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ORIGINAL PAPER

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INFLUENCE OF PROHEXADIONE-CALCIUM ON VEGETATIVE GROWTH AND REPRODUCTION OF '0900 ZIRAAT' SWEET CHERRY

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ABSTRACT

Effects of prohexadione-calcium (ProCa, 125 and 250 mgL^{-1}) and ProCa + ammonium sulfate (AMS, 500 mgL^{-1}) treatments on vegetative characteristics (shoot growth, branch diameter, trunk cross-sectional area (TCSA), canopy volume, number of flower per cm^2, number of fruits per cm^2) and quality parameters (fruit size, flesh firmness, color, titratable acidity and soluble solids content (SSC)) of '0900 Ziraat' sweet cherry were investigated in this study. Solutions were sprayed when the shoots were just 10 cm long in 2014 and 2015. As compared to control, in 2015, TCSA decreased only with ProCa treatments (125 and 250 mgL^{-1}) and canopy volume and shoot length decreased with all treatments. On the other hand, number of flower and fruit per cm^2 significantly increased with all treatments. While there were not significant differences in fruit size, flesh firmness, color, SSC and titratable acidity values were significantly lower in 250 mgL^{-1} ProCa and 250 mgL^{-1} ProCa + AMS treatments. It was concluded that ProCa treatments could be used as an efficient tool for suppression of shoot growth and to increase the number of flower and fruits per cm^2 in '0900 Ziraat' sweet cherry cultivar.

Key words: canopy volume, *Prunus avium* L., Regalis, shoot length, trunk cross-sectional area

INTRODUCTION

It is essential to create a balance between vegetative and generative growth in fruit trees. Such a balance is required to accelerate flowering in young trees and to prevent excessive shoot growth-induced shading in productive trees. The balance is often disrupted by climate conditions or improper cultural practices. The imbalance may promote shoot growth and increase shading and consequently negatively influence flower bud formation and yield. Dwarf rootstocks or proper training systems can be used to control the growth and development of trees, but dwarf rootstocks alone are not sufficient to eliminate the problem fully [Guak et al. 2005]. Turkey has a significant position in world cherry production and dwarf rootstocks are not sufficiently widespread in Turkey. Strong rootstocks (*P. avium)* and semi-dwarf rootstocks (*P. mahaleb*) are still widely used in commercial cheery orchards in Turkey. Thus, high tree vigor creates various problems especially in harvest and other cultural practices.

Some growth regulators are used to control vegetative growth in apple and cherry trees [Edgerton 1986; Webster and Quinlan 1986]. The mode of action of these growth regulators is related to their inhibitory effects on gibberellin biosynthesis [Rademacher 2000]. Efficiency of gibberellin synthesis-inhibitors like paclobutrazol and daminozide in controlling vegetative growth was reported in several studies. However, registration of such substances were withdrawn for use on apples in several countries

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since they may accumulate in soil and plants and may have adverse impacts on environment and human health [Greene 1999]. ProCa without any negative impacts on human health is another growth regulator used to control vegetative growth. ProCa was identified and reported as an efficient growth regulator in different apple cultivars [Greene 1999; Rademacher and Kober 2003; Başak 2004; Byers et al. 2004; Medjdoub et al. 2005]. On the other hand, varying results were reported in cherries based on the cultivars [Manriquez et al. 2001; Elfving et al. 2003].

There are some previous researches reporting efficiency of ProCa in flower bud formation. It was reported that this growth regulator promoted flower bud formation in apples [Greene 2005], did not have significant effects on flower bud formation in cherries when used alone, but improved flower bud formation when combined with Ethephon [Elfving et al. 2003]. Negative impacts of ProCa on fruit quality haven't been reported before, but inconsistent results have been reported about positive impacts of ProCa on fruit quality. It was reported in some previous studies that ProCa was inactivated through interactions with mineral salts in water, especially with Ca, thus the impacts of ProCa could be improved through admixture with ammonium sulphate [Byers et al. 2004; Cline et al. 2008].

