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Does plant growth and yield affected by Prohexadione Ca cause changes in chemical fruit composition of 'Loch Ness' and 'Triple Crown' blackberries?

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Summary

Two semi-erect blackberry cultivars, 'Loch Ness' and 'Triple Crown', were used to investigate the effect of Prohexadione-Ca (ProCa) on vegetative growth, generative potential and fruit quality parameters. ProCa reduced the cane and internode length, but increased the node number per meter of cane length in both cultivars. ProCa application enhanced the number of fruiting branches and yield per bush in the following year. Fruit weight, number of drupelets per fruit, fruit shape index, content of soluble solids, shikimic acid and some phenolics were significantly increased by ProCa. ProCa-cultivar interaction was significant regarding the content of quercetin-3-rutinoside and procyanidin trimer. 'Loch Ness' had approximately 40% higher yields per bush and contained abundant quantities of anthocyanins and flavonols, whereas larger fruits and higher content of primary metabolites, some flavanols and total hydroxycinnamic acid derivatives were found in 'Triple Crown'. According to obtained results, the ProCa application at the rate from 100 to 200 mg L^{-1} is able to establish acceptable growth control without negative effects on yield and chemical fruit composition.

Keywords
growth retardant, fruit quality, organic acids, phenolic compounds, productivity, *Rubus* Watson, sugars, vegetative potential

Significance of this study

What is already known on this subject?

- Prohexadione-Ca (ProCa) is applied to agronomic and horticultural crops to reduce unwanted longitudinal shoot growth without lowering productivity.

What are the new findings?

- The application of this compound is able to establish growth control of blackberries without negative effects on yield and fruit quality.

What is the expected impact on horticulture?

- Modification of vegetative primocane growth during the first season, aiming at increased quantity and quality of fruits in the next season.

Introduction

Semi-erect thornless blackberries (*Rubus* subgenus *Rubus* Watson), such as the two cultivars investigated in this study, have a clearly defined growth cycle. These biennial-fruiting cultivars possess an intensive vegetative primocane growth in the first year. Primocanes grow in length and buds develop in the leaf axils. In temperate regions, cane extension growth diminishes in late summer or fall and process of 'flower initiation' begins in axillary buds on the primocanes (Takeda et al., 2003). The following year, when reproductive growth resumes, these canes produce flowers and become floricanes. Fruiting branches emerge from axillary buds on main and lateral branches of floricanes, which die out soon after fruiting and should be removed at any time before spring.

As both types of cane simultaneously exist in the hedgerow, there is a large competition between the vegetative growth of primocanes and the reproductive phase of floricanes. Primocanes, which begin to grow first, could reduce both sunlight interception and its within-hedgerow distribution. Sunlight distribution influences both the flower initiation in primocanes and the level of productivity and fruit quality on floricanes, particularly size, colour, and soluble solids content (Miller and Tworkoski, 2003).

Plant growth retardants are applied to agronomic and horticultural crops to reduce unwanted longitudinal shoot growth without lowering plant productivity. Prohexadione-Ca (ProCa) is a new-generation gibberellin biosynthesis inhibitor that has low toxicity and persistence in the plant (Mandemaker et al., 2005). Recent studies have highlighted the growth retardants application on different fruit crops, such as apple (Ramírez et al., 2010), pear (Smit et al., 2005), sweet cherry (Jacyna and Lipa, 2010), avocado (Mandemaker et al., 2005), strawberry (Black, 2006; Hytönen et al., 2009) and raspberry (Poledica et al., 2012). The application of ProCa on blackberries has not been studied yet. Research of Poledica et al. (2012) indicates that ProCa significantly reduced cane length and diameter of raspberry cv. Willamette, whereas node number per meter of cane length and yield per cane were significantly increased. In the same aspect, planting year ProCa application was studied in strawberries to reduce the runnering and increase the number of crown branches which was correlated with increased number of inflorescences and, consequently, berry yield in the following year (Hytönen et al., 2009). According to Mikulic-Petkovsek et al. (2009), ProCa treatment also affected the metabolism of phenolic substances in the developing fruit and leaves of two apple varieties ('Jonagold' and 'Florina'). In their study, the content of some phenolic compounds, which play an active part in resistance to apple scab, increased after the treatment, while the level of total phenolics in the skin and pulpa decreased resulting in lower antioxidant activity of the apples.

