	***	*	**

Different letters in rows denote significant differences among treatments within each sampling date and cultivar (LSD test, $P < 0.05$). *, statistically significant differences at P value <0.05; **, statistically significant differences at P value <0.01; ***, statistically significant differences at P value <0.001.

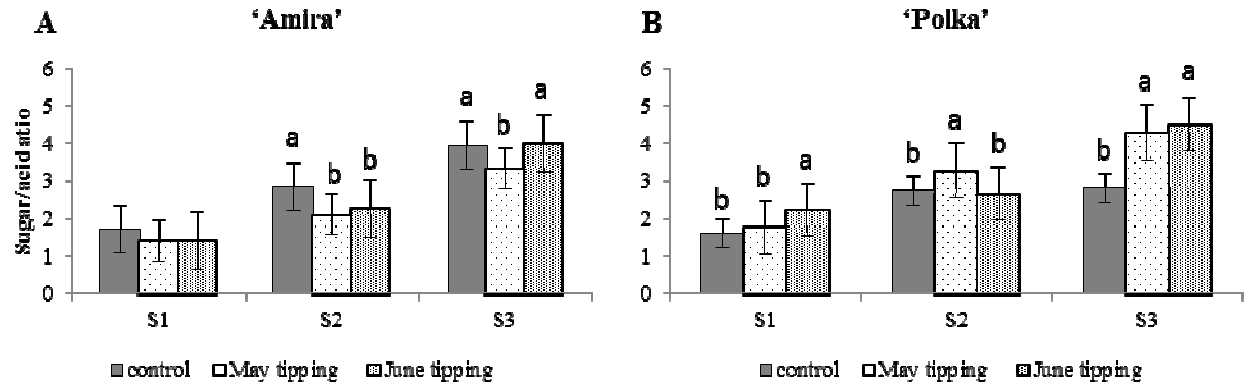

Fig. 2. Sugar/organic acid ratio of 'Amira' (A) and 'Polka' (B) raspberry cultivars, harvested in different dates (S1-S3). Different letters denoted statistically significant differences among treatments within sampling date

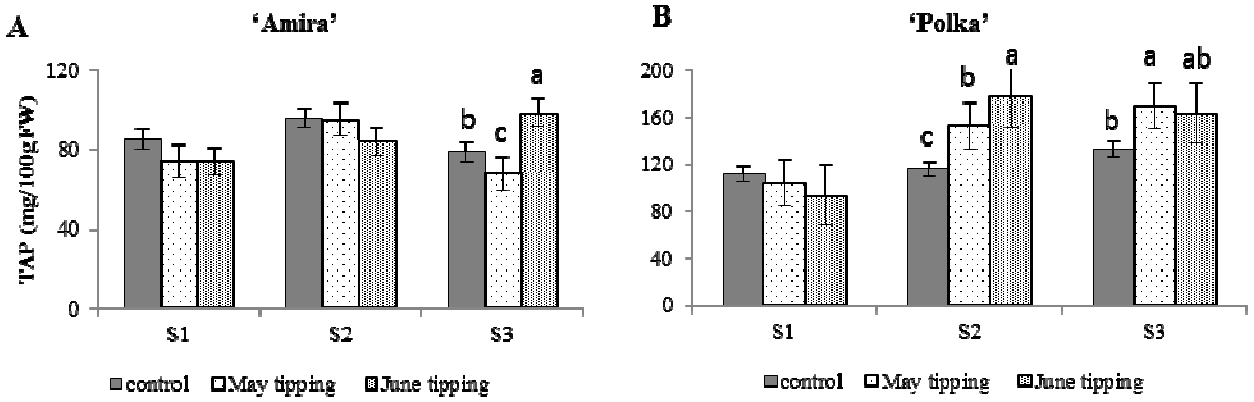

Fig. 3. Total analyzed phenolics (TAP) (mg 100 g^{-1} FW) of 'Amira' (A) and 'Polka' (B) raspberry cultivars, harvested in different dates (S1-S3). Different letters denoted statistically significant differences among treatments within sampling date

Table 3. Content of total anthocyanins, ellagitannins and ellagic acid derivatives, flavanols, flavonols and hydroxycinnamic acid derivatives (HCA) (mg 100 g^{-1} FW) of raspberry fruits and Two-Way ANOVA of sampling date (S1-S3), treatment (control, May and June tipping), and their interaction (S × T)

