

## Reduction of apple vegetative shoot growth cv. Starcrimson Delicious/MM 111 with prohexadione calcium application does not decrease fruit quality

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### Summary

Prohexadione-calcium % 10 (Pro-Ca) has been shown to effectively reduce the vigor of apple trees. However, it is important that the reduction of vegetative shoot growth does not decrease yield and fruit quality. The primary objective of this study was to determine the response of Starcrimson Delicious/MM111 apple trees to foliar Pro-Ca application and its effect on vegetative growth and fruit quality. For this reason, trees were sprayed twice within a three weeks interval with 62.5, 125, 250 g/100 L water Pro-Ca when annual shoots reached 5 cm. Pro-Ca applications decreased the growth of annual shoots and shoot length of apple trees. Shoot length was decreased by 31% for shoots treated with P-Ca. The number of nodes and average internode length were significantly reduced for P-Ca-treated shoots, conferring a higher node density relative to control shoots. Three weeks intervals of application Pro-Ca applied shoot 125 and 250 g/100 L water dosage is more effective in terms of reducing the development. Fruits from Pro-Ca treated trees did not significantly differ in size or other observed quality characteristics (firmness, soluble solids content (SSC), titratable acidity (TA), and fruit color) from fruits from the control group. As a result, application Pro-Ca of 125 g/100 L dosages for Starcrimson/MM111 apple varieties can be recommended.

### Introduction

World production of apples (*Malus domestica* Borkh) is close to 81 million tonnes, with a surface area under cultivation of 5.2 million ha. Production in Turkey is about 3.3 million tonnes, distributed over 173 thousand ha (FAO, 2013). A high percentage of orchards are situated on semi-vigorous or semi-dwarf rootstocks. Some 70% of the plants produced in nurseries in Turkey are on MM 106 (48%) or more vigorous rootstocks (22%). Approximately 4% of the apple seedling production in Turkey constitutes the Starcrimson Delicious apple (BUGEM, 2014).

Starcrimson Delicious/MM 111 gardens are mostly found in the Isparta-Eğirdir region. The 'Starcrimson Delicious' is an important apple in Eğirdir and is a high yield and fruit and flavor quality apple. However, the use of tall trees results in lower labor efficiency (harvest, pruning, and thinning), and a higher use of agro-chemicals per hectare. For this reason, it is important that reduction of this apple tree's vegetative shoot growth does not decrease the yield and fruit quality. Plant height reduction is playing an important role in promoting both yield and quality, and in decreasing cost, space, and labor (HAYASHI et al., 2001; KARLOVIC et al., 2004). One of the many methods used in the world are various foliar sprays with plant growth retardants for plant height reduction. One such plant growth retardant is Pro-Ca (STEFFENS et al., 1993; KIM et al., 2010; DUYVELSHOFF and CLINE, 2013). Pro-Ca has been found to reduce the growth of various plants such as rice, apples, pomes, petunias, and various vegetables and grain crops (LEE et al., 1998; COSTA et al., 2004; ILIAS and RAJAPAKSE, 2005; ERGUN et al., 2007; KIM et al., 2007).

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Pro-Ca is a recently developed plant growth retardant used in apple and other fruit trees (GREENE, 1999; ROEMMELT et al., 2003). It was first used on rice and small grains in Japan and France, and will be introduced for use on apples (*Malus × domestica*) in the United States and several European countries (YODER et al., 1999; COSTA et al., 2001). Pro-Ca is an acylcyclohexanedione, and acts on the blocking of 3 $\beta$ -hydroxylation (RADEMACHER, 2000) and prevents the biosynthesis of active GA's. This process is known to happen late in the bio-synthesis pathway of gibberellins (BARCEL, 2005). High rates of gibberellin biosynthesis stimulate excessive vegetative growth. The inhibitors of the gibberellin biosynthesis retard growth and improve plant productivity and/or performance (RADEMACHER, 1991). Paclobutrazol, a gibberellin biosynthesis inhibitor, was used successfully on fruits trees to control growth and improve fruit quality – although it is not commercially available in the United States for this purpose (GREENE, 1991; 1999). Of this, prohexadione-calcium appears to be particularly effective, and has the potential to improve performance (GREENE, 1999).

The purpose of this investigation was to determine the optimum concentrations in the application of prohexadione-calcium 10% for controlling the vegetative growth of apple trees, as well as the effects of Pro-Ca 10% on fruit quality. In this work, we studied the effect of reducing the plant height on fruit quality, vegetative growth, and labor efficiency at harvest, using cv. 'Starcrimson Delicious' grafted on semi-vigorous MM 111 rootstocks in the successive two years (from 2013/2014 to 2014/2015).