The present study was conducted to determine the effects of ProCa treatments on vegetative and generative growth of "0900 Ziraat" sweet cherry cultivar, which has a significant place in Turkish cherry production, and to investigate possible changes in efficiency of ProCa when used together with ammonium sulphate.

MATERIAL AND METHODS

Plant material. Experiments were conducted in Suşehri town of Sivas Province, Turkey (40°10'09.67"N latitude, 38°06'37.14"E longitude and 952 m altitude) during the years 2014 and 2015. Five-years old "0900 Ziraat" sweet cherry trees grafted on SL 64 rootstocks (a semi-dwarf rootstock belonging to *P. mahaleb* species) were used as the plant material of the study. Trees were planted at 4 × × 4 m spacing and trained with Steep Leader system. Standard cultural practices (irrigation, fertilization, disease control) were regularly performed throughout the experimental period.

Treatments. Thirty trees were selected for experiments and trees were divided into 6 blocks with 5 trees in each. A tree in each block was used for each treatment. Regalis® (containing 10% ProCa a.i, BASF, Turkey) was sprayed to trees at specified doses (125 and 250 mg L^{-1} ProCa) calculated based on active ingredient. ProCa was also combined with ammonium sulfate (AMS, 500 mg L^{-1}) to improve the efficiency. Treatments were arranged as the control without any sprayd (0 mgL^{-1} ProCa and AMS), 125 mgL^{-1} ProCa, 250 mgL^{-1} ProCa, 125 mgL^{-1} ProCa + AMS and 250 mgL^{-1} ProCa + AMS. Solutions were sprayed to trees when the annual shoots reached to a length of 10 cm with a hand-gun sprayer as to drip the entire tree early in the morning of a non-rainy day.

TCSA, canopy volume and shoot growth. Trunk diameters were measured 30 cm above the ground before the treatments and at the end growing season of experimental years. Then these values were used to calculate trunk cross-sectional areas (TCSA) (πr^2). Canopy heights and widths were also measured to calculate canopy volumes ($\pi r^2 h/2$). At the end of growing season of each year, shoot lengths, shoot diameters and internode lengths were measured over 20 annual shoots of each tree.

Number of flowers and fruits per cm². Three branches were selected from each tree. Branch stem diameters were measured to calculate branch cross sectional area (BCSA). Number of flowers was counted in flowering period and number of fruits was counted at harvest period. These counts were used to calculate the number of flowers and fruits per cm^2 of branch area and to determine fruit-set ratios.

Fruit quality parameters. To determine fruit quality parameters, 50 fruits were randomly sampled from each tree at harvest period. Harvest was performed based on CTIFL chart, developed by the Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL) and by taking fruit size and fruit epidermis and mesocarp color into consideration.

Flesh firmness was measured as the maximum force (Newton, N) required to vertically penetrate

into the fruits. Measurements were performed with Zwick Z0.5 (Zwick/Roell Z0.5, Germany) universal test device able to apply maximum 500 N force with 1.8 mm diameter stainless-steel penetrating tip at 0.5 mm s^{-1} test speed and maximum 10 mm penetration. For other quality parameters, stones of 25 fruits harvested from each tree were removed. Fruit flesh was blended in a hand blander. Resultant juice was used to measure soluble solids content (SSC) with a digital refractometer (%, (PAL-1, McCormick Fruit Tech., Yakima, Wash)). For titratable acidity (TA), 10 ml of each fruit juice sample was supplemented with 10 ml distilled water, samples were then titrated with 0.1 N sodium hydroxide (NaOH) until they reach to a pH value of 8,1. Amount of NaOH consumed to increase pH level was expressed in malic acid equivalent (g malic acid 100 g^{-1}).

Statistical analyses

Experiments were conducted in randomized complete blocks design with six blocks (replicate), each consisting of five trees. Each experimental plot contained a single tree for treatments. Data were analyzed by analysis of variance and means separation was carried out using Duncan's Multiple Test. All analyses were conducted using the SAS statistical package (SAS, 9.1 version, Cary N.C.).