Sampling date	S1			S2			S3			Factors		
Treatment	Control	May tipping	June tipping	Control	May tipping	June tipping	Control	May tipping	June tipping	S	T	S × T
'Amira'												
Anthocyanins	35.6 ± 1.68 ns	31.5 ± 1.74 ns	37.5 ± 1.70 ns	35.6 ± 2.18 ns	36.9 ± 2.60 ns	36.7 ± 1.96 ns	28.3 ± 2.43 ab	24.1 ± 2.10 b	32.8 ± 2.17 a	***	**	NS
Ellagitannins	35.2 ± 4.02 ns	30.0 ± 2.35 ns	24.1 ± 2.20 ns	42.9 ± 4.03 ns	40.9 ± 3.85 ns	32.2 ±2.55 ns	35.6 ± 1.52 b	29.2 ± 2.13 b	50.2 ± 3.00 a	*	NS	**
Ellagic acid derivatives	2.77 ± 0.18 ns	2.34 ± 0.10 ns	2.44 ± 0.14 ns	3.45 ± 0.09 ns	3.09 ± 0.27 ns	2.79 ± 0.18 ns	2.37 ± 0.14 ns	3.12 ± 0.12 ns	2.72 ± 0.11 ns	*	NS	*
Flavanols	9.29 ± 0.70 ns	8.11 ± 0.45 ns	7.78 ± 0.65 ns	10.8 ± 0.83 ns	11.4 ± 0.71 ns	9.62 ± 0.85 ns	10.4 ± 0.90 ns	9.25 ± 0.65 ns	9.80 ± 0.53 ns	**	NS	NS
Flavonols	2.21 ± 0.10 ns	1.95 ± 0.09 ns	2.13 ± 0.06 ns	2.98 ± 0.08 ns	2.76 ± 0.12 ns	2.51 ± 0.12 ns	2.15 ± 0.02 ns	2.37 ± 0.06 ns	2.82 ± 0.09 ns	**	NS	NS
'Polka'												
Anthocyanins	45.0 ± 3.90 ns	40.8 ± 4.08 ns	44.3 ± 4.09 ns	46.5 ± 2.90 b	52.8 ± 3.09 a	43.3 ± 2.58 b	39.5 ± 2.13 b	47.2 ± 2.39 a	41.2 ± 3.94 ab	**	NS	***
Ellagitannins	45.8 ± 3.23 a	43.8 ± 2.54 ab	32.1 ± 3.07 b	47.0 ± 1.73 c	76.1 ± 2.82 b	110 ± 4.17 a	77.1 ± 5.90 ns	99.7 ± 6.12 ns	99.7 ± 9.25 ns	***	***	***
Ellagic acid derivatives	4.56 ± 0.34 ns	4.47 ± 0.27 ns	4.21 ± 0.35 ns	5.65 ± 0.37 ns	6.51 ± 0.11 ns	6.27 ± 0.28 ns	4.12 ± 0.25 b	7.74 ± 0.63 a	7.40 ± 1.28 a	***	*	*
Flavanols	9.35 ± 0.09 ns	9.09 ± 0.59 ns	7.67 ± 0.34 ns	9.94 ± 0.31 ns	10.3 ± 0.32 ns	11.3 ± 0.41 ns	8.09 ± 0.54 ns	8.93 ± 0.17 ns	8.95 ± 0.56 ns	**	NS	*
Flavonols	3.11 ± 0.14 ns	2.84 ± 0.09 ns	2.65 ± 0.24 ns	3.28 ± 0.04 ns	3.49 ± 0.11 ns	3.22 ± 0.13 ns	2.26 ± 0.11 b	3.13 ± 0.09 a	2.61 ± 0.26 b	***	*	**
HCA	3.87 ± 0.15 a	3.02 ± 0.18 b	2.71 ± 0.15 b	3.17 ± 0.06 ns	3.47 ± 0.12 ns	3.01 ± 0.14 ns	2.35 ± 0.13 b	3.08 ± 0.06 a	2.98 ± 0.16 a	***	*	**