Factors defining outer and inner fruit quality, such as fruit weight, the content of soluble solids, organic acids, and phenolics, are also indirectly affected by vegetative growth and light distribution. It is well known that blackberries are characterized by a high content and wide diversity of the phenolic compounds (Määttä-Riihinen et al., 2004; Mikulic-Petkovsek et al., 2012; Milivojević et al., 2013; Wang and Lin, 2000) which play an important role in controlling oxidative reactions in the human body and exhibit anticarcinogenic activities. Unfortunately, almost no information is currently available on the effects of plant growth and yield affected by growth retardants on both chemical fruit composition and antioxidant capacity of blackberries.

Therefore, the aim of the current study was to establish whether it would be possible to use the tested rates of ProCa to optimize the vegetative growth, not affecting the productivity and fruit quality of blackberries in commercial plantations. Additional aim was to determine whether the response to ProCa application is cultivar-specific in semi-erect blackberries.

Materials and methods

Plant material and experimental design

The current study was conducted over two consecutive years (2013 and 2014) in a blackberry plantation of semi-erect cultivars 'Loch Ness' and 'Triple Crown' located near Šabac, Republic of Serbia (44°45'N, 19°41'E, 94 m a.s.l.). This region has temperate continental climate, with an average annual temperature of 12.3°C for 2013 and 12.9°C for 2014. Average annual precipitation in the experimental period ranged from 608.6 mm (2013) to 735.2 mm (2014). The orchard was planted in the spring of 2009 in the form of vertical 3-wire trellis system. The planting distance was 1.5 m in the row and 3 m between the rows (2,200 bushes per ha).

The blackberry canes were treated in 2013 with foliar sprays of Regalis®, containing as an active ingredient ProCa (BASF 125 10 W–10% Pro-Ca, BASF, Germany). Foliar application of ProCa was carried out twice. The first spraying was performed on 25 April when the primocane growth reached 30 cm in height. A second application of ProCa was done 3 weeks later. The following concentrations of ProCa were applied: 100 mg L^{-1} (the first application) and 200 mg L^{-1} (the second application). In both terms, removal was done when the primocanes were 30 cm high. Lower parts of floricanes and the whole length of young canes were treated with ProCa solutions. Spray volumes of 300 L ha^{-1} were applied.

The experimental design was completely randomized with 4 replications per treatment. The treatments consisted of bushes treated with ProCa and untreated/control bushes. Six randomly selected bushes per replication were investigated (24 bushes per treatment).

Vegetative potential

Measurement of vegetative characteristics [number of canes per bush, cane length (cm), cane diameter (mm), internode length (cm) and number of nodes per meter of cane length] was performed on primocanes at the end of the 2013 growing season.

Generative potential

Generative characteristics were investigated in 2014 by counting the number of fruiting branches and fruits per floricane, and weighing the harvested fruit to determine yield per cane at commercial maturity (kg). Yield per bush (kg) was calculated as a product of cane number per bush and yield obtained per cane.

Fruit quality traits

1. Biometrical fruit traits. To assess biometrical fruit properties (fruit weight, index of fruit shape and number of drupelets per fruit), fully ripe fruit samples were collected in 4 replications on 20th of July, 2014. In laboratory, the fruit weight was determined by weighing 50 fruit (± 0.1) per replication (200 per treatment). For drupelets counting, the same fruit samples were used within each replication. Index of fruit shape was also determined as the ratio of the maximum height and width.

2. Chemical analyses. The soluble solids content (SSC %) was determined by digital refractometer (Pocket PAL-1, Atago, Japan). Primary metabolites (glucose, fructose, citric, malic, tartaric, quinic, shikimic and fumaric acid) were analysed in four replications (n = 4) per cultivar. For the extraction of primary metabolites, 5 g of fruit were homogenized in 25 mL of double distilled water using Ultra-Turrax T-25 (Ika-Labortechnik) and left for 30 min at room temperature. Extraction protocol was performed as reported by Mikulic-Petkovsek et al. (2012). The HPLC (Thermo Scientific, San Jose, USA) analysis of sugars was performed using a Rezex RCM-monosaccharide Ca+ (2%) column (Phenomenex, 300 mm × 7.8 mm) operated at 65°C. The mobile phase was double distilled water, and the flow rate was 0.6 mL min^{-1}; the total running time was 30 min, and a refractive index (RI) detector was used as previously described (Mikulic-Petkovsek et al., 2012). Organic acids were analysed with the same HPLC system, equipped with a UV detector set at 210 nm, using a Rezex ROA – organic acid H+ (8%) column (Phenomenex, 300 mm × 7.8 mm) (Mikulic-Petkovsek et al., 2012). The concentrations were calculated with the help of the corresponding external standard and expressed as g kg^{-1} fresh weight (FW) for sugars and mg 100 g^{-1} FW for organic acids, respectively. The content of all analysed sugars and organic acids was summed up and presented as their total amount.

