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The Influence of Early Yield on the Accumulation of Major Taste and Health-Related Compounds in Black and Red Currant Cultivars (*Ribes* spp.)

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ABSTRACT: The focus of our study was to investigate the effect of crop load on the accumulation and composition of primary metabolites (sugars and organic acids), selected groups of flavonoids (anthocyanins and flavonols), and total phenolics in two subsequent years in four black currant cultivars ('Titania', 'Triton', 'Tsema', and 'Cacanska crna') and three red currant cultivars ('Junifer', 'Rolan', and 'Stanza'). For the determination and quantification of compounds, high-performance liquid chromatography−photodiode array with a mass spectrometer was used. Significant differences among cultivars were detected in all analyzed compounds. Anthocyanins were the predominant phenolic group and were more abundant in black currant cultivars as compared to red ones. Similar amounts of sugars and organic acids were measured in both *Ribes* species; however, vitamin C was 3-fold higher in black currants. A larger crop load in the second year had a negative effect on the sugar content of berries and promoted a higher degree of acids, with the exception of vitamin C, which was higher in the year with a lower crop load. On the other hand, the content of anthocyanins and flavonols was higher in the year with a larger crop load, while there were no differences in total phenolic content.

KEYWORDS: *Ribes, crop load, sugars, organic acids, anthocyanins, flavonols*

INTRODUCTION

Currants (*Ribes* spp.) are perennial shrubs best known for their tart-tasting fruit. The *Ribes* genus is important in the world production of berry fruits, especially as currants are ranked immediately after strawberries.[1] Although they are grown all over the world in the cooler climates, most of the commercial currant production occurs in northern Europe.[2] In the *Ribes* genus, the most important species are black currant (*Ribes nigrum* L.) and red currant (*Ribes rubrum* L.). Fruits of both species can be consumed fresh or processed into jams, jellies, liquors, and extracts for nutritional supplements.

Fresh berries are highly perishable, and their quality and shelf life can be greatly affected by different pre- and postharvest factors. A number of studies have reported the effect of preharvest factors, including climate conditions and cultural practices, on different phenolic compounds and antioxidant values at harvest and also on shelf life during storage.[3] Häkkinen and Törrönen[4] measured the effect of geographical origin and cultivation technique on fruit phenolic acids in strawberries. Stopar et al.[5] indicated that the polyphenol concentration in *Malus* (apple) was increased by reducing crop load, although there was no effect of thinning on flavonoid and chlorogenic acid concentrations.[6]

The quality of fruits is also influenced by the amount of primary metabolites, specifically different sugars and organic acids. Concentrations of sugars are most commonly measured in relation to the organoleptic factors of sweetness, acidity, astringency, and overall flavor perception. Additionally, the sugar/acid ratio can have an important impact on perceiving the fruits as sweet or sour. In this ratio, the organic acid content as well as the composition of individual metabolites play a crucial role in fruit taste perception. Berries with pleasant sensory characteristics often have high contents of sugars and relatively low contents of organic acids.[7,8] There seems to be a lack of information addressing how genotype and crop load affect the concentration of sugars and acids in the fruit, since they can act as an index of consumer acceptability.

The red coloration of the fruits can be attributed to high contents of anthocyanins, a subclass of phenolic compounds, situated in the vacuoles of the berry tissue, mostly located in the fruit skin.[9] Beside these compounds, many other phenolics are present in currants; however, they mostly have no influence on the fruit color with the exception of the class of flavonoids yielding yellow copigments. Nevertheless, phenolics perform other functions like UV protection, act as antioxidative agents, deter pathogens, and influence the taste of the fruits.[10]

Therefore, the aim of the present study was to evaluate how the quality of the fruits differs on young and full fruiting bushes of red and black currant cultivars. Also, the differences in the content of primary and secondary metabolites between this two species were evaluated. Studies conducted so far focused mainly on the composition of metabolites in the *Ribes* species as compared to other fruits and lack data on orchard aspects like yield of the bush. It has previously been demonstrated on nectarines that crop load can have a substantial effect on the

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Table 1. Average Monthly and Yearly Values of Temperature (°C) and Precipitation (mm) for the Period of 1951−2007 and Two Studied Years

	temperature						precipitation					
period	March	April	May	June	July	year	March	April	May	June	July	year
1951−2007	5.7	11.3	16.4	19.3	20.9	*10.8*	42.2	51.5	68.0	78.7	69.7	*665.1*
2008	9.1	13.8	19.3	23.0	23.7	*14.0*	79.7	34.9	60.6	43.3	53.0	*586.5*
2009	7.9	15.8	19.9	21.0	24.1	*13.6*	64.9	6.1	34.7	151.0	80.0	*804.4*

Table 2. Flowering and Ripening Dates (Beginning and End) of Black and Red Currants in 2008 and 2009

		flowering time				ripening time			
		2008		2009		2008		2009	
species	cultivar	beginning	end	beginning	end	beginning	end	beginning	end
black currant	'Cacanska crna'	26.03	10.04	09.04	24.04	16.06	04.07	08.06	01.07
	'Titania'	30.03	19.04	07.04	30.04	06.06	26.06	06.06	28.07
	'Triton'	01.04	20.04	06.04	21.04	09.06	27.06	10.06	01.07
	'Tsema'	28.03	20.04	09.04	27.04	15.06	04.07	13.06	10.07
red currant	'Junifer'	20.03	05.04	04.04	20.04	01.06	18.06	04.06	23.06
	'Rolan'	08.04	18.04	15.04	03.05	15.06	02.07	10.06	28.06
	'Stanza'	03.04	20.04	15.04	07.05	08.06	26.06	10.06	26.06

quantity of fruit metabolites.[11] Over the past years, currants have been increasingly consumed as table fruit and not just processed into juices and other products; therefore, the data presented in this study are useful for nutritionist's purposes as well as studies of the impacts of production technologies on fruit quality. The research also supports the concept of an extended view of a consumer oriented quality in which health-promoting bioactive compounds are highly desired quality attributes. This is gaining importance among currant growers and actors involved in the food distribution chain.

