

## Prohexadione-Ca and young cane removal treatments control growth, productivity, and fruit quality of the Willamette raspberry

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**Abstract:** Plant growth retardants' applications have a practical use in fruit production, generally resulting in better vegetative growth and cropping level balance. Foliar applications of the gibberellin synthesis inhibitor prohexadione-Ca (ProCa), removal of young canes, and the combination of these treatments were evaluated in the Willamette raspberry (*Rubus idaeus* L.). The experiment consisted of 5 treatments, including 1 untreated control. ProCa was applied during the period of April–May, as a foliar spray at active ingredient concentrations of 100 ppm (first application) and 200 ppm (second application) at a 3-week interval, either in treatment with ProCa alone or in combination with young cane removal (R+ProCa). The first series of young primocanes were removed for the first time in mid-April (R and R+ProCa) and for the second time at the beginning of May (2R). Application of a growth retardant resulted in the inhibition of the cane's growth, followed by increasing the number of nodes per meter of cane. Positive effects of ProCa and R+ProCa on the number of fruiting laterals and yields per floricane were found without negative consequences on fruit quality, except for a reduction of total acidity. All treatments increased yield per cane. Total antioxidant capacity, total phenolics, and anthocyanin contents were significantly increased using ProCa.

**Key words:** Antioxidant compounds, cane removal, generative potential, growth retardant, raspberry

### Introduction

*Rubus* is accepted among the most diverse genera in the plant kingdom; it comprises over 400 species and is subdivided into 12 subgenera (Jennings 1988). However, only a few subgenera of *Rubus*, such as raspberries, blackberries, arctic fruits, and flowering raspberries, have been domesticated and utilized in breeding programs (Ercisli et al. 2008).

The place of origin of the red raspberry (*Rubus idaeus* L.) has been postulated to be the Ida

Mountains of Turkey (Ercisli 2004). Currently the Russian Federation, Poland, and Serbia are the main raspberry producers in the world; Serbia supplies about 20% of world raspberry production (FAO 2010). Raspberry is the most important agricultural crop in Serbia, providing large economic value.

One of the primary requirements to maintain the current production level and optimize fruit quality is the careful balancing of the vegetative and generative growth of raspberry plants (Nikolić and Milivojević

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2010). Raspberries form biennial canes that are vegetative the first year (primocanes) and fruitful the second (floricanes). Young canes (primocanes), arising in the spring from roots or the basal buds of old canes, have intensive growth during the spring and the first half of summer. The following year, these canes (floricanes) produce fruiting laterals without cane extension growth. Floricanes die soon after fruiting and should be removed at any time before the spring. As both types of cane simultaneously exist in the hedgerow, it seems likely that there is a large competition between the vegetative growth of primocanes and the reproductive phase of floricanes (Nenadić 1986). Primocanes, which begin to grow first, can be longer than 2 m with large leaves that reduce sunlight interception and within-hedgerow distribution. Sunlight distribution influences both the flower initiation in primocanes and the level of productivity and fruit quality in floricanes, particularly size, color, and soluble solids content (Miller and Tworowski 2003).

Previously, the selection of growing systems and adequate pruning to regulate the amount of biomass has been included in vegetative growth control. As reported before (Waister and Cormack 1977), the removal of young raspberry canes appeared to be useful for achieving yield increase in the fruiting canes. Nenadić (1986) confirmed that completely removing the first series of primocanes until flowering starts on the floricanes has a positive influence on yield, fruit quality, and disease prevention. Primocanes developing later do not have excessive vigor, but great fruiting potential for the following season can be expected. However, managing the vegetative growth by removing the first series of primocanes is a very labor-intensive and expensive management practice in commercial raspberry production.

Plant growth retardants are also applied in 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), which also has been proven to be a useful tool for the control of excessive vegetative growth and to improve fruit quality in apple (Ramírez et al. 2010). Recent studies

have highlighted the possibilities of application growth retardants on different fruit crops, such as apple, *Malus domestica* Borkh. (Medjdoub et al. 2004; Ramírez et al. 2010); pear, *Pyrus communis* L. (Southwich et al. 2004; Smit et al. 2005); grapevine, *Vitis vinifera* L. (Lo Giudice et al. 2004); avocado, *Persea americana* Mill. (Mandemaker et al. 2005); strawberry, *Fragaria ananassa* Duch. (Black 2006); and sweet cherry, *Prunus avium* L. (Jacyna and Lipa 2010). Although there is a need for intensifying the raspberry production, the application of ProCa growth retardant on this berry fruit has not been studied extensively. Palonen and Mouhu (2008) found that the application of ProCa strongly reduced cane growth and internode length in primocane fruiting in the raspberry cultivar Ariadne.