The phenolic extraction of blackberry fruits was performed as described by Mikulic-Petkovsek et al. (2010). All phenolic compounds were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionization (ESI) operating in positive (anthocyanins) and negative (other phenolics) ion mode. The analyses were carried out using full scan data-dependent MSn scanning from m/z 115 to 1,500. The injection volume was 10 µL and the flow rate maintained at 1 mL min^{-1}. The capillary temperature was 250°C, the sheath gas and auxiliary gas were 20 and 8 units respectively, and the source voltage was 4 kV for negative ionization and 0.1 kV for positive ionization. Spectral data were elaborated using the Excalibur software (Thermo Scientific). The identification of compounds was confirmed by comparing retention times and their spectra, by adding the standard solution to the sample and by fragmentation. Concentrations of phenolic compounds were calculated from peak areas of the sample and the corresponding standards and expressed in µg g^{-1} FW of the fruit.

The amount of total phenolics (TPC) in extracts was determined employing Folin-Ciocalteu spectrophotometric

(2501 PC Shimadzu, Kyoto, Japan) procedure (Singleton and Rossi, 1965), using gallic acid (GA) as a standard for the calibration curve. Results were read at 724 nm and expressed as milligrams of GA equivalent per gram of FW (mg GA eq g^{-1} FW).

Determination of total antioxidant capacity (TAC) was done following the ABTS method (Arnao et al., 1999). The reactions were monitored at 730 nm (2501 PC Shimadzu, Kyoto, Japan) at 25°C until a stable absorbance was obtained due to ABTS radical formation. Afterwards, different concentrations (0.1–0.8 mM) of ascorbic acid were added for a standard curve set-up. Adding of methanolic extracts to the reaction mixture resulted in absorbance decreasing caused by ABTS radical depletion. Results were expressed as milligrams of ascorbic acid equivalent per gram of fresh weight (mg AsA eq g^{-1} FW).

Statistical analysis

Statistical analysis was performed using software Statistica 8.0 for Windows (StatSoft Inc., Tulsa, OK, USA). The data of vegetative parameters was analysed for 2013, whereas data of generative potential and fruit quality parameters were analysed for 2014. A two-way analysis of variance (ANOVA) was carried out to determine the significance of cultivar and ProCa treatment. Mean separation was performed using LSD test ($P < 0.05$).

Results

Effect on vegetative growth traits

As shown in Table 1, significantly higher mean value of cane length ($P < 0.001$) was observed in cultivar 'Loch Ness' (3.7 m) than in 'Triple Crown' (3.1 m), whereby ProCa treatment significantly reduced cane length ($P < 0.001$) in both cultivars, compared to the control. Cane diameter was reduced by ProCa application ($P < 0.05$), without significant effect of the cultivar itself and ProCa-cultivar interaction. Number of nodes per meter of cane length and internode length were significantly affected by ProCa application ($P < 0.001$), and also affected by the cultivar alone ($P < 0.01$ and $P < 0.001$, respectively). Internode length was almost 35% reduced under ProCa treatment in 'Loch Ness', whereas in 'Triple Crown' reduction was 50% compared to the control.

Effect on generative traits

A positive impact of ProCa application on number of fruiting branches per floricane and yield per bush ($P < 0.05$) in 2014 was observed (Table 2). ProCa-cultivar interaction did not have a significant effect on tested parameters. The cultivar significantly influenced the number of fruiting branches ($P < 0.001$), fruits per floricane ($P < 0.01$), and yield per bush ($P < 0.05$). 'Loch Ness' was more productive cultivar with approximately 40% higher yields per bush than 'Triple Crown'. However, none of the factors influenced yield per floricane.

Effect on fruit quality attributes

1. Biometrical fruit traits. 'Triple Crown' had significantly higher fruit weight and number of drupelets per fruit (7.5 g and 78.6, respectively) than 'Loch Ness' (6.0 g and 67.1, respectively). ProCa treatment significantly increased the fruit weight ($P < 0.01$), number of drupelets per fruit and fruit shape index ($P < 0.05$), whereby ProCa-cultivar interactions were not significant (Table 3).

2. Chemical fruit traits. SSC significantly varied with cultivars ($P < 0.01$), treatments ($P < 0.001$), and the influence of

Table 1. Vegetative traits of Prohexadione Ca (ProCa) treated and untreated blackberry primocanes in 2013. Means ± S.E.M. ($n = 4$).