■ MATERIALS AND METHODS

Plant Material. Berries of four black currant cultivars ('Titania', 'Triton', 'Tsema', and 'Cacanska crna') and three red currant cultivars ('Junifer', 'Rolan', and 'Stanza') were harvested in 2008 (third year after planting with early, not yet full yield) and 2009 (first year with full yield) at the experimental plantation of the Faculty of Agriculture located in Belgrade region (Serbia). The orchard was planted in 2005 with 3 m × 1 m spacing (3.330 bushes per ha). Each cultivar was replicated three times in a randomized complete block design. The meteorological data (temperatures and precipitation) are presented in Table 1. Both years were warmer as compared to the long-term average, especially the year 2008. In the ripening period in June 2008, the average temperatures were 2.0 °C higher as compared to 2009 and 3.7 °C higher as compared to the long-term average. In the same period, the precipitation was lowest in 2008 as compared to 2009 as well as the long-term average.

During the research period, the technological properties such as fruit yield per bush, berry weight, bunch weight, and number of berries per bunch were monitored. Investigation of generative characteristics was carried out on samples of 30 bushes in three replicates. Each replicate consisted of 10 bushes selected for their uniformity. Fruit bunches were counted on each bush and weighted to determine yield at commercial maturity. Because the examined period is characterized by increased productivity of studied black and red currant cultivars, the results were shown in tables for each year separately. Beside the technological properties, chemical characteristics (sugars, organic acids, and phenolic content) were determined. The fruits were sampled at optimal ripening stage (Table 2), and 250 g of fruit per sampling was taken for biochemical analysis and stored at −20 °C for subsequent extraction.

Extraction and Determination of Sugars and Organic Acids. Primary metabolites (glucose, fructose, sucrose, citric, tartaric, and malic acid) were analyzed in the whole fruit without seeds. For each cultivar, five repetitions per sampling date were carried out ($n = 5$); each repetition included several fruit. For the extraction of primary metabolites, 10 g of fruit was homogenized in 50 mL of bidistilled water using Ultra-Turrax T-25 (Ika-Labortechnik) and left for 30 min at room temperature. After the extraction, the homogenate was centrifuged (Eppendorf Centrifuge 5810 R) at 12000 rpm for 7 min at 10 °C. The supernatant was filtered through a 0.45 μm cellulose ester filter (Macherey-Nagel) and transferred into a vial, and 20 μL of the sample was used for the analysis. The analysis of primary metabolites was carried out using high-performance liquid chromatograph (HPLC) of Thermo Separation Products. The separation of sugars was carried out using a Rezex RCM-monosaccharide column (300 mm × 7.8 mm) from Phenomenex operated at 65 °C. The mobile phase was bidistilled water, and the flow rate was 0.6 mL min^{-1}; the total run time was 30 min, and a refractive index (RI) detector was used to monitor the eluted carbohydrates as described by Dolenc-Sturm et al.[12] Organic acids were analyzed on a HPLC using an Aminex HPX-87H column (300 mm × 7.8 mm) with a UV detector set at 210 nm, as described by Dolenc-Sturm et al.[12] The column temperature was set at 65 °C. The elution solvent was 4 mM sulphuric acid in bidistilled water at a flow rate of 0.6 mL min^{-1}. The duration of the analysis was 30 min. The concentration of an individual metabolite was calculated according to a calibration curve of corresponding standard solutions. The content of all analyzed sugars was summed up and presented as total analyzed sugars. In a similar way, total analyzed organic acids were calculated. Both values were used for the determination of total sugar/organic acid ratio. The sweetness index was calculated by multiplying the sweetness coefficient of each individual sugar (glucose = 1, fructose = 2.3, and sucrose = 1.35), as described by Keutgen and Pawelzik.[13]

Extraction and Determination of Vitamin C. Vitamin C was quantified using the reflectometer set of Merck Co (Merck RQflex) as described by Pantelidis et al.[14] Fruit sample (5 g) and 20 mL oxalic acid (1%) were mixed, homogenized for 1 min, and filtered. PVPP (polyvinylpolypyrrolidone) (500 g) was added to 10 mL of the filtered sample to remove phenols, and 6−7 drops of H_2SO_4 (25%) were added, to reduce the pH level below 1.[14] Results were expressed as mg ascorbic acid 100 g^{-1} fresh weight (FW).

Extraction and Determination of Individual Phenolic Compounds. The extraction of individual phenolic compounds in whole fruit samples without the seeds was performed as described by Mikulic Petkovsek et al.[15] Five grams of the sample was extracted with 10 mL of methanol containing 1% 2,6-di-*tert*-butyl-4-methylphenol (BHT) and 3% formic acid in a cooled water bath using sonification. After they were centrifuged at 10000 rpm for 10 min at 4 °C, the supernatants were filtered through a 0.45 μm membrane filter (Macherey-Nagel), prior to injection into the HPLC system. The phenolic compounds were analyzed on a Thermo Finnigan Surveyor HPLC system, using a diode array detector at 350 (flavonols) and 530 nm (anthocyanins). The spectra of the compounds were also recorded between 200 and 800 nm. The column used was a Phenomenex Gemini C18 (150 mm × 4.5 mm, 3 μm) operated at 25 °C. The elution solvents were 1% formic acid in bidistilled water (A) and 100% acetonitrile (B). The samples were eluted according to the gradient described by Marks et al.,[16] with an injection amount of 20 μL and a flow rate 1 mL min^{-1}. The identification of compounds was achieved by comparing retention times and spectra. All phenolic compounds were also confirmed using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionization (ESI) operating in negative (flavonols)/positive (anthocyanins) ion mode. Analysis was carried out using MS/MS scanning m/z from 115 to 1000 (Table 3).