The research reported here was designed to evaluate the effects of both ProCa alone and in combination with young cane removal treatment on vegetative growth, yield components, and fruit quality of the Willamette raspberry. In addition, the aim of this study was to establish whether application of ProCa can be an appropriate method in commercial production instead of young cane removal as a labor-intensive management practice.

## Materials and methods

Studies were conducted at a commercial raspberry plantation of the cultivar Willamette located in Western Serbia (Krupanj), in the period of 2010–2011. The orchard was established in 2000 in the form of a vertical trellis system with columns of 2 m in height and 2 lines of single wire, to which canes are fastened by a plastic rope. The planting distance was 0.25 m in the row and 3 m between the rows.

The raspberry canes were treated in 2010 with foliar sprays of Regalis®, containing as an active ingredient ProCa. Where applicable, removal of the first series of young canes was also performed. More specifically, the experiment consisted of 5 treatments, as follows: untreated control, receiving no treatment at all; ProCa, treated with 2 ProCa applications; R, the first removal of young canes; R+ProCa, using both young cane removal and 2 ProCa applications; and 2R, a second young cane removal. Foliar application of ProCa was carried out twice during the period of

April–May. 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 ppm (first application) and 200 ppm (second application). Lower parts of floricanes and the whole length of young canes were treated with ProCa solutions. Spray volumes of 300 L ha<sup>-1</sup> were applied. The first series of young canes were removed for the first time in mid-April (R, R+ProCa, and 2R) and for the second time at the beginning of May (2R). In both terms, removal was done when the primocanes were 30 cm high.

The experimental design was completely randomized with 4 replications per treatment. The experimental area was established in 4 rows of the commercial raspberry plantation, where each row represents 1 replication including a 10-m length of hedgerow. Five randomly selected canes per replication were investigated (20 canes per treatment). All treatments were performed in each row. The untreated space between various treatments was 2 m. ProCa solution was applied at rate of 1 L treatment<sup>-1</sup>.

Investigation of vegetative characteristics (length of canes (cm), cane diameter (mm), number of nodes per meter of cane, and total number of nodes per cane) was carried out on primocanes at the end of the 2010 growing season. Generative characteristics were investigated in 2011 by counting the number of fruiting laterals, inflorescences, and fruits per cane and weighing the harvested fruit to determine yield per cane (g). Yield per meter of hedgerow (kg) was obtained during the harvest season from 10 June to 17 July 2011. It was calculated as a product of cane number per meter of hedgerow and yield obtained per cane for each harvest date.

To assess fruit quality, fruits were picked at the commercial maturity stage on 20 June 2011. A sample of 50 fruits per replication (10 fruits per cane) was used for the fruit quality assessment. In the laboratory, fruits were weighed and the number of drupelets per fruit was counted. A part of the fresh fruits was stored at -20°C for further analysis of phenolic compounds and antioxidative capacity. The rest of the samples were extracted with a hand press and filtered through cheesecloth. The soluble solids content (SSC) was

determined using a digital refractometer (Pocket PAL-1, Atago, Japan). Titratable acidity (TA) was measured using a digital burette and 0.1 M NaOH to titrate samples to an endpoint of 8.1. Acidity was based as a percentage of malic acid equivalent. Vitamin C was quantified using a reflectometer set (Merck RQflex, Merck KGaA, Germany) as described by Pantelidis et al. (2007). Results were expressed as mg ascorbic acid 100 g<sup>-1</sup> of fresh weight (FW).

For extraction of phenolic compounds, 5 g of frozen fruits were homogenized in 15 mL of extraction solution containing acetone, water, and hydrochloric acid at a ratio of 70:30:5 by volume. The homogenates were centrifuged at 10,000 × g for 20 min and supernatants were used for further analyses. Triplicate extractions were prepared for each sample. Total anthocyanin content (TACY) was measured with the pH differential method (Cheng and Breen 1991). Results were expressed as milligram of cyanidin-3-glucoside equivalent per 100 grams of fresh weight (mg eq cyanidin-3-glucoside 100 g<sup>-1</sup> FW). The amount of total phenolics (TPH) in extracts was determined according to the Folin-Ciocalteu spectrophotometric (2051 PC Shimadzu, Japan) procedure (Singleton and Rossi 1965). Phenol content was estimated from a standard curve of gallic acid (GA) and results were expressed as milligram of GA equivalent per gram of fresh weight (mg GAE g<sup>-1</sup> FW). Total antioxidant capacity (TAC) was assessed by following the ABTS method of Arnao et al. (1999). Results were expressed as milligram of ascorbic acid equivalent per gram of fresh weight (mg eq ascorbic acid g<sup>-1</sup> FW). A statistical analysis was performed by ANOVA using software Statistica 6.0 for Windows (StatSoft Inc., USA). Significant differences among the means were determined by a least significant difference (LSD) test at a level of P ≤ 0.05.