Cultivar (C)	Treatment (T)	No of cane per bush	Cane diameter (mm)	Cane length (m)	Internode length (cm)	No. of nodes per m of cane length
'Loch Ness'	ProCa	4.7±0.29	17.2±0.95	2.4±0.07	3.4±0.04c	29.7±0.37
	Control	4.5±0.47	20.1±0.48	3.7±0.06	7.5±0.02a	13.3±0.06
'Triple Crown'	ProCa	4.2±0.12	19.0±1.36	1.5±0.13	3.0±0.18c	33.2±1.93
	Control'	3.5±0.24	22.4±0.76	3.1±0.07	5.2±0.14b	19.5±0.50
Factors	C	*	ns	***	***	***
	T	ns	**	***	***	***
	C×T	ns	ns	ns	***	ns

Different letters in column denote significant differences (LSD test, P ≤ 0.05). Statistically significant differences at * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001; ns = not significant.

Table 2. Generative traits of Prohexadione Ca (ProCa) treated and untreated blackberry primocanes in 2014. Means ± S.E.M. ($n = 4$).

Cultivar (C)	Treatment (T)	No. of fruiting branches per floricane	No. of fruits per floricane	Yield per floricane (kg)	Yield per bush (kg)
'Loch Ness'	ProCa	27.5±1.48	213±22.9	1.43±0.203	6.7±0.95
	Control'	23.8±1.47	219±4.4	1.17±0.043	5.3±0.40
'Triple Crown'	ProCa	19.0±0.99	164±12.7	1.24±0.095	5.2±0.57
	Control'	14.5±0.99	149±17.1	1.09±0.166	3.8±0.40
Factors	C	***	**	ns	**
	T	*	ns	ns	*
	C×T	ns	ns	ns	ns

Statistically significant differences at * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001; ns = not significant.

Table 3. Biometrical traits of fruit harvested from Prohexadione Ca (ProCa) treated and untreated blackberry canes in 2014. Means ± S.E.M. (n = 4).

Cultivar (C)	Treatment (T)	Fruit weight (g)	Fruit shape index	No. of drupelets per fruit
'Loch Ness'	ProCa	6.6±0.35	1.20±0.009	73.0±3.60
	Control	5.4±0.16	1.17±0.009	61.1±0.98
'Triple Crown'	ProCa	7.8±0.34	1.19±0.006	80.6±2.86
	Control	7.2±0.10	1.17±0.015	76.6±1.89
Factors	C	***	ns	**
	T	**	*	*
	C×T	ns	ns	ns

Statistically significant differences at * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001; ns = not significant.

ProCa-cultivar interaction ($P < 0.01$). ProCa treatment considerable increased SSC in both cultivars ranging from 12.5% ('Loch Ness') to 14.1% ('Triple Crown'). Glucose and fructose were the main sugars present in almost equal ratio in tested cultivars. The levels of individual and total sugars were significantly affected by the cultivar ($P < 0.01$), whereby 'Triple Crown' contained higher amounts of both sugars in comparison to 'Loch Ness' (Table 4).

The predominant organic acid was citric, followed by tartaric and quinic acids. The portion of tartaric and quinic acids was almost equivalent in 'Loch Ness', while 'Triple Crown' contained almost 1.5-fold higher amount of quinic acid (501 mg 100 g^{-1} FW). Malic acid was represented up to one third in 'Loch Ness' and half in 'Triple Crown' of citric acid content. Fumaric and shikimic acids were represented in far lower quantities. Statistically, the cultivar significantly influenced the content of malic, shikimic, fumaric ($P < 0.01$) and quinic acids ($P < 0.05$), whereby higher levels were determined in the fruit of 'Triple Crown'. ProCa treatment only had a significant effect on shikimic acid content ($P < 0.05$).

Several anthocyanins have been identified in this study (Table 5). Four individual cyanidin glycosides, cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xyloside and cyanidin-3-6" malonylglucoside accounted for 93.2% of all analysed anthocyanins in 'Loch Ness', resp. 92.6% in 'Triple Crown'. The levels of identified anthocyanins were significantly affected by the cultivar except for cyanidin-3-6" malonylglucoside. 'Loch Ness' had more than 2-fold higher amount of cyanidin-3-glucoside and cyanidin-3-rutinoside compared to 'Triple Crown', whereas reverse pattern of cyanidin-3-xyloside was observed. ProCa treatment and ProCa-cultivar interaction did not have a significant effect on the total and individual anthocyanins.

The most prominent flavanols detected in 'Loch Ness' were procyanidin dimer 2, procyanidin dimer 3 and procyanidin dimer 1, while only procyanidin dimer 1 and procyanidin trimer were detected in 'Triple Crown'. Statistically, the cultivar significantly influenced the content of procyanidin dimer 1 and procyanidin trimer ($P<0.001$), as well as epicatechin content ($P<0.01$). However, their amounts were much higher in 'Triple Crown' compared to 'Loch Ness'. ProCa treatment and ProCa-cultivar interaction had a significant effect only on procyanidin trimer content ($P<0.01$). Specifically, ProCa application caused a significant decrease of procyanidin trimer content in 'Triple Crown'.