Table 3. Identification of Phenolic Compounds in Black Currants (*R. nigrum* L.) and Red Currants (*R. rubrum* L.) in Negative and Positive Ions with HPLC-MS and MS^2

					present in currants	
peak no.	λ (nm)	$[M - H]^-$ $(m/z)^a$	MS^2 (m/z)	tentative identification	black	red
1	350	625	316	myricetin 3-rutinoside	+	+
2		479	316	myricetin 3-galactoside	+	
3		479	316	myricetin 3-glucoside	+	+
5		565	521, 316	myricetin 3-malonylglucoside	+	
6		609	301	quercetin 3-rutinoside	+	+
7		463	301	quercetin 3-galactoside	+	
8		463	301	quercetin 3-glucoside	+	+
9		593	285	kaempferol 3-rutinoside	+	+
10		549	301	quercetin 3-malonylglucoside	+	+
11		447	301	quercetin 3-rhamnoside		+
11		447	285	kaempferol 3-galactoside	+	
12	530	465	303	delphinidin 3-glucoside	+	
13		611	303	dephinidin 3-rutinoside	+	
14		449	287	cyanidin 3-glucoside	+	+
12		611	287	cyanidin 3-sophoroside		+
15		595	449, 287	cyanidin 3-rutinoside	+	+
16		625	317	petunidin 3-rutinoside	+	
17		609	301	peonidin 3-rutinoside	+	
13		757	287	cyanidin 3-glucosylrutinoside		+
15		581	287	cyanidin 3-sambubioside		+
16		727	581, 287	cyanidin 3-xylosylrutinoside		+
18		611	303	delphinidin 3-(6″-coumaroyl) glucoside	+	
19		595	287	cyanidin 3-(6″-coumaroyl) glucoside	+	

[a] $[M + H]^+$ (m/z) anthocyanins were obtained in the positive ion mode.

The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 20 and 7 units, respectively, and the source voltage was 4 kV for negative ionization and 0.1 kV for positive ionization. Quantification was achieved according to the concentrations of a corresponding external standard.

Concentrations of phenolic compounds were calculated from the peak areas of the sample and the corresponding standards. Concentrations were expressed in mg kg^{-1} FW. For compounds lacking standards, quantification was carried out using similar compounds as standards. Thus, quercetin malonylglucoside, kaempferol 3-galactoside, kaempferol 3-rutinoside, and myricetin glycosides were quantified in equivalents of quercetin-3-galactoside. Cyanidin glycosides, delphinidin glycosides, and peonidin 3-rutinoside were quantified in equivalents of cyanidin 3-glucoside.

Determination of Total Phenolic Content (TPC). The extraction of total phenolics was made according to the same protocol as for phenolics, with the difference that no BHT was added. The TPC of extracts was assessed using the Folin−Ciocalteu phenol reagent method.[17] To 100 μL of the sample extracts, 6 mL of bidistilled water and 500 μL of Folin−Ciocalteu reagent were added; after resting between 8 s and 8 min at room temperature, 1.5 mL of sodium carbonate (20% w/v) and 1.9 mL of bidistilled water was added. The extracts were mixed and allowed to stand for 30 min at 40 °C before measuring the absorbance on a spectrophotometer (Perkin-Elmer, UV/visible Lambada Bio 20) at 765 nm. A mixture of water and reagents was used as a blank. The TPC was expressed as gallic acid equivalents (GAE) in mg kg^{-1} FW of fruit. Absorption was measured in three replicates.

Statistical Analysis. The data were analyzed with the Statgraphics Plus 4.0 program (Manugistics, Inc.) using one-way analysis of variance (ANOVA). The differences between the cultivars of each *Ribes* species for an individual year were tested using the Duncan test at the 0.05 significance level. The difference between the 2 years was tested with least significant difference (LSD) at the 0.05 significance level. The means and the standard errors of the means are reported (mean ± SE). Multivariate statistical analysis (hierarchical cluster analysis, discriminate analysis, and classification) was conducted to interpret the differences in phenolic compounds among analyzed *Ribes* species and cultivars. Ward's method based on square Euclidean distance[18] was used to interpret the differences in primary and secondary metabolites in fruits.

■ RESULTS AND DISCUSSION

The most fruitful cultivar of black currants (Table 4) was 'Cacanska crna', and the cultivars with the least yield per bush were 'Triton' and 'Tsema', respectively. 'Triton' also had the lowest number of berries per bunch and therefore a low bunch weight. Regarding all cultivars of black currants, the berry and bunch weight did not differ between the 2 years; however, the number of berries per bunch as well as the yield per bush increased in year 2009. This is an expected result since 2009 was the year with first full yield, while in 2008, the bushes were still in the juvenile phase. Among the red currants, 'Rolan' was the most productive cultivar with highest yield, number of berries per bunch, and the highest bunch weight (Table 4). Similar to black currants, no differences in bunch weight and number of berries per bunch were recorded between the years in red currant cultivars. The yield in the first year of the trial was higher in black currant cultivars as compared to red currant cultivars due to the fact that 3 year old black currant shoots are more fruitful. However, in the next year, the red currant yield per bush was much higher as compared to black currants due to a higher yield potential of *R. rubrum*.[19]

The total content of sugars as well as their composition did not differ between the two currant species (Table 5). Sugars in black and red currant fruit are mainly mono- and disaccharides (glucose, fructose, and sucrose), and the relative proportion of these individual sugars is important for the perception of sweetness.[20] In the present study, the main sugars in black currant cultivars were glucose and fructose, present in ratio from 1:0.7 in 2009 to 1:1.9 in year 2008. Similarly, Zheng et al.[8] reported values between 1:0.7 and 1:1.1 (glucose to fructose ratio) for the juice of three black currant cultivars. The ratio between these sugars was also similar in red currant cultivars. A high content of monosaccharides is also typical for other berry fruit species like *Fragaria* and *Rubus*.[21] The obtained data confirm that numerous factors such as cultivar, ecological conditions, maturity stage, and crop load can influence