### Materials and methods

#### Orchard layout and experimental design

The experiments were carried out in the Fruits Research Station in Eğirdir, and Isparta in an orchard located at 37°49' N latitude and 30°52' E longitude and 926 m above sea level, the Eğirdir Province in the Mediterranean region of Turkey. To determine the effect of Pro-Ca %10 on vegetative growth control and the fruit quality parameters of apples, uniform 5-years old 'Starcrimson Delicious' apple (*Malus domestica* Borkh) trees grafted on MM 111 rootstocks (spaced 5 × 4 m) were selected and grouped into four blocks with 32 trees in each, based on proximity in the orchard and crop load. The experimental design was a randomized block, with 4 treatments and 4 replicates, using a double tree for each treatment. The trees were trained to a central leader and pruned in winter, and standard cultural practices had been used on the trees for several years.

The trees were sprayed twice with 0 (water + surfactant), 62.5, 125, 250 g/100 L water Pro-Ca %10 in the annual shoots 5 cm within a three week interval in the spring (I. and II. years) (Fig. 1a, b). All the spray solutions contained 'Spur' as a surfactant [0.01%, v/v (Sumi-Agro, Turkey)]. The pulverized treatments were applied with a low pressure hand sprayer.

#### Assessment of fruit quality and vegetative growth

##### Fruit Quality

The fruits were harvested at the commercial stage of maturity. The pro-Ca-treated fruits and untreated fruits were harvested separately



**Fig. 1:** Application times (a) Annual shoots 5 cm, (b) After the three weeks

and picked into specially designated bins. After each harvest pick, the fruit was transported to the Fruit Research Station Laboratory in the Horticulture Department.

The fruit width (mm), fruit length (mm) and fruit weight (g) were determined by using a digital scale sensitive to 0.01 g. The Fruit color was determined with a colorimeter (Model CR-300, Minolta) using the  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C$ ,  $h^\circ$  scale. Minolta color measurement apparatus was calibrated according to the standard white calibration plate ( $Y = 93.9$ ,  $x = 0.313$  and  $y = 0.321$ ). The flesh firmness was measured on two sides of the equatorial line of each fruit using a press-mounted Effegi penetrometer (FT 327; McCormick Fruit Tech. Torino, Italy) with a 11.1 mm tip. The SSC [digital refractometer (Palette PR-32 Atago)] were measured. The TA was measured using a digital buret (Digitrate Isolab 50 ml) by titration with 0.1 N NaOH up to pH 8.1, using 10 ml of diluted juice, and the TA was converted to malic acid.

#### Vegetatif growth

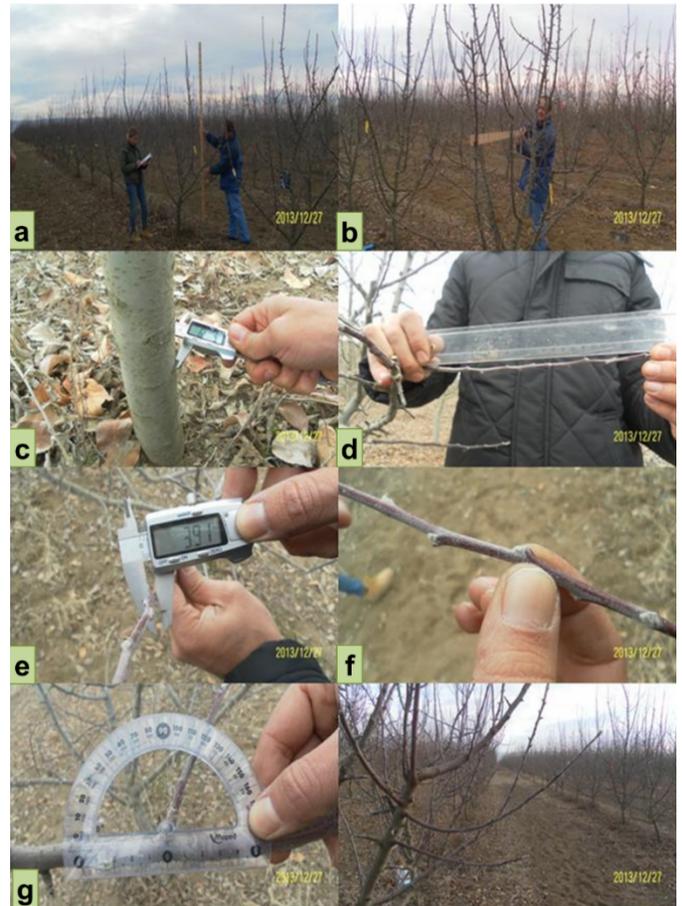
Tree height (cm), (Fig. 2a) canopy width (cm) (Fig. 2b) and trunk diameter (mm) (Fig. 2c) was measured in meters. Average shoot length (Fig. 2d) and average shoot width (Fig. 2e) for all treatments was measured on 10, one-year-old shoots, in the end of the season. The number of nodes was counted on all tagged shoots at the end of the growing season. Derived from these data, average inter-node length (cm) (Fig. 2f) and number of nodes per cm of shoot length were calculated. Shoots crotch angle ( $\circ$ ) was as measured with a protractor (Fig. 2g).