## Results

### Effect on vegetative growth characteristics

The lowest value of cane length was observed in ProCa treatment (211 cm) compared to other treatments as well as the untreated control (Table 1). An increase in node number per meter of cane length was also detected when ProCa was applied alone (31.7) or in combination with removal of young primocanes

Table 1. Effects of prohexadione-Ca (ProCa) and young cane removal treatment once (R) or twice (2R) on vegetative characteristics of Willamette raspberry primocanes in 2010.

Treatment	Length of canes (cm)	Cane diameter (mm)	Number of nodes per meter of cane	Total number of nodes per cane
Control	266 ± 4 a	6.65 ± 0.02 a	24.5 ± 0.3 c	65.3 ± 4.1
ProCa	211 ± 6 b	5.60 ± 0.03 b	31.7 ± 1.1 a	66.1 ± 5.7
R	249 ± 12 a	6.80 ± 0.02 a	25.3 ± 0.4 c	63.0 ± 12.0
R+ProCa	250 ± 5 a	6.80 ± 0.02 a	27.2 ± 0.1 b	68.0 ± 5.3
2R	253 ± 9 a	6.85 ± 0.02 a	25.4 ± 0.2 c	64.2 ± 8.6
P-value	*	*	*	ns

Data are the means of 4 replications ± standard error. Values within column followed by the same letter are not significantly different at  $P \leq 0.05$  (LSD test). \*Significant at  $P \leq 0.05$ ; ns – not significant.

(27.2). No differences were found in the total number of nodes per cane in all treatments. Finally, cane diameter was reduced by ProCa application (5.60 mm) but not when ProCa was combined with cane removal.

#### Effect on generative characteristics

A positive impact of ProCa application in terms of generative characteristics of floricanes in the subsequent year (2011) was also observed (Table 2). An increase in the number of fruiting laterals per cane was recorded in ProCa (14.3) and R+ProCa (14.1) in relation to other examined treatments. However, none of the treatments influenced the number of inflorescences per floricanes. On the other hand, ProCa, R, and R+ProCa had a positive effect on fruit number per cane with the highest value noted in treatment R+ProCa (218). All strategies were able to increase yield per cane compared to untreated control (537 g).

#### Effect on fruit quality attributes

All applied treatments had no effect on average fruit weight, whereas a higher value of drupelet number per fruit was observed in the ProCa treatment (106) (Table 3). SSC was slightly lower in the control treatment (10.5%) than in ProCa or R, whereas a very high TA was obtained in the control treatment (2.14%). No differences in vitamin C content were found among the treatments.

ProCa treatment promoted an increase in TPH content that achieved higher values in treatments ProCa and R+ProCa (16.0 and 16.7 mg eq GAE g<sup>-1</sup> FW, respectively), and consequently the highest levels of TAC were recorded in the same treatments (0.70 and 0.65 mg eq ascorbic acid g<sup>-1</sup> FW, respectively) (Table 4). Furthermore, ProCa and R+ProCa enhanced TACY content in the raspberry fruit (24.6 and 31.0 mg eq cyanidin-3-glucoside 100 g<sup>-1</sup> FW,

Table 2. Effects of prohexadione-Ca (ProCa) and young cane removal treatment once (R) or twice (2R) on generative characteristics of Willamette raspberry in 2011.

Treatment	Number of fruiting laterals per cane	Number of inflorescences per cane	Fruit number per cane	Yield per cane (g)	Yield per meter of hedgerow (kg)
Control	11.3 ± 0.6 b	37.6 ± 2.0	147 ± 18 c	537 ± 78 b	3.48 ± 0.19 b
ProCa	14.3 ± 0.3 a	44.2 ± 3.6	205 ± 14 ab	881 ± 31 a	4.38 ± 0.28 a
R	12.3 ± 0.8 b	41.7 ± 2.9	193 ± 11 ab	800 ± 49 a	4.13 ± 0.16 a
R+ProCa	14.1 ± 0.1 a	45.3 ± 4.3	218 ± 23 a	864 ± 40 a	4.27 ± 0.15 a
2R	12.1 ± 0.7 b	42.0 ± 2.5	173 ± 5 bc	766 ± 15 a	4.01 ± 0.16 ab
P-values	*	ns	*	*	*

Data are the means of 4 replications ± standard error. Values within column followed by the same letter are not significantly different at  $P \leq 0.05$  (LSD test). \*Significant at  $P \leq 0.05$ ; ns – not significant.

Table 3. Effects of prohexadione-Ca (ProCa) and young cane removal treatment once (R) or twice (2R) on fruit quality characteristics of Willamette raspberry in 2011.

Treatment	Fruit weight (g)	Number of drupelets per fruit	Soluble solids content (%)	Titrateable acidity (%)	Vitamin C content (mg 100 g <sup>-1</sup> FW)
Control	4.2 ± 0.3	90 ± 5 b	10.5 ± 0.3 b	2.14 ± 0.01 a	52.5 ± 0.6
ProCa	4.5 ± 0.1	106 ± 6 a	11.3 ± 0.1 a	1.96 ± 0.03 b	51.9 ± 1.1
R	4.5 ± 0.2	98 ± 2 ab	11.3 ± 0.1 a	2.02 ± 0.03 b	49.7 ± 1.3
R+ProCa	4.4 ± 0.3	97 ± 2 ab	11.0 ± 0.3 ab	1.97 ± 0.01 b	52.8 ± 0.7
2R	4.6 ± 0.1	99 ± 4 ab	10.7 ± 0.3 ab	2.00 ± 0.07 b	52.8 ± 1.9
P-values	ns	*	*	*	ns

Data are the means of 4 replications ± standard error. Values within column followed by the same letter are not significantly different at  $P \leq 0.05$  (LSD test). \*Significant at  $P \leq 0.05$ ; ns – not significant; FW – fresh weight.

respectively). In the control treatment, TACY was as low as 9.9 mg eq cyanidin-3-glucoside 100 g<sup>-1</sup> FW.

## Discussion

This study indicates that ProCa (Regalis®) can significantly decrease the vegetative cane growth of the Willamette raspberry. The research of Palonen and Mouhu (2008) also confirmed that the reduction of cane length was affected by ProCa treatment. The average cane length recorded in our experiment was generally greater than that reported by Eydurán et al. (2008) for the Willamette raspberry grown in Turkey, which could be explained as a consequence of different environmental conditions. These authors also found an almost 2-fold greater cane diameter than that obtained in our study. Reduction of the

cane diameter was associated with the use of ProCa, which is in accordance with earlier findings (Palonen and Mouhu 2008).

The positive impact of ProCa and R+ProCa treatments on the number of fruiting laterals and yields per cane observed in the subsequent year (2011) could be explained by better growth conditions created by reduction in hedgerow volume and density achieved using both ProCa alone and ProCa accompanied with the removal of young canes. Some other studies on apple (Medjdoub et al. 2004) and pear (Southwich et al. 2004) did not report any significant effect of ProCa on flower initiation. In addition, Palonen and Mouhu (2008) found that a reduction in return bloom was associated with the use of ProCa in primocane fruiting raspberries. Our results showed that the increase of fruit number per

Table 4. Effects of prohexadione-Ca (ProCa) and young cane removal treatment once (R) or twice (2R) on total anthocyanins (TACY), total phenolics (TPH), and total antioxidant capacity (TAC) in fruit of Willamette raspberry in 2011.

Treatment	TACY (mg eq cyanidin-3-glucoside 100 g <sup>-1</sup> FW)	TPH (mg GAE g <sup>-1</sup> FW)	TAC (mg eq ascorbic acid g <sup>-1</sup> FW)
Control	9.9 ± 3.0 c	13.2 ± 1.2 c	0.50 ± 0.04 b
ProCa	24.6 ± 3.4 ab	16.0 ± 0.6 ab	0.70 ± 0.03 a
R	18.7 ± 3.4 bc	13.8 ± 0.3 bc	0.53 ± 0.03 b
R+ProCa	31.0 ± 5.9 a	16.7 ± 0.3 a	0.65 ± 0.03 a
2R	11.5 ± 1.0 c	13.4 ± 0.3 c	0.56 ± 0.01 b
P-values	*	*	*

Data are the means of 4 replications ± standard error. Values within column followed by the same letter are not significantly different at  $P \leq 0.05$  (LSD test). \*Significant at  $P \leq 0.05$ ; ns – not significant; FW – fresh weight.

cane observed in ProCa, R, and R+ProCa treatments (205, 193, and 218, respectively) could be attributed to higher production of fruiting laterals as a result of both individual application of ProCa and its application combined with removal of young canes. In a previous study of the Willamette raspberry grown in Turkey, Eyduran et al. (2008) obtained lower yield per cane (96 g) compared to our control treatment (537 g), which can be explained by the favorable environmental conditions for raspberry-growing in Serbia.

The obtained values of fruit weight were not affected by ProCa and young cane removal in our study. However, ProCa alone without cane removal had a positive influence on drupelet number per fruit, indicating that ProCa treatment contributed to better physical fruit properties. Although the fruit weight was not changed by ProCa application, the number of smaller-sized drupelets per fruit was increased. Milivojević et al. (2010) reported a lower fruit size (3.4 g) for the Willamette raspberry, but their drupelet number per fruit (85.7) was comparable to that in our study. Willamette raspberry is also known for its nutritional qualities, which are characterized by the very high contents of sugars, acids, and vitamin C (Milivojević et al. 2011a). Values of SSC obtained in our experiment showed higher levels in all treatments, ranging from 10.5% to 11.3%, than the value of 9.7% previously reported for the Willamette cultivar grown in northeastern Turkey (Gülçin et al. 2011). TA was found to be similar among the examined treatments, but a significantly higher value was achieved in the control treatment, accompanied by low SSC. Tosun et al. (2009) found lower TA levels in different raspberry genotypes grown in Turkey.

Red raspberry is a great source of natural antioxidant substances that provide high nutritional, dietary, and health value. Vitamin C content in raspberry fruit was previously reported to be between 17 and 37 mg 100<sup>-1</sup> g FW (Pantelidis et al. 2007). In our study, higher levels of vitamin C (49.7 to 52.8 mg 100<sup>-1</sup> g FW) in raspberry fruit were consistently found, and no significant differences were expressed among the treatments. Conversely, the review of Ramírez et al. (2010) showed that ProCa increased the content of vitamin C in ripe apple fruits. Vitamin C has been shown to play an important role in

controlling oxidative reactions in the human body and it exhibits anticarcinogenic activities (Sun et al. 2002).

Currently, there is increasing interest in raspberries as an important source of bioactive compounds with antioxidant activity (Pantelidis et al. 2007). In particular, phenolic compounds contribute substantially to the antioxidant ability of raspberry fruit (Heinonen et al. 1998; Liu et al. 2002). Our results showed that the average content of TPH for the cultivar Willamette was in the range of 12.7 to 16.7 mg GAE g<sup>-1</sup> FW. In general, treatments with ProCa and R+ProCa were found to greatly promote an increase in TPH. Milivojević et al. (2011b) evaluated TPH content in 8 primocane fruiting red raspberry cultivars, and they found values ranging from 2.34 to 5.08 mg GAE g<sup>-1</sup> FW. Many factors are known to affect the raspberry fruit's nutritional quality, including cultivar and genotype, altitude, environmental conditions, and more (Ercisli et al. 2007). Anthocyanins, the pigments responsible for the red and blue color, also have an important contribution to the total antioxidant activity of raspberry fruit (Wang and Lin 2000). In the present study, the amounts of TACY varied greatly among the examined treatments and expressed a similar trend to that of TPH. This suggests that it is very likely that raspberry selections with greater concentrations of anthocyanins will have high antioxidant activity. Slightly higher results for TACY in raspberry fruit were reported by Pantelidis et al. (2007), which could be explained as a consequence of diverse environmental conditions, postharvest treatment, and storage, as well as different extraction procedures.

Antioxidant status in fruit is an important parameter of quality and plays a significant role as a health-protecting factor (Scalzo et al. 2005). The highest TAC level obtained in the Willamette raspberry was affected by ProCa, as previously reported for apple by Ramírez et al. (2010).

In conclusion, ProCa application leads to some alterations in the physiological and metabolic pathways that resulted in growth, productivity, and fruit quality changes of raspberry plants. ProCa was effective in reducing the vegetative growth, increasing the number of inflorescences per cane, and promoting the yield and nutritional fruit

quality of the Willamette raspberry. On the other hand, young cane removal did not affect vegetative growth or nutritional fruit quality, but instead enhanced the fruit yield. Therefore, ProCa provides more advantages than young cane removal and its application in the concentrations tested in this study could be recommended as appropriate in commercial raspberry production.

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