Table 4. Yield and Bunch Properties of Different Red and Black Currant Cultivars in Years 2008 and 2009[a]

		black currant					red currant			
properties	year	'Titania'	'Triton'	'Tsema'	'Cacanska crna'	sig.	'Junifer'	'Rolan'	'Stanza'	sig.
berry weight (g)	2008	0.9 ± 0.0 a	1.1 ± 0.0 b	1.0 ± 0.0 b	0.8 ± 0.0 a	NS	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	***
	2009	0.7 ± 0.0 a	0.9 ± 0.0 b	0.7 ± 0.0 a	1.2 ± 0.1 c		0.8 ± 0.0 c	0.7 ± 0.0 b	0.6 ± 0.0 a	
bunch weight (g)	2008	5.6 ± 0.3 a	6.1 ± 0.3 a	7.2 ± 0.3 b	5.8 ± 0.2 a	NS	8.9 ± 0.6 a	11.6 ± 0.4 b	9.3 ± 0.2 a	NS
	2009	4.8 ± 0.2 a	5.3 ± 0.2 b	7.2 ± 0.1 b	10.1 ± 0.1 c		10.7 ± 0.5 b	12.1 ± 0.3 c	5.8 ± 0.2 a	
no. of berries per bunch	2008	6.9 ± 0.4 b	5.4 ± 0.2 a	7.1 ± 0.2 b	7.3 ± 0.2 b	***	11.1 ± 0.6 a	14.4 ± 0.1 b	13.2 ± 0.2 b	NS
	2009	7.3 ± 0.2 b	6.0 ± 0.2 a	10.9 ± 0.1 d	9.2 ± 0.1 c		13.5 ± 0.5 b	16.3 ± 0.3 c	10.1 ± 0.4 a	
yield per bush (g)	2008	766.7 ± 54.2 b	605.0 ± 36.2 a	572.3 ± 49.9 a	1010.0 ± 17.7 c	***	435.0 ± 28.4 b	557.0 ± 26.5 c	238.0 ± 25.3 a	***
	2009	809.3 ± 115.7 a	1413.3 ± 157.1 a	1633.3 ± 304.8 a	3393.3 ± 477.2 b		3360.0 ± 391.0 a	7510.0 ± 756.3 b	3020.0 ± 135.3 a	

[a] Mean values ± standard errors are presented. Different letters in rows denote significant differences between cultivars in an individual year ($P \leq 0.05$). Asterisks in the column sig. (significance) indicate statistically significant differences between 2 years for each compound at: NS, not significant; *$P \leq 0.05$; **$P \leq 0.005$; and ***$P \leq 0.001$.

chemical fruit composition. It is expected that berries on plants that are not yet in full yield ripen a bit earlier than those on full fruiting plants, which can be demonstrated by a higher sugar content obtained in the first year of investigation. In the future, the glucose to fructose ratio can also be evaluated as a potential ripeness indicator in *Ribes* species.[22]

In addition to glucose and fructose, sucrose was determined in black and red currants. Glucose accounted up to 14.2% of total sugars in black currants in 2009 and only up to 3.9% in red currants in the same year. In the previous year, those shares were lower. The sucrose content in red currants was somewhat lower as compared to the data obtained for juice of the same red currant cultivars published by Djordjevic et al.[1] Also, the average amount of sucrose per g of fresh weight in red currants was lower as compared to black currants, which could present valuable information for the consumers. Because fruit taste depends not only on the total sugar and organic acid contents but also on the type and the quantity of individual compounds, their composition may reflect changes in fruit quality.

According to differences in the sugar distribution between black and red currant cultivars in the two subsequent years, the sweetness index showed a similar tendency achieving the highest value in cultivar 'Junifer' (181.3) in 2008. Much lower relative units of the sweetness index were recorded in the second year of investigation in all tested red currant cultivars due to lower amounts of individual sugars contained in their fruits. Variation in the sweetness index between the 2 years can be observed in black currant cultivars with lower relative units obtained by 'Titania' and 'Cacanska crna' in 2009 as compared to the previous year, whereas 'Triton' and 'Tsema' expressed similar values in both investigated years.

The predominant organic acid in both *Ribes* species was citric acid (Table 5) with 2.9-fold lower values to that reported for black currant juice[8] and 3.8-fold lower values for black currant berries.[23] Again, in the year 2008, the fruits contained less citric acid than in the following year, suggesting that their ripeness was more advanced in 2008 since organic acids tend to degrade with the ripening processes. Also, the recorded rainfall levels in the ripening period of 2008 were much lower as compared to 2009. A lower content of organic acids with the advanced ripening stage was also recorded in grape fruit.[24] Malic and tartaric acids were present in 1.9- (malic) to 12.4-fold (tartaric) lower quantities as compared to citric acid, similar to the research by Bordonaba and Terry.[23] Comparing total analyzed organic acids among the black currants, the 'Titania' cultivar contained the highest amounts, while 'Triton' and 'Cacanska crna' cultivars contained the lowest, respectively. Among red currants, the 'Junifer' cultivar contained the highest amount of total organic acids and the 'Stanza' cultivar the lowest. A good measure for the perception of sweet and sour taste is the sugar/organic acid ratio.[7] Because of a high amount of sugars and low amount of organic acids, this ratio was highest in 'Cacanska crna' among black currant cultivars and 'Stanza' among red currants. Both cultivars were therefore sweeter tasting as compared to others where the ratio was lower.