#### Phytotoxicity and side effects

Starting from a few days after the application to the end of the research, to test the phytotoxicity effects of the Pro-Ca applications on leaf, shoot and fruit macroscopic observations were carried out on the shoot, leaf and fruit. In addition to that, the application effect on bee activities was observed.

#### Statistical analysis

The experiment was set up according to the Split Plot experimental design with 4 replications, and was assigned to two trees at each replication with each plot having 20 apple fruits. The differences between the mean of the groups was determined by using the Duncan multiple range test. All the analyses were performed with the SPSS software package v.18.0 for Windows with the General Linear Model (GLM) univariate test. The significance was accepted at a  $P < 0.05$  level. The variety was the only independent variable. Statistical analysis.



**Fig. 2:** Illustration of observations and measurements (a) Tree height, (b) Canopy width, (c) Trunk diameter, (d) Average shoot length, (e) Average shoot width, (f) Average internode length on shoots, (g) Shoots crotch angle

## Results

#### Effect of Pro-Ca on the vegetative growth

The effects of Pro-Ca on the vegetative growth of the 'Starcrimson Delicious' apple cultivar are given in Tab. 1. Only in the second experiment year, were the effects of the Pro-Ca dosage on the tree height (cm) and trunk diameter (mm) statistically significant at the 5% level. In the first year, the Pro-Ca treatments did not affect the reduction in the tree height, canopy width, or trunk diameter. However, in the second year, it affected the height of the tree being clearly defined. The lowest was obtained with 250 g/100 L (283 cm) (Tab. 1). The control trees' tree height (323 cm) is the longest.

In both years of this study, the effects of the Pro-Ca dosage on the average number of shoots, average shoot length and average shoot width, average number of internode on the shoot, and the average internode length on shoot were statistically significant ( $P < 0.05$ ). The effects of the Pro-Ca treatments on the shoots crotch angle were determined as having no difference statistically in the first and second year (Tab. 2). The lowest average number of shoots were obtained with 250 g/100 L Pro-Ca (53.50 number in the first year) and 125 g/100 L Pro-Ca (6.13 number in the second year). 250 g/100 L Pro-Ca with the shortest average shoots [16.40 cm (in first year), 14.49 cm (in the second year)] were obtained in both experiment years (Fig. 3) (Tab. 2). In the first experiment year, the average shoot width was reduced by the Pro-Ca application, while it increased in the second experiment year. The highest average shoot width was found in the control (3.91 mm) (in the first year)