Different letters in rows denote significant differences among treatments within each sampling date and cultivar (LSD test, $P < 0.05$). *, statistically significant differences at P value <0.05; **, statistically significant differences at P value <0.01; ***, statistically significant differences at P value <0.001.

content in various investigated red fruited cultivars ranged around 30 mg 100 g^{-1} FW (Remberg *et al*., 2010; Mazur *et al*., 2014a,b), although higher contents have also been reported (Bobinaitė *et al*., 2012). As noted previously, anthocyanins mainly consisted of cyanidin, followed by pelargonidin-based pigments (Veberic *et al*., 2015; Kula *et al*., 2016), while peonidin glycosides (detected only in the minor contents) have not previously been identified in raspberries. Interaction between sampling date and treatment in total anthocyanins was shown only for cv. 'Polka', although sampling date had high influence on their contents in both cultivars (Table 3). This may be connected with various environmental conditions within the season, since anthocyanin accumulation is dependent on solar radiation and temperature, whereas high light and temperatures around 25 °C increase the content of anthocyanins, while much lower or higher temperatures (which also occurred in our study) retard ripening and anthocyanin accumulation (Jaakola *et al*., 2013; Zoratti *et al*., 2015). Mazur *et al*. (2014b), on the other hand, showed

that different photoperiods did not significantly affect total anthocyanins in raspberry fruit, although total monomeric anthocyanins increased with the successive week of harvest. Differences in total anthocyanins among treatments were measured on the third sampling date in both cultivars and in cv. 'Polka' additionally on the second date. 'Amira' had a 1.4-fold higher content with the later (June) tipping than with the earlier (May) primocane tipping, while 'Polka' had a 1.2-fold higher content on both dates with the earlier (May) tipping than with the control or June tipping. In a study by Poledica *et al*. (2012), no effect of young cane removal on total anthocyanins was noted.

Ellagitannins, with ellagic acid derivatives, is the major group of polyphenolics in raspberry fruit, followed by anthocyanins (Kula *et al*., 2016). Their total levels depend on many factors and vary from 90 to 164 mg 100 g^{-1} FW (Sójka *et al*., 2016), which is somewhat higher than our results. In both raspberry cultivars, the interaction of different sampling dates and treatment had a significant effect on their levels. Total ellagitannins and ellagic acid derivatives in cv. 'Amira' were only influenced by sampling date, while 'Polka' was additionally affected by different treatment regimes (Table 3). Total ellagitannins content was 1.4-fold higher on the first harvest date in the untipped control than with the June primocane tipping, in contrast to the second harvest date, in which June tipping showed a 2.3-fold higher content. Total ellagic acid derivatives showed differences in their contents on the third harvest date, in which tipping treatments contained around 1.8-fold higher levels of ellagic acids than the control.

Flavanols, consisting of catechin, epicatechin and procyanidin dimers and trimers, is another important group of raspberry phenolics (Sójka *et al*., 2016), although some studies have found flavanols in minor amounts (Remberg *et al*., 2010; Kula *et al*., 2016). Total flavanol contents were significantly influenced by sampling date in both cultivars in our study, while no differences were shown among treatments (Table 3). Flavanols are mainly characterized by cultivar (Määttä-Riihinen *et al*., 2004; Kula *et al*., 2016) and ripening stage of berries, while there are no reports of different agricultural practices on flavanol content in raspberries. Their levels usually decrease in various fruits with ripening (Zorenc *et al*., 2017), but may increase as a defense response to fungal infection or some other kind of stress (Mikulic-Petkovsek *et al*., 2014).

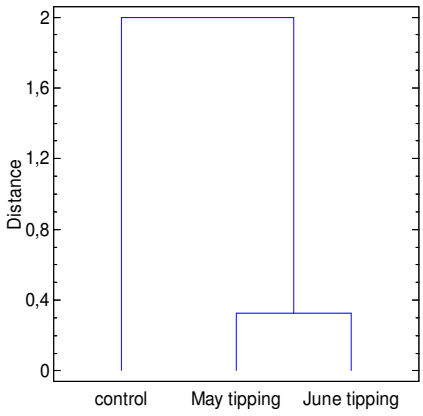

Fig. 4. Dendrogram of analyzed secondary metabolites of different treatments (regardless of the sampling date and cultivar), using Wards method, based on square Euclidean distance