This research and years of experience on this field stress the importance of the ideal picking time when the berries are in fully ripe stage. Therefore, the determination and application of a ripening stage scale for *Ribes* species are significant to improve the uniformity of sensory attributes and to pick berries with optimal content of sugars, organic acids, and sugars/organic acids ratio. The correlations between the chemical attributes and the sensory evolution of peach and nectarine fruit reported

Table 5. Content of Individual and Total Sugars and Organic Acids (g kg^{-1} FW) as Well as Content of Vitamin C (mg 100 g^{-1} FW), Sugar/Acid Ratio and Sweetness Index in Different Red and Black Currant Cultivars in Years 2008 and 2009[a]

		black currant					red currant			
compound	year	'Titania'	'Triton'	'Tsema'	'Cacanska crna'	sig.	'Junifer'	'Rolan'	'Stanza'	sig.
fructose	2008	25.5 ± 0.9 c	16.4 ± 0.9 a	21.2 ± 0.6 b	40.9 ± 0.9 d	NS	40.2 ± 2.5 b	30.3 ± 1.7 a	31.8 ± 2.1 a	***
	2009	27.7 ± 0.8 b	18.3 ± 1.2 a	29.0 ± 0.5 b	34.2 ± 0.5 c		20.8 ± 0.6 a	26.0 ± 0.8 b	22.0 ± 0.4 a	
glucose	2008	49.4 ± 3.2 c	21.4 ± 1.1 a	36.3 ± 1.0 b	78.9 ± 3.8 d	***	86.8 ± 5.0 b	44.3 ± 2.4 a	57.3 ± 5.5 a	***
	2009	19.6 ± 0.2 b	14.4 ± 0.8 a	20.1 ± 0.3 b	24.9 ± 0.3 c		16.8 ± 0.4 a	24.5 ± 0.5 b	17.3 ± 0.3 a	
sucrose	2008	4.1 ± 0.0 d	2.4 ± 0.0 b	2.9 ± 0.0 c	1.8 ± 0.0 a	***	1.5 ± 0.2	1.6 ± 0.1	1.7 ± 0.1	NS
	2009	7.8 ± 0.3 c	5.3 ± 0.1 b	5.9 ± 0.6 b	1.0 ± 0.1 a		0.5 ± 0.1 a	1.8 ± 0.0 c	1.6 ± 0.0 b	
total sugars	2008	78.9 ± 3.8 c	40.2 ± 1.9 a	60.4 ± 0.6 b	121.5 ± 4.7 d	***	128.5 ± 7.7 b	76.2 ± 2.7 a	90.8 ± 6.7 a	***
	2009	55.1 ± 1.3 b	37.9 ± 2.1 a	55.1 ± 0.7 b	60.1 ± 0.9 c		38.2 ± 0.9 a	52.3 ± 1.3 b	40.9 ± 0.6 a	
citric acid	2008	7.1 ± 0.2 c	5.7 ± 0.2 a	7.2 ± 0.2 c	6.5 ± 0.2 b	***	9.8 ± 0.4 b	5.9 ± 0.2 a	5.8 ± 0.0 a	***
	2009	9.3 ± 0.2 a	9.3 ± 0.4 a	11.7 ± 0.2 b	11.5 ± 0.4 b		14.7 ± 0.6 c	11.4 ± 0.4 b	9.6 ± 0.4 c	
malic acid	2008	5.1 ± 0.3 c	1.9 ± 0.1 a	2.7 ± 0.1 b	2.2 ± 0.2 ab	***	3.4 ± 0.1 b	3.8 ± 0.2 b	2.9 ± 0.0 a	*
	2009	7.3 ± 0.3 d	5.2 ± 0.2 c	3.7 ± 0.2 b	2.4 ± 0.1 a		2.6 ± 0.1 a	5.8 ± 0.3 c	4.5 ± 0.2 b	
tartaric acid	2008	0.5 ± 0.0 b	0.4 ± 0.0 b	0.2 ± 0.0 a	0.5 ± 0.0 b	***	0.9 ± 0.1 b	0.5 ± 0.0 a	0.3 ± 0.0 a	*
	2009	1.2 ± 0.0 c	1.0 ± 0.0 b	0.6 ± 0.0 a	0.5 ± 0.1 a		0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	
total organic acids	2008	12.7 ± 0.5 c	7.6 ± 0.0 a	10.3 ± 0.3 b	9.0 ± 0.8 ab	***	15.6 ± 1.2 b	9.8 ± 0.5 a	9.3 ± 0.4 a	***
	2009	17.7 ± 0.5 c	15.6 ± 0.6 ab	16.7 ± 0.8 bc	14.4 ± 0.4 a		17.7 ± 0.7 b	17.6 ± 0.7 b	14.5 ± 0.5 a	
sugars/acid ratio	2008	6.2 ± 0.1 a	5.3 ± 0.3 a	5.8 ± 0.1 a	13.6 ± 0.8 b	***	8.3 ± 0.4 ab	7.8 ± 0.6 a	9.7 ± 0.3 b	***
	2009	3.1 ± 0.1 b	2.4 ± 0.1 a	3.3 ± 0.1 b	4.2 ± 0.1 c		2.2 ± 0.1 a	3.0 ± 0.1 b	2.8 ± 0.1 b	
sweetness index	2008	113.8 ± 4.6 a	62.4 ± 8.0 a	89.0 ± 7.2 a	172.2 ± 8.8 b	**	181.3 ± 13.0 b	116.2 ± 9.7 a	132.7 ± 11.7 a	*
	2009	93.7 ± 2.5 a	63.5 ± 3.7 a	94.9 ± 1.3 b	104.9 ± 1.7 c		65.4 ± 1.7 a	86.7 ± 2.2 b	69.9 ± 1.1 a	
vitamin C	2008	147.8 ± 6.7 a	176.8 ± 1.9 b	202.3 ± 3.4 c	182.33 ± 5.4 b	***	45.8 ± 2.1 a	60.4 ± 2.8 b	55.8 ± 2.0 b	***
	2009	117.8 ± 7.5 a	138.6 ± 3.9 b	175.0 ± 3.2 c	143.93 ± 7.5 b		35.2 ± 1.6 a	45.8 ± 0.8 b	36.9 ± 1.0 a	

[a] Mean values ± standard errors are presented. Different letters in rows denote significant differences between cultivars in an individual year ($P \leq 0.05$). Asterisks in the column sig. (significance) indicate statistically significant differences between 2 years for each compound at: NS, not significant; *$P \leq 0.05$; **$P \leq 0.005$; and ***$P \leq 0.001$.

by Colaric et al.[25] provide a good tool in the quick assessment of fruit quality.