**Tab. 1:** The effect of Pro-Ca dosage on some vegetative growth in the 'Starcrimson Delicious' apple cultivar

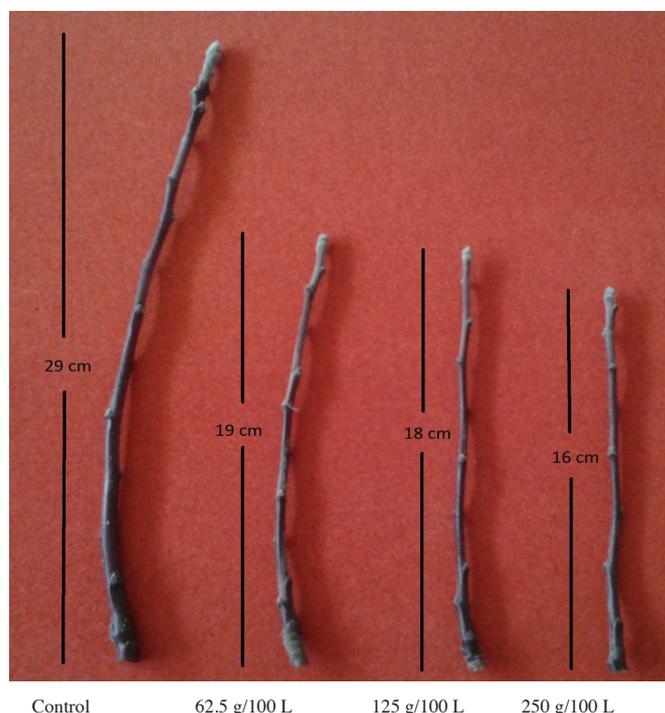
Treatments	Tree height (cm)		Canopy width (cm)		Trunk diameter (mm)	
	I. Year	II. Year	I. Year	II. Year	I. Year	II. Year
Control	291	323a	158	191	59.89	68.89ab
62.5 g/100 L	289	294ab	167	172	54.00	61.25b
125 g/100 L	296	306ab	154	166	60.82	72.45a
250 g/100 L	279	283b	183	186	55.29	61.39b
<i>P</i> values	0.798	0.012	0.310	0.430	0.357	0.031

ab: Values in a same column for each effect followed by different letters are significantly different (Duncan,  $P < 0.05$ )

and 62.5 g/100 L Pro-Ca (4.84 mm) (in the second year). In both years, the average number of internode on the shoot and the average internode length on the shoot were significantly reduced Pro-Ca applications. The lowest average internode length on the shoot was obtained with 250 g/100 L Pro-Ca (1.34 cm in the first year and 1.32 cm in the second year) (Tab. 2).

#### Effect of Pro-Ca on the fruit quality

The effects of the Pro-Ca dosage on the fruit width and fruit weight were statistically significant ( $P < 0.05$ ) in both years, but the effects on fruit length were statistically significant ( $P < 0.05$ ) in the second experiment year only. The highest fruit width (80.71 and 79.34), fruit length (72.04 in the second year), fruit weight (222.68 g in the second year) were observed in the 250 g/100 L Pro-Ca dose (Tab. 3). The Pro-Ca applications did not significantly affect fruit firmness, and the titratable acidity (TA) in both experiment years (Tab. 3). Nevertheless, the soluble solid content (SSC) was significantly ( $P < 0.05$ ) increased by the 250 g/100 L (14.15%) Pro-Ca dose. While the effects of the Pro-Ca dosage on the fruit color characteristics were not found statistically significant in the first year, the difference in fruit color ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $h^\circ$ ) in the second experiment

**Fig. 3:** The effect of Pro-Ca on average shoot length

year was significant ( $P < 0.05$ ) (Tab. 4). In the second year, the Pro-Ca applications reduced the fruit color characteristics. In this year, the highest  $L^*$  value (38.75),  $a^*$  value (27.25),  $b^*$  value (17.00),  $C^*$  value (32.50) and  $h^\circ$  value (31.00) were with the control fruits.

#### Effect of Pro-Ca phytotoxicity and side effects

In both of the two years, as a result of the macroscopic observations conducted on the shoots, the leaves and fruits a few days after application and until end of trial no phytotoxicity or side effect of Pro-Ca applications could be observed. Besides, it was also determined that the application has no negative effect on bee activity.

**Tab. 2:** The effect of Pro-Ca dosage on shoot growth in the 'Starcrimson' apple cultivar

Treatments	Average number of shoots	Average shoot length (cm)	Average shoot width (mm)	Average number of internode on shoot	Average internode length on shoots (cm)	Shoots crotch angle (°)
<i>I. Year</i>						
Control	77.25a	29.15a	3.91a	13.83a	2.09a	57.50
62.5 g/100 L	55.50ab	18.20b	3.27b	10.75b	1.67b	56.88
125 g/100 L	67.50ab	19.48b	3.88a	12.68ab	1.45c	62.68
250 g/100 L	53.50b	16.40b	3.61ab	11.85ab	1.34d	63.03
<i>P</i> values	0.048	0.000	0.019	0.043	0.000	0.224
<i>II. Year</i>						
Control	14.13a	33.48a	4.10b	14.73a	2.31a	62.50
62.5 g/100 L	11.25ab	29.58a	4.84a	13.88b	1.61b	59.25
125 g/100 L	6.13c	14.59b	4.25b	11.23c	1.28c	62.31
250 g/100 L	9.63b	14.49b	4.09b	11.13c	1.32c	65.76
<i>P</i> values	0.000	0.000	0.025	0.000	0.000	0.429

ac: Values in a same column for each effect followed by different letters are significantly different (Duncan,  $P < 0.05$ )