RESULTS

As compared to control treatment, in 2014, 125 mgL^{-1} ProCa, 250 mgL^{-1} ProCa or 125 mgL^{-1} ProCa + AMS treatments yielded lower increase ratios in TCSA (tab. 1). The 250 mgL^{-1} ProCa + AMS treatment did not create a significant change in increase ratios in TCSA values as compared to the control treatment. In 2015, ProCa treatments alone had lower increase ratios in TCSA values than the control treatment and the effects of ProCa + AMS treatments were not found to be significant (tab. 1).

In the first year of experiments, both ProCa treatments alone and combined ProCa + AMS treatments did not have significant effects on increase in canopy volumes. On the other hand, in the second year of experiments, significant differences were observed between the control and the other treatments. ProCa treatments alone or in combination with AMS created a significant decrease in increase ratios in canopy volume and the greatest effect in this decrease was observed in 125 mgL^{-1} ProCa treatment alone. In 2015, while 82.01% increase was observed in canopy volume of the control treatment, the ratio was observed as 49.53% in 125 mgL^{-1} ProCa treatment (tab. 1).

Table 1. Effects of ProCa and AMS on TCSA and canopy volume of "0900 Ziraat" sweet cherry

Treatments	TCSA (cm^2)				
	2013*	2014	increase (%)	2015	increase (%)
Control	49.15	61.23	24.57 a	94.98	55.12 a
125 mgL^{-1} ProCa	50.12	60.42	20.55 b	83.28	37.83 b
250 mgL^{-1} ProCa	51.45	61.10	18.75 b	73.52	20.32 c
125 mgL^{-1} ProCa + AMS	50.94	59.89	20.56 b	90.98	51.91 a
250 mgL^{-1} ProCa + AMS	49.23	60.12	22.12 a	92.58	53.99 a
	Canopy volume (m^3)				
Control	3.85	5.17	34.28 b	9.41	82.01 a
125 mgL^{-1} ProCa	3.87	5.31	37.20 a	7.94	49.53 c
250 mgL^{-1} ProCa	3.95	5.35	35.44 ab	8.61	60.93 b
125 mgL^{-1} ProCa + AMS	3.89	5.28	35.73 ab	8.67	64.20 b
250 mgL^{-1} ProCa + AMS	3.91	5.39	37.85 a	8.68	61.08 b

* Data obtained without treatments. The difference between mean values shown on the same column with the same letter is not significant according to Duncan's multiple range test at $P < 0.05$. ProCa: Prohexadione-calcium, AMS: Ammonium sulfate

Table 2. Effects of ProCa and AMS on shoot diameter, internode and shoot length of '0900 Ziraat' sweet cherry in 2014–2015

Treatments	Internode length (mm)	Shoot length (cm)	Shoot diameter (mm)
Control	35.64 a	37.35 a	5.50 a
125 mgL^{-1} ProCa	13.29 c	30.26 b	5.30 a
250 mgL^{-1} ProCa	22.09 b	31.21 b	5.50 a
125 mgL^{-1} ProCa + AMS	19.08 b	32.70 b	4.05 b
250 mgL^{-1} ProCa + AMS	20.52 b	33.38 b	5.19 a

The difference between mean values shown on the same column with the same letter is not significant according to Duncan's multiple range test at $P < 0.05$

Table 3. Effects of ProCa and AMS on generative growth of '0900 Ziraat' sweet cherry in 2014–2015

Treatments	Branch diameter	BCSA (m^2)	Number of flowers	Number of flowers per cm^2	Number of fruits	Number of fruits per cm^2	Fruit set (%)
Control	4.30	14.51	176.30	12.15 c	35	2.41c	24.76
125 mgL^{-1} ProCa	3.46	9.40	156.66	16.67 b	32	3.40 b	25.67
250 mgL^{-1} ProCa	3.90	11.94	293.00	24.54 a	60	5.02 a	25.75
125 mgL^{-1} ProCa + AMS	4.00	12.56	256.83	20.44 ab	53	4.22 ab	26.02
250 mgL^{-1} ProCa + AMS	3.88	11.81	295.00	24.97 a	62	5.24 a	26.60