Amongst the flavonols (Table 6), quercetin-3-rutinoside represented the greatest share of all identified flavonols in tested cultivars ('Loch Ness' 53.6%; 'Triple Crown' 92.3%), followed by quercetin-3-glycoside 2 detected only in 'Loch Ness' (15.1%). Namely, quercetin-3-glycoside 1, quercetin-3-glycoside 2, quercetin-pentoside hexoside, kaempferol-3-galactoside and kaempferol glycoside were not detected in 'Triple Crown'. The cultivar significantly influenced the content of kaempferol-3-glucoside and quercetin-3-glucoside ($P<0.001$), as well as kaempferol-3-rutinoside ($P<0.01$) and quercetin-3-rutinoside ($P<0.05$). 'Loch Ness' had considerable higher content of kaempferol-3-glucoside, quercetin-3-rutinoside and quercetin-3-glucoside than 'Triple Crown', whereas higher level of kaempferol-3-rutinoside was detected in 'Triple Crown'. ProCa treatment was not significant for flavonols, but the content of quercetin-3-rutinoside was significantly ($P<0.05$) affected by ProCa-cultivar interaction. ProCa application significantly increased quercetin-3-rutinoside content in 'Loch Ness'.

From the group of hydroxycinnamic acids and their derivatives (Table 7), *p*-coumaric acid hexoside 1 and 2 were only found in 'Loch Ness' (45.5 and 138 µg g^{-1} FW, respectively). In 'Triple Crown', the greatest portion was represented by neochlorogenic acid (62.4% of total analysed hydroxycinnamic acids), followed by 3-*p*-coumaroylquinic and 4-*p*-coumaroylquinic acids (15.4% and 12.6%, respectively). None of identified hydroxycinnamic acids and their derivatives were significantly influenced by ProCa treatment.

The cultivar significantly influenced ($P<0.01$) the total phenolics content (TPC), whereas none of the treatments had significant effect on the antioxidant capacity (Table 8). Cultivar 'Loch Ness', rich in individual anthocyanins and flavonols, contained significantly higher amounts of TPC compared to 'Triple Crown' (9.6 mg and 7.7 mg GA eq g^{-1} FW, respectively).

Table 8. Total phenolic content (TPC, mg GA eq g^{-1} FW) and antioxidant capacity (TAC, mg AsA eq g^{-1} FW) in the fruit harvested from Prohexadione Ca (ProCa) treated and untreated blackberry canes in 2014. Means ± S.E.M. (n = 4).

Cultivar (C)	Treatment (T)	TPC	TAC
'Loch Ness'	ProCa	10.1±0.33	1.54±0.09
	Control	9.1±0.29	1.44±0.30
'Triple Crown'	ProCa	7.5±0.71	1.34±0.13
	Control	8.0±0.39	1.29±0.11
Factors	C	**	ns
	T	ns	ns
	C×T	ns	ns

Statistically significant differences at ** P ≤ 0.01; ns = not significant.

Table 4. Content of soluble solids (SSC, %), individual and total sugars (g kg^{-1} FW), individual and total organic acids (mg 100 g^{-1} FW) in the fruit harvested from Prohexadione Ca (ProCa) treated and untreated blackberry canes in 2014. Means ± S.E.M. (n = 4).

Cultivar (C)	Treatment (T)	SSC	Glucose	Fructose	**Total sugars**	Citric acid	Malic acid	Tartaric acid	Quinic acid	Shikimic acid	Fumaric acid	**Total acids**	Ratio sugars/acids
'Loch Ness'	ProCa	12.5±0.31b	20.7±1.95	21.2±1.77	41.9±3.72	748±139.9	272±41.2	322±45.2	312±22.9	2.3±0.37	1.4±0.15	1658±208	2.6±0.15
	Control	11.6±0.15c	20.7±2.94	20.9±2.96	41.6±5.90	814±33.1	273±4.3	352±26.1	348±66.7	3.0±0.34	1.6±0.09	1791±115	2.6±0.14
'Triple Crown'	ProCa	14.1±0.09a	24.4±2.40	24.7±2.40	49.1±4.80	720±59.3	399±24.8	251±23.8	427±52.9	3.5±0.49	2.1±0.34	1909±132	2.6±0.20
	Control	11.7±0.03c	33.5±2.15	34.1±2.24	67.6±4.39	864±36.1	375±37.5	303±16.4	575±52.3	4.7±0.23	2.6±0.08	2125±124	3.2±0.22
Factors	C	***	**	**	**	ns	**	ns	**	**	**	ns	ns
	T	***	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
	C×T	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Different letters in column denote significant differences (LSD test, P ≤ 0.05). Statistically significant differences at * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001; ns = not significant.