Currants, particularly black currant cultivars, are a rich source of vitamin C.[26] Our results confirmed these findings as the highest vitamin C level was measured in the 'Tsema' cultivar. Our results are somewhat higher than those reported by Milivojevic et al.[27] Although the content of vitamin C was on average approximately 3-fold lower in red currant cultivars as compared to black ones, the average amount of 41 mg 100 g^{-1} FW is still sufficient to consider red currants as a rich source of vitamin C among the fruits.[28]

As expected, a greater variation of anthocyanins was detected in black currants as compared red currant cultivars (Table 6). Similar findings have been reported by previous researchers who confirmed the presence of aglycones of delphinidin, cyanidin, petunidin, and peonidin in *R. nigrum*.[29] This has also been confirmed by our results, where delphinidin glycosides were most representative and accounted for the largest part of total anthocyanins present, resulting in purple and blue colors of the fruit. Cyanidin rutinoside was also quantified in high amounts, but we were not able to confirm the presence of malvidin glycosides, which have previously been reported;[30] however, different extraction techniques were used. The anthocyanidin composition was quite different in red currant cultivars. In this *Ribes* species, the cyanidin aglycone is bound to different sugar moieties. Cyanidin 3-xylosylrutinoside appeared to be the prevailing anthocyanin; however, its amounts were only somewhat higher as compared to other anthocyanins, not like the proportion of the prevailing anthocyanins in *R. nigrum* cultivars.

The total amount of anthocyanins in red currants was lower as compared to black currants, a well-known fact from literature.[31] The total content of anthocyanins as well as their composition visually results in the high intensity of the black currant color. The authors report that the reddish black color is mainly located in the berry skin. The content of all anthocyanins significantly increased from the first to the second year. Among black currants, the cultivars with low amounts of anthocyanins measured in one or both years were 'Cacanska crna' and 'Triton'. On the other hand, 'Tsema' and 'Titania' cultivars contained high amounts of total anthocyanins. Cultivars rich in anthocyanins also contained high amounts of total phenolics since anthocyanins represent a large share of them. However, despite an increase in total anthocyanins from the year 2008 to 2009, there was no significant increase of total phenolics. The content of phenolic compounds is affected by different factors such as the degree of maturity at harvest, genetic differences between the cultivars, and preharvest environmental conditions.[22] Although environmental conditions during ripening time as well as yield per bush in both years were quite different, no significant influence of the year was noticed in the amount of total phenolics.

The red currant cultivar with the highest anthocyanin content was 'Stanza' in both years. Meanwhile, no significant differences between the other two cultivars were recorded in years 2008 and 2009. Interestingly, cyanidin 3-glucosylrutinoside, detected only in red currants,[29] could not be confirmed in the 'Junifer' cultivar. On the other hand, this cultivar contained high amounts of cyanidin 3-xylosylrutinoside, especially in the second experimental year. A similar occurrence has previously been reported by Gavrilova et al.[31] Cyanidin 3-sophoroside was the only anthocyanin in which contents did not change significantly between the 2 years. It is interesting that an increase of other anthocyanins was measured in the second year, especially if we consider that the yield in this year was much higher as compared to the previous one. The increase of total anthocyanins in 2009 was 1.8−2.4-fold higher in different red currant cultivars. However, this increase was not detected in cultivars where the values were even lower in the second year, although the differences were not significant.

Beside anthocyanins, the *Ribes* genus is a rich source of other flavonoids, especially flavonols, which have also been quantified in our study (Table 7). In the samples of black currant cultivars, a greater variation of flavonol glycosides composed of the aglycones of kaempferol, myricetin, and quercetin was determined. These three aglycones have previously been reported by Milivojevic et al.[27] in three black currant cultivars; yet, in the study of Gavrilova et al.,[31] only glycosides of quercetin and myricetin were reported. Sojka et al.[32] also reported the presence of isorhamnetin in black currant pomace. In red currants, the same aglycones as in black currants were detected in our study; however, they were bound to different sugars and less abundant. In general, the content of flavonoids increased from the first to the second year in both *Ribes* species. The main exception was the 'Junifer' cultivar where a slight decrease was measured in the second year. The most prominent flavonols in black and red currant cultivars were quercetin glycosides. However, the amounts were much higher in black currants as compared to the red ones.

The differences between the cultivars were established with the use of cluster analysis of mean values of the 2 year's data on primary and secondary compounds (Figure 1). The results show a good separation of red and black currant cultivars, which was already noted for individual compounds where generally the values of black currants were superior to those of red ones. This was especially evident in the group of anthocyanins and has previously been reported by Gavrilova et al.[31] Within the group of red currants, the highest similarity was achieved between the 'Rolan' and 'Stanza' cultivars. Meanwhile, in the group of black currants, a higher degree of similarity was detected between the 'Titania' and the 'Tsema' cultivars. These were distinguished from the other black currant cultivars, especially by their high average values of total anthocyanins.

The hypothesis in the present work aimed to answer how productivity influences the content of primary and secondary metabolites in currant species. Genotype had a profound influence on the content of analyzed flavonoids and primary metabolites in red and black currant species and contributed significantly to the separation into two groups with black currants being superior in the content of anthocyanins and flavonols. The differences were less prominent when comparing the content of sugars and organic acids with the exception of vitamin C content, which was significantly higher in black currants.