Hydroxycinnamic acids and flavonols are minor phenolic classes in raspberry fruits, representing only around 2% of TAP. In cultivar 'Amira' we could not detect any hydroxycinnamic acids, while in cv. 'Polka' various caffeic and *p*-coumaric acid derivatives were measured (data not shown). Total hydroxycinnamic acids vary around 0.9-1.9 mg 100 g^{-1} FW (Määttä-Rihiinen, 2004), which is somewhat less than our results. Total hydroxycinnamic acid derivatives were influenced by sampling date, as well as by treatment regime and their interaction. On the first sampling date, a 1.3 or 1.4-fold higher content was detected in the untipped control than in the primocane tipping treatments, which was in contrast to the results on the third harvest date. Total flavonols were the sum of quercetin, isorhamnetin and kaempferol glycosides in our study (data not shown). Quercetin glycosides, the main flavonols in raspberry fruits, ranged from 0.32–1.55 mg 100 g^{-1} FW in a study by Anttonen and Karjalainen (2005), while Wang *et al*. (2009) reported similar total flavonols content to our results. Of others phenolic groups, total flavonol glycosides were also predominantly influenced by different sampling dates, while only cv. 'Polka' was additionally affected on the third harvest date by different treatments. The highest content was for the May primocane tipping (1.2 and 1.6-fold higher than for the other two treatments).

TAP showed similar content as results by Mikulic-Petkovsek *et al*. (2012b) (107.6 mg 100 g^{-1} FW), although some other studies have reported higher contents (Wang *et al*., 2009; Remberg *et al*., 2010). TAP detected in raspberry fruit showed a significant interaction between different sampling dates and treatments. In both raspberry cultivars, lower TAP contents were measured on the first sampling date than on to the two subsequent dates (Fig. 3), which is in contrast to results by Mazur *et al*. (2014b), in which higher levels were noticed at the beginning of the harvest season. It is likely that photoperiod and other light-related factors influence the phenolic compounds of berries via light receptors, which interact directly or indirectly with certain anthocyanin biosynthesis-related MYB transcription factors to induce transcription of different flavonoid pathway genes (Jaakola *et al*., 2013). Wang *et al*. (2009), for instance, showed that raspberry fruits exposed to higher light intensities had higher total phenolics than those exposed to lower intensities. Remberg *et al*. (2010) also suggested that temperature fluctuations may enhance the concentration of secondary metabolites in raspberry fruits, as compared to constant temperatures. Differences in TAP among treatments were significant only on the last sampling date (S3) in cultivar 'Amira', while in cv. 'Polka' the second date was additionally effected by primocane tipping. 'Amira' had an almost 1.4-fold higher TAP content with the June tipping compared to the May primocane tipping. 'Polka' had similarly a 1.4-fold higher TAP with the June tipping than the control on the second sampling date, while on the last date, a 1.3-fold higher content was measured than in the control. These differences may be partly linked to changed light conditions due to lateral branches, since cane density (and therefore number of leaves) is an important factor influencing light penetration into the canopy (Oliveira *et*

al., 2004; Qiu *et al*., 2016). In a previous study by Poledica *et al*. (2012), however, no effect of cane removal on total phenolics was reported. Although differences in TAP among treatments for each sampling date and cultivar were small, the dendrogram clustering TAP showed some dissimilarities in different cultivation techniques, regardless of the sampling date and cultivar (Fig. 4). The dendrogram was characterized by two distinct branches, clustering the control in one cluster and May and June tipping treatments in the other, indicating that the untipped control differed from tipping treatments in analyzed secondary metabolites.

Conclusions

This study showed that higher differences in raspberry fruit quality were measured with the progress of the harvest season, than in relation to the different cultivation technique. Fruit color parameters, shape and yield were not affected by primocane tipping, while a small influence on other berry quality parameters was observed. Some differences in primary and secondary metabolites that were shown were not consistent and were more prominent on the second and third sampling dates than on the first, showing that multiple factors may affect their contents. Primocane tipping was shown to be a good cultivation practice for delaying the production season of raspberries, but this technique demands more hand-work, so growers should calculate its economic value.

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