The difference between mean values shown on the same column with the same letter is not significant according to Duncan's multiple range test at $P < 0.05$

Table 4. Effects of ProCa and AMS treatments on fruit quality of '0900 Ziraat' sweet cherry in 2014–2015

Treatments	Fruit size (CTIFL)	Firmness (N)	Color index (CTIFL)	TA (%)	SSC (%)
Control	29.2 a	3.06 a	4.2 a	0.80 a	15.10 a
125 mgL^{-1} ProCa	28.7 a	3.09 a	4.5 a	0.67 ab	15.00 a
250 mgL^{-1} ProCa	28.8 a	3.05 a	4.6 a	0.58 b	14.80 a
125 mgL^{-1} ProCa + AMS	29.3 a	3.10 a	4.3 a	0.76 a	15.09 a
250 mgL^{-1} ProCa + AMS	29.5 a	3.06 a	4.3 a	0.60 b	14.80 a

The difference between mean values shown on the same column with the same letter is not significant according to Duncan's multiple range test at $P < 0.05$

As compared to control treatment, ProCa treatments alone or in combined with AMS had lower internode and shoot lengths (tab. 2). The 125 mgL^{-1} ProCa treatment alone, creating 168% decrease in internode length as compared to control treatment, was identified as the most effective treatment on internode length. A decrease was observed in shoot diameters with ProCa 125 + AMS treatments, but the differences between the control and the other treatments were not found to be significant (tab. 2).

An increase was observed in number of flowers and fruits per cm^2 BCSA (branch cross sectional area) values with ProCa treatments alone or in combination with AMS. The 250 mgL^{-1} ProCa alone or in combination with AMS was more effective in increasing the number of flower and fruit per cm^2 BCSA than 125 mgL^{-1} ProCa alone (tab. 3).

The effects of all ProCa treatments on fruit size, fruit color, firmness and SSC were not found to be significant. However, significant decreases were observed in titratable acidy with 250 mgL^{-1} ProCa and 250 mgL^{-1} ProCa + AMS treatments (tab. 4).

DISCUSSION

High tree sizes bring about various problems in cherry culture. Thus, the present study was conducted to investigate the effects of ProCa treatments on vegetative and generative growth of cherry trees and to assess potential changes in such effects with combined ProCa + AMS treatments. Present findings revealed that ProCa was effective in controlling vegetative growth of cherry trees. Complying with the current findings, a decrease was reported in vegetative growth with ProCa treatments in apples [Greene 1999; Unrath 1999], pears [Basak and Rademacher 2000; Costa et al. 2001, 2004; Elfving et al. 2002, 2003; Rademacher et al. 2004; Smit et al. 2005; Asin et al. 2007] and cherries [Manríquez et al. 2001; Elfving et al. 2003; Guak et al. 2005; Jayna et al. 2012; Cares et al. 2014]. Such a decreasing effect of ProCa on vegetative growth may be attributed to inhibition of gibberellin synthesis promoting cell elongation. Evans et al. [1999] and Rademacher [2000] reported that ProCa treatments applied at the beginning of growth stage decreased gibberellin levels in plant tissues.

Byers et al. [2004] and Cline et al. [2008] reported that ProCa was inactivated by the mineral salts dissolved in water, but such a negative case could be eliminated and turned into a positive case when combined with AMS. However, combined treatments of ProCa and AMS of this study did not create significant differences in their effects on vegetative growth. Nitrogen and sulphur in structure of AMS might have played a role in various biochemical processes in plants and thus promoted plant growth. Thusly, Ergle and Eaton [1951] reported that sulphur deficiency resulted in decreased leaf sizes, shoot lengths, protein and water-soluble sugar contents, chlorosis and negatively influenced chloroplasts. Some plant basic processes like net photosynthesis production, plant growth and yield are regulated by nitrogen [Nurzynska-Wierdak et al. 2013]. Weinbaum et al. [1992] and Tagliavini et al. [1995] reported that nitrogen promoted shoot development in various plants.