Important differences in the content of secondary metabolites between the 2 years were detected, indicating that the increased yield in 2009 did not reduce the content of anthocyanins and flavonols in berries. Probably the weather conditions played an important role on the phenolic content. Higher yield, however, decreased the amounts of sugars and vitamin C and increased the amounts of organic acids, making fruits of both species less sweet for the consumer. The obtained data are of high relevance for both nutritional scientists as well as researchers striving to optimize the production technologies. Further research on the impact of growing conditions and

Table 6. Content of Individual and Total Anthocyanins (mg kg^{-1} FW) as Well as Total Phenolics in Different Cultivars of Red and Black Current in Years 2008 and 2009[a]

		black currant					red currant			
compound	year	'Titania'	'Triton'	'Tsema'	'Cacanska crna'	sig.	'Junifer'	'Rolan'	'Stanza'	sig.
delphinidin 3-rutinoside	2008	325.9 ± 20.7 c	81.0 ± 2.7 a	234.1 ± 18.7 b	63.5 ± 6.5 a	***				
	2009	615.7 ± 54.9 d	212.5 ± 16.9 b	391.6 ± 15.1 c	95.8 ± 9.3 a					
delphinidin 3-glucoside	2008	69.1 ± 0.6 b	54.2 ± 4.8 a	123.1 ± 4.9 c	46.2 ± 5.1 a	***				
	2009	121.3 ± 4.2 b	137.4 ± 4.8 b	233.5 ± 16.8 c	63.3 ± 4.5 a					
delphinidin 3-(6″-coumaroyl)glucoside	2008	11.4 ± 1.1 c	5.5 ± 0.3 b	8.8 ± 1.2 c	0.7 ± 0.0 a	***				
	2009	14.8 ± 2.8 b	13.7 ± 1.0 b	18.0 ± 1.3 b	2.0 ± 0.1 a					
delphinidin 3-xyloside	2008	1.9 ± 0.1 c	0.3 ± 0.0 a	1.0 ± 0.1 b	0.5 ± 0.0 ab	**				
	2009	2.6 ± 0.1 b	1.2 ± 0.0 a	3.5 ± 0.4 b	0.7 ± 0.4 a					
cyanidin 3-glucoside	2008	16.2 ± 1.2 a	14.2 ± 0.8 a	25.8 ± 1.1 b	51.3 ± 8.1 c	***				
	2009	22.2 ± 1.1 a	38.2 ± 2.0 b	44.6 ± 0.7 bc	52.1 ± 3.4 c					
cyanidin 3-rutinoside	2008	178.5 ± 7.7 d	64.9 ± 2.5 a	134.1 ± 9.2 c	98.2 ± 7.6 b	**	17.6 ± 0.6 a	15.9 ± 1.5 a	27.2 ± 1.4 b	***
	2009	263.7 ± 10.9 b	151.9 ± 13.3 a	265.2 ± 4.9 b	178.5 ± 18.0 a		45.7 ± 0.4 b	22.8 ± 1.2 a	56.4 ± 2.6 c	
cyanidin 3-(6″-coumaroyl) glucoside	2008	2.9 ± 0.1 d	1.4 ± 0.1 b	2.1 ± 0.1 c	0.7 ± 0.0 a	***				
	2009	3.8 ± 0.4 bc	2.5 ± 0.2 ab	4.4 ± 0.4 c	1.5 ± 0.1 a					
cyanidin 3-glucosylrutinoside	2008						ND[b]	30.1 ± 3.0 a	40.5 ± 0.9 b	***
	2009						ND	59.2 ± 3.5 a	82.9 ± 5.1 b	
cyanidin 3-sambubioside	2008						10.9 ± 0.6 a	10.7 ± 0.8 a	22.4 ± 1.9 b	***
	2009						14.9 ± 0.5 a	22.0 ± 0.4 b	27.4 ± 2.0 c	
cyanidin 3-sophoroside	2008						5.8 ± 0.1 a	11.1 ± 0.5 b	15.6 ± 0.8 c	NS
	2009						5.6 ± 0.5 a	14.2 ± 0.2 b	15.7 ± 0.6 b	
cyanidin 3-xylosylrutinoside	2008						48.0 ± 2.6 b	30.4 ± 1.2 a	47.4 ± 0.3 b	***
	2009						128.9 ± 2.1 c	59.7 ± 3.2 a	96.4 ± 7.3 b	
peonidin 3-rutinoside	2008	8.9 ± 0.4 c	3.5 ± 0.4 b	4.0 ± 0.2 b	1.6 ± 0.6 a	***				
	2009	9.2 ± 0.3 b	8.4 ± 0.4 b	6.5 ± 0.8 b	2.4 ± 0.9 a					
petunidin 3-rutinoside	2008	19.0 ± 1.7 c	5.0 ± 0.4 b	7.0 ± 0.6 b	1.3 ± 0.5 a	**				
	2009	26.1 ± 0.4 b	16.5 ± 1.0 b	20.0 ± 1.1 b	3.2 ± 1.4 a					
total anthocyanins	2008	633.8 ± 33.6 b	230.0 ± 12.2 a	540.0 ± 36.2 b	264.0 ± 28.8 a	***	82.4 ± 3.6 a	98.2 ± 8.9 a	153.1 ± 13.2 b	***
	2009	1079.4 ± 75.1 b	582.3 ± 39.6 a	987.3 ± 41.7 b	399.5 ± 38.6 a		195.2 ± 1.9 a	177.9 ± 7.4 a	278.8 ± 21.7 b	
TPC	2008	1443.1 ± 68.7 b	1171.6 ± 16.7 a	1331.6 ± 46.1 ab	1225.8 ± 88.8 a	NS	1167.9 ± 82.5 b	643.9 ± 45.2 a	551.4 ± 37.2 a	NS
	2009	1265.2 ± 40.7 b	831.6 ± 77.2 a	1651.6 ± 117.0 c	1178.0 ± 106.4 b		547.2 ± 42.2 a	694.0 ± 8.9 b	671.1 ± 2.5 b	

[a] Mean values ± standard errors are presented. Different letters in rows denote significant differences between cultivars in an individual year ($P \leq 0.05$). Asterisks in the column sig. (significance) indicate statistically significant differences between 2 years for each compound at: NS, not significant; *$P \leq 0.05$; **$P \leq 0.005$; and ***$P \leq 0.001$. [b] ND, not detectable.