Table 5. The content of anthocyanins and flavanols (µg g^{-1} FW) in the fruit harvested from Prohexadione Ca (ProCa) treated and untreated blackberry canes in 2014. Means ± S.E.M. (n = 4).

Cultivar (C)	Treatment (T)	Cyanidin-3-glucoside	Cyanidin-3-rutinoside	Cyanidin-3-xyloside	Cyanidin-3-6" malonylglucoside	Pelargonidin-3-glucoside	**Total anthocyanins**	Procyanidin dimer 1	Procyanidin dimer 2	Procyanidin dimer 3	Procyanidin trimer	Catechin	Epicatechin	**Total flavanols**
'Loch Ness'	ProCa	401±39.5	110±9.6	4.9±0.37	0.95±0.210	35.9±3.71	553±52.8	37.6±2.38	73.1±7.47	50.6±7.5	4.8±0.44c	16.3±1.95	19.7±2.50	202±15.8
	Contro	401±55.9	116±18.1	5.2±1.13	1.16±0.253	39.7±8.17	563±82.9	37.4±7.763	81.2±5.99	54.0±5.2	3.8±0.63c	16.0±1.33	21.2±2.92	212±16.4
'Triple Crown'	ProCa	200±50.0	48±7.1	13.2±2.31	0.60±0.073	21.8±4.65	284±55.5	102.8±12.9	-	-	66.0±3.57b		27.2±2.26	196±16.2
	Control	229±14.2	42±3.7	7.4±0.64	0.73±0.236	21.6±1.40	301±20.0	108.8±1.81	-	-	80.3±0.88a		31.6±1.52	221±2.9
Factors	C	**	***	**	ns	*	**	***	-	-	***	-	**	ns
	T	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
	C×T	ns	ns	ns	ns	ns	ns	ns	-	-	**	-	ns	ns

Different letters in column denote significant differences (LSD test, P ≤ 0.05). Statistically significant differences at * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001; ns = not significant.

Table 6. The content of individual and total flavonols (µg g^{-1} FW) in the fruit harvested from Prohexadione Ca (ProCa) treated and untreated blackberry canes in 2014. Means±S.E.M. (n=4).

Cultivar (C)	Treatment (T)	Quercetin-3-glycoside 1	Quercetin-3-glycoside 2	Quercetin-pentoside hexoside	Quercetin-3-rutinoside	Quercetin-3-glucoside	Kaempferol-3-glucoside	Kaempferol-3-rutinoside	Kaempferol-3-galactoside	Kaempferol glycoside	**Total flavonols**
'Loch Ness'	ProCa	16.4±2.49	40.4±7.41	30.0±4.51	159±4.2a	9.8±1.85	9.0±1.36	2.8±0.42	0.091±0.0183	7.8±0.78	276±14.3
	Control	20.7±8.31	35.1±5.12	33.5±5.06	109±21.0b	9.0±1.31	8.6±2.47	2.0±0.33	0.065±0.0104	6.2±1.04	225±39.5
'Triple Crown'	ProCa	-	-	-	88±10.6b	2.9±0.11	0.8±0.10	3.6±0.42	-	-	95±10.7
	Control	-	-	-	107±0.9b	3.8±0.54	0.9±0.10	4.0±0.36	-	-	116±1.0
Factors	C				*	***	***	**	-	-	***
	T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	C×T				*	ns	ns	ns	-	-	ns

Different letters in column denote significant differences (LSD test, P ≤ 0.05). Statistically significant differences at * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001; ns = not significant.

Table 7. The content of hydroxycinnamic acid derivatives (μg g⁻¹ FW) in the fruit harvested from Prohexadione Ca (ProCa) treated and untreated blackberry canes in 2014. Means ± S.E.M. (n = 4).