**Tab. 3:** The effect of Pro-Ca dosage on some fruit characteristics in the ‘Starcrimson’ apple cultivar

Treatments	Fruit width (mm)	Fruit length (mm)	Fruit weight (g)	Firmness (lb)	SSC (%)	TA (%)
<i>I. Year</i>						
Control	79.52a	80.91	239.17a	15.74	12.93b	0.31
62.5 g/100 L	75.77b	76.25	211.60b	15.32	13.40ab	0.30
125 g/100 L	79.45a	78.99	235.98a	15.32	13.45ab	0.29
250 g/100 L	80.71a	78.43	238.10a	15.85	14.15a	0.32
<i>P</i> values	0.006	0.062	0.024	0.338	0.049	0.132
<i>II. Year</i>						
Control	75.75b	70.04ab	201.58b	12.20	12.20	0.30
62.5 g/100 L	76.65b	68.76b	201.16b	12.55	12.55	0.31
125 g/100 L	77.84ab	70.60ab	208.68ab	11.86	11.86	0.30
250 g/100 L	79.34a	72.04a	222.68a	12.10	12.10	0.29
<i>P</i> values	0.012	0.037	0.033	0.555	0.555	0.755

ab: Values in a same column for each effect followed by different letters are significantly different (Duncan,  $P < 0.05$ )

**Tab. 4:** The effect of Pro-Ca dosage on some fruit color characteristics in the ‘Starcrimson’ apple cultivar

Treatments	L*	a*	b*	C*	h°
<i>I. Year</i>					
Control	40.12	24.01	16.95	29.76	34.67
62.5 g/100 L	38.57	24.00	16.05	29.19	33.27
125 g/100 L	39.23	24.32	16.50	29.76	33.61
250 g/100 L	38.11	22.97	15.95	28.33	33.95
<i>P</i> values	0.066	0.199	0.305	0.071	0.654
<i>II. Year</i>					
Control	38.75a	27.25a	17.00a	32.50a	31.00a
62.5 g/100 L	37.50a	27.75a	15.50b	32.25a	29.25ab
125 g/100 L	37.25a	26.75a	14.50bc	30.75a	28.25b
250 g/100 L	35.75b	24.50b	13.50c	28.75b	28.25b
<i>P</i> values	0.013	0.002	0.001	0.001	0.018

ac: Values in a same column for each effect followed by different letters are significantly different (Duncan,  $P < 0.05$ )

## Discussion

The Pro-Ca application to the Starcrimson Delicious/MM 111 significantly reduced the extension shoot length in both years of application by up 31% compared to the control. Similar to our findings, DUYVELSHOFF and CLINE (2013) found that the 125 mg L<sup>-1</sup> Pro-Ca application reduced the shoot length in the ‘Northern Spy/M9’ apple trees. As in many studies, our study showed that the shoot growth was slower than that of control trees (GREENE, 1999; YODER, 1999; COSTA, 2001). During the 5 years of MILLER (2002)’s study, the reduction in shoot growth ranged from 21% to 71% from the control trees depending on the dose applied, time of application, and the vigor of cultivar treated. Parallel to the decrease in shoot development, the distance between the internodes also decreased. The 125 and 250 g/L dosages of Pro-Ca application which were applied in an interval of 3 weeks were found to be more effective

in terms of decreasing the shoot development. However, the 250 g/100 L dosage was not found appropriate as it limits shoot development more than is necessary.

The effect on fruit quality was neither positive nor negative in our study. In our study, it was determined to be related to the fruit size that the Pro-Ca applied fruits had the same characteristic as the control fruits, while significant differences could not be observed in other quality properties (firmness, SSC, TA and fruit color). On the other hand, in a study performed by SCHUPP et al. (2003) it was stated that the 250 mg/l of Pro-Ca applied to Empire apple reduced fruit size and caused damage in fruit and that Pro-Ca should not be used with the empire apple under certain conditions (such as usage together with ammonium sulphate). Different researchers (BLANCO et al., 2005) found that the slight decrease in fruit size occurred when the Pro-Ca was applied in dosages of 125, 175, and 250 ppm to Fuji and Royal Gala. In another study carried out on pears, a significant weight change in the fruit could not be detected. (COSTA et al., 