Table 7. Content of Individual and Total Flavonols (mg kg^{-1} FW) in Different Cultivars of Red and Black Currant in Years 2008 and 2009[a]

		black currant					red currant			
compound	year	'Titania'	'Triton'	'Tsema'	'Cacanska crna'	sig.	'Junifer'	'Rolan'	'Stanza'	sig.
kaempferol 3-galactoside	2008	4.0 ± 0.4 b	1.7 ± 0.0 a	9.2 ± 0.5 c	1.5 ± 0.4 a	***				
	2009	5.7 ± 0.6 a	4.3 ± 0.2 a	19.1 ± 1.8 b	4.2 ± 0.5 a					
kaempferol 3-rutinoside	2008	5.2 ± 0.8 b	1.6 ± 0.2 a	4.8 ± 0.7 b	1.8 ± 0.4 a	***	1.8 ± 0.1 b	1.2 ± 0.2 a	1.1 ± 0.1 a	*
	2009	5.5 ± 0.7 b	2.8 ± 0.1 a	15.3 ± 1.2 c	3.6 ± 0.1 ab		1.3 ± 0.0 a	1.9 ± 0.1 b	3.5 ± 0.1 c	
myricetin 3-galactoside	2008	3.5 ± 0.2 b	4.1 ± 0.1 c	3.1 ± 0.0 b	0.3 ± 0.0 a	**				
	2009	2.9 ± 0.3 b	6.6 ± 0.4 d	5.6 ± 0.1 c	1.0 ± 0.1 a					
myricetin 3-glucoside	2008	10.3 ± 0.3 c	8.3 ± 0.3 b	9.2 ± 0.2 b	7.0 ± 0.4 a	***	1.5 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	NS
	2009	14.9 ± 0.5	13.0 ± 1.0	20.3 ± 3.3	15.1 ± 0.5		1.4 ± 0.1 a	1.8 ± 0.0 a	2.6 ± 0.4 b	
myricetin 3-malonylglucoside	2008	11.8 ± 0.6 d	4.7 ± 0.2 b	0.1 ± 0.0 a	8.8 ± 0.9 c	**				
	2009	10.1 ± 1.6 a	8.3 ± 0.1 a	21.3 ± 1.9 b	12.2 ± 1.1a					
myricetin 3-rutinoside	2008	32.0 ± 3.8 b	5.3 ± 0.3 a	9.5 ± 0.7 a	9.7 ± 1.2 a	***	1.8 ± 0.1 c	0.6 ± 0.0 a	1.2 ± 0.2 b	NS
	2009	55.7 ± 6.0 a	8.3 ± 0.4 a	18.8 ± 0.9 b	14.9 ± 0.8 ab		1.5 ± 0.0 b	1.1 ± 0.1 a	0.9 ± 0.1 a	
quercetin 3-galactoside	2008	2.1 ± 0.2 b	2.3 ± 0.1 b	4.1 ± 0.2 c	0.5 ± 0.0 a	**				
	2009	2.2 ± 0.1 b	4.4 ± 0.1 c	6.5 ± 0.3 d	0.7 ± 0.1 a					
quercetin 3-glucoside	2008	15.9 ± 1.4 b	4.8 ± 0.4 a	18.4 ± 1.2 b	4.7 ± 0.6 a	**	1.5 ± 0.1	1.8 ± 0.2	1.6 ± 0.1	**
	2009	23.5 ± 1.2 b	11.8 ± 0.6 a	34.9 ± 1.1 c	17.3 ± 1.2 ab		1.3 ± 0.0 a	2.8 ± 0.1 b	3.1 ± 0.2 b	
quercetin 3-rutinoside	2008	34.8 ± 1.7 c	4.7 ± 0.4 a	19.7 ± 1.6 b	17.1 ± 1.2 b	***	10.3 ± 0.4	10.8 ± 0.7	8.8 ± 0.4	***
	2009	49.7 ± 1.9 c	9.0 ± 0.2 a	45.4 ± 1.4 c	29.6 ± 1.2 b		10.9 ± 0.3 a	19.3 ± 1.5 b	23.8 ± 1.1 c	
quercetin 3-malonylglucoside	2008	7.4 ± 0.4 b	1.3 ± 0.0 a	6.5 ± 0.2 b	8.6 ± 0.5 c	***	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	*
	2009	7.6 ± 0.3 a	6.9 ± 0.2 a	17.5 ± 0.6 c	10.8 ± 0.4 b		0.5 ± 0.0 a	1.1 ± 0.1 b	1.0 ± 0.0 a	
quercetin 3-rhamnoside	2008						1.3 ± 0.1 a	3.1 ± 0.1 b	1.2 ± 0.1 a	*
	2009						1.3 ± 0.0 a	4.5 ± 0.0 b	1.1 ± 0.1 a	
total flavonols	2008	127.0 ± 9.8 d	38.8 ± 1.9 a	84.5 ± 5.4 c	60.0 ± 4.6 b	***	18.9 ± 0.9	19.9 ± 1.4	16.5 ± 1.0	**
	2009	177.8 ± 13.2 c	75.4 ± 3.3 a	204.7 ± 12.6 d	109.4 ± 6.0 b		18.2 ± 0.4 a	32.5 ± 1.9 b	36.0 ± 2.0 c	

[a] Mean values ± standard errors are presented. Different letters in rows denote significant differences between cultivars in an individual year ($P \leq 0.05$). Asterisks in the column sig. (significance) indicate statistically significant differences between 2 years for each compound at: NS, not significant; $*P \leq 0.05$; $**P \leq 0.005$; and $***P \leq 0.001$.

```
                            Rescaled Distance Cluster Combine

     C A S E        0         5        10        15        20        25
Label          Num  +---------+---------+---------+---------+---------+

Rolan            6   -+---------+
Stanza           7   -+         +-------------------------------------+
Junifer          5   -----------+                                     |
Titania          1   -----------+-------------+                       |
Tsema            3   -----------+             +-----------------------+
Triton           2   ---------------------+---+
Cacanska crna    4   ---------------------+
```

Figure 1. Dendrogram using an average linkage (between groups) dendrogram for a 2 year mean value of primary and secondary metabolites of different currant cultivars, using Ward's method based on square Euclidian distance.

cultural practices is needed to produce currant fruit with high amounts of beneficial phytochemicals, especially with an increasing trend of fresh currant consumption.

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Notes

The authors declare no competing financial interest.

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