Cultivar (C)	Treatment (T)	*p*-coumaric acid hexoside 1	*p*-coumaric acid hexoside 2	Chlorogenic acid	Isochlorogenic acid	Feruloylquinic acid	4-*p*-coumaroylquinic acid	5-*p*-coumaroylquinic acid	Neochlorogenic acid	3-*p*-coumaroylquinic acid	Total hydroxycinnamic acid derivatives
'Loch Ness'	ProCa	46.1±5.31	148±13.4	-	-	-	-	-	-	-	195±18.7
	Control	44.9±5.54	128±7.5	-	-	-	-	-	-	-	173±12.9
'Triple Crown'	ProCa	-	-	18.8±1.52	8.2±0.7	7.3±1.50	52.5±5.35	4.5±1.04	256±25.6	67.1±11.76	414±37.8
	Control	-	-	15.8±2.53	10.2±0.40	11.5±0.45	56.8±3.26	7.1±0.08	287±21.7	66.8±2.03	455±16.1
Factors	C	-	-	-	-	-	-	-	-	-	***
	T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	C×T	-	-	-	-	-	-	-	-	-	ns

Statistically significant differences at *** P ≤ 0.001; ns = not significant.

Discussion

Effect on vegetative potential

The current results indicate that ProCa can significantly decrease the cane extension growth of both 'Loch Ness' and 'Triple Crown' by approximately 35 to 50.3%, respectively. The research of Palonen and Mouhu (2008) and Poledica et al. (2012) also confirmed that ProCa reduced cane length in raspberries. Since ProCa has been classified as an inhibitor of the synthesis of gibberellins (Rademacher, 2000), changes in the cane length observed in blackberries in response to ProCa application support this finding. Moreover, Poledica et al. (2012) found significant reduction of cane diameter and an increase of node number per meter of cane length in 'Willamette' raspberry, which is in accordance with results currently obtained. The cultivars differed in their number of canes per bush, node number per meter of cane length, as well as cane and internode length. Effect of the cultivar was more prominent when combined with ProCa causing shorter internode length ('Loch Ness' – 3.4 cm and 'Triple Crown' – 3.0 cm). Changes in internode length could be attributed to a higher number of nodes per meter of cane length under ProCa treatment. In general, the growth response to ProCa application indicated that the differences between the two cultivars are more likely associated with genetic and environmental influences.

Effect on generative potential

The number of fruiting branches per floricane and yield per bush in 2014 were positively affected by ProCa application in the previous year, which could be explained by better growth conditions created by reduction in hedgerow volume and density. 'Loch Ness' was the more productive cultivar with higher number of fruiting branches and fruits per floricane, and consequently yield per bush compared to 'Triple Crown'. According to study conducted in Poland (Wójcik-Seliga and Wójcik-Gront, 2013), 'Loch Ness' produced over 6 kg per plant. ProCa application at tested rates had no significant influence on the fruit number per floricane in the current research. The opposite was observed in 'Willamette' raspberry (Poledica et al., 2012) and 'Royal Court TM' apple (Byers et al., 2004), as well as in 'Honeoye' and 'Polka' strawberries (Hytönen et al., 2009) where ProCa application increased the fruit set and yields.

Effect on fruit quality traits

1. Biometrical fruit traits. Blackberries harvested from ProCa-treated bushes tended to have a greater fruit weight, number of drupelets per fruit and fruit shape index than those from the control bushes. It is well known that ProCa hardly affects firmness, size and mass of apple (Basak, 2004; Cline et al., 2008) and sweet cherry fruits (Jacyna and Lipa, 2010), whereas no data are available on the relation of ProCa treatment vs. blackberry fruit quality. According to Clark and Finn (2011), the ideal berry weight for fresh market use ranges from 8 to 10 g. In the current study, 'Triple Crown' had fruit weight close to 8 g, which makes this cultivar more acceptable for fresh market. The fruit shape index was not cultivar-dependent, but ProCa application significantly increased the values. Contrary, Milošević et al. (2012) reported much higher values of fruit shape index for 'Loch Ness' grown in Serbia. Observed differences are probably due to diverse cultivation and environmental factors.

2. Chemical fruit traits. SSC increased in ProCa treatment and differed significantly between the cultivars. 'Triple

Crown' had significantly higher SSC compared to 'Loch Ness', but the differences were much prominent in interaction effect of ProCa and cultivar. In 'Triple Crown', SSC was significantly increased in ProCa treatment, whereas this increment was lower in 'Loch Ness'. The two tested cultivars also differed significantly in glucose, fructose and total sugars content, without significant effect of ProCa application. Glucose and fructose were present in almost equal ratio, and similar proportion of individual sugars in wild and cultivated blackberries was previously reported (Mikulic-Petkovsek et al., 2012). 'Triple Crown' had higher amounts of each analysed sugar and appeared sweeter than 'Loch Ness'. However, higher sugar content does not automatically mean a sweeter-tasting blackberry fruit, because the amount of organic acids is also important in the perception of sweetness (Milivojević et al., 2013). The main organic acid in the present study was citric, followed by tartaric and quinic acids. A high level of citric acid in blackberries was also reported (Mikulic-Petkovsek et al., 2012) where the level of fumaric acid was just in traces, whereas tartaric acid was not detected. Interestingly, abundant quantities of tartaric acid currently found was not significantly influenced by cultivar, ProCa treatment and their interaction. 'Triple Crown' contained higher levels of malic, quinic, shikimic and fumaric acids, but ProCa application showed a significant effect only on shikimic acid content. The sugar/acid ratio is often utilized as an index of sweetness for specific fruit. Fruits that taste sweet do not necessarily have high sugar content but they generally contain low levels of organic acids, especially malic acid (Mikulic-Petkovsek et al., 2012). Although malic acid content was distinguished, calculated sugar/acid ratio did not differ significantly among the treatments.

Phenolics, as secondary metabolites, also contribute to the sweet, bitter or astringent taste of fruit (Veberic et al., 2007). These compounds are mainly represented by flavonoids, phenolic acids, and tannins, which also provide health benefits as dietary antioxidants. In our study, cyanidin derivatives were the most frequently occurring pigments in both cultivars analysed, similarly reported by Hassimotto et al. (2008). The share of cyanidin derivatives in five blackberry cultivars grown in Brazil represented 66–80% of the flavonoids. In Veberic et al. (2015) two acylated glycosides were determined and corresponded to cyanidin 3-malonylglucoside and cyanidin 3-(6"-dioxalylglucoside), whereas four individual cyanidin glycosides were detected (cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xyloside and cyanidin-3-6" malonylglucoside); the latter was the only one not affected by cultivar. ProCa treatment and ProCa-cultivar interaction did not have a significant effect on the total and individual anthocyanin content.

Besides anthocyanins, flavonols and flavanols have also been explored. Quercetin-3-rutinoside represented the greatest share of all identified flavonols in both cultivars, followed by quercetin-3-glycoside 2 detected only in 'Loch Ness'. This has already been reported by Jakobek and Seruga (2012), who confirmed the presence of quercetin derivatives as a most prominent flavonols in blackberries. Additionally, treatment with ProCa significantly increased quercetin-3-rutinoside content in 'Loch Ness' which was also characterized by a considerable higher content of kaempferol-3-glucoside and quercetin-3-glucoside. Myricetin was not detected in the current study, which confirms the findings of Cho et al. (2004).

Procyanidin dimer 2, procyanidin dimer 3 and catechin were detected only in 'Loch Ness'. Treatment with ProCa significantly decreased procyanidin trimer content in 'Triple Crown'. It was also reported (Mikulic-Petkovsek et al., 2009) that ProCa treatment significantly decreased the flavan 3-ol content in apples. Some hydroxycinnamic acids and their derivatives proved to be of interest in blackberry fruits, in particular *p*-coumaric acid hexoside 2 detected in 'Loch Ness' and neochlorogenic acid detected in 'Triple Crown'. None of identified hydroxycinnamic acids and their derivatives were significantly influenced by ProCa treatment. As opposed to current results, ProCa treated apple fruits contained much more chlorogenic acid than untreated fruits during the major part of growing season, whereas there were no differences in concentration of other hydroxycinnamic acids at technologically ripe fruits (Mikulic-Petkovsek et al., 2009). This variation in the responses may in part be fruit species-dependent, but it is also affected by different time of ProCa application. ProCa treatment of raspberry primocanes caused an increase of TPC in the fruits from floricanes (Poledica et al., 2012) contrary to the current results obtained on blackberries. The determination of the TAC showed no significant effect of cultivar, ProCa treatment, and ProCa-cultivar interaction. Ramirez et al. (2010) also reported that ProCa did not express significant effect on the antioxidant capacity of apple fruit; moreover significant reduction of the antioxidant activity of ProCa treated fruits was observed by Mikulic-Petkovsek et al. (2009). The influence of two autumn preharvest applications of ProCa on the polyphenol metabolism in apple peel during the advanced maturation has previously been reported by Bizjak et al. (2013). Temporary effect of ProCa on the intensity of red coloration was found, which was not detected anymore at the technological maturity of apples.

In the current study conducted on blackberries, ProCa was primarily used as a plant growth regulator which expressed a positive effect on fruiting of floricanes, and even on biometrical and some chemical fruit traits.

From the present observations it can be generally concluded that ProCa may be a valuable tool, controlling vegetative growth in orchard technology of 'Loch Ness' blackberry. The application of this compound at the rate from 100 to 200 mg L^{-1} is able to establish acceptable growth control without negative effects on yield and chemical fruit composition. Due to an increasing commercial importance of 'Triple Crown' in the Serbian blackberry industry, further studies should be undertaken to determine optimal rates and time of ProCa application.

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