Improved flowering and fruit-set are desired in cherries, especially in "0900 Ziraat" like low-yield cultivars. Flower bud formation is a complex physiological process carried out by meristems located at places where flower buds are formed over the shoots or spurs [Zhang et al. 2015]. Such a process is regulated by a complex cooperation of different plant hormones [Tromp 1987; Lavee 1989; Curry and Greene 1993]. In perennial plants, GA_3 generally directs assimilation substances from apex to rapidly growing shoots [Mutasa-Göttgens and Hedden 2009] and inhibits flowering [Wilkie et al. 2008; Mutasa-Göttgens and Hedden 2009]. Therefore, ProCa-like treatments inhibiting gibberellin synthesis are recommended to improve flowering. In present study, ProCa treatments increased number of flowers per cm^2 and consequently increased number of fruits per cm^2. Such effects of ProCa increased with increasing treatment doses. Cares et al. [2014] reported increased number of buds, bud sizes and number of flowers in cherries with ProCa treatments. According to Guak et al. [2005], ProCa treatments did not have significant effects on flowering. Elfving et al. [2003] indicated that ProCa treatments alone did not pro--

mote flower bud formation, but combined treatments of ProCa and Etephon twice in 3-week intervals tripled flower bud intensity and product efficiency. However, it was reported in previous studies carried out with pears that ProCa treatments did not have significant effects on flower bud formation and thus on fruit-set [Byers et al. 2004; Cline et al. 2008] or decreased flower bud formation [Einhorn et al. 2014]. According to Koutinas et al. [2010], flower bud formation in mild climate fruits was a complex biological process related to fruit species and cultivars, environmental conditions and cultural practices. Inconsistent impacts of ProCa treatments on flowering might have resulted from contributions of such factors.

The addition of AMS to ProCa did not cause an increase in effectiveness of ProCa on internode and shoot length (tab. 2). Such a finding contradicts with the findings of Byers et al. [2004] for apple trees indicating inhibition of shoot growth by ProCa combined with AMS.

Reduced vegetative growth and increased fruit intensity with ProCa treatments may result in nutrition-related problems in fruit quality. On the other hand, decreased shading rates through reduced vegetative growth may result in more efficient light intake and distribution and thus improve the fruit quality. Combination of such effects may then result in inconsistent outcomes in fruit quality parameters with ProCa treatments. In present study, ProCa treatments did not have significant effects on fruit quality parameters (fruit size, color, firmness and SSC). Complying with the current findings, several other researchers also indicated that ProCa treatments did not have significant effects on fruit quality parameters. Thusly, Unrath [1999] reported that ProCa treatments did not have negative effects on fruit quality, but the effects were inconsistent. Cares et al. [2014] reported that ProCa treatments increased flesh firmness of cherries, but did not have significant effects on SSC and fruit sizes. Guak et al. [2005] indicated that ProCa treatments again did not have significant effects on fruit quality parameters of cherries. Hawerroth et al. [2012] reported that fruit diameter and weights of ProCa-treated Housui pear trees increased through increasing assimilation substances in fruits. Elfving et al. [2003], Sugar et al. [2004] and Smit et al. [2005] on the other hand reported decreased fruit sizes in pears with ProCa treatments and indicated that ProCa treatments should be limited with the control of vegetative growth and fire blights.

CONCLUSIONS

In brief, it was observed in this study that ProCa treatments reduced vegetative growth and increased number of flowers and fruit-set ratios in "0900 Ziraat" cherry cultivar without any negative influences on fruit quality parameters. Therefore, it was concluded based on present findings that ProCa treatments could be used as a potential and efficient method to control strong growth of cherry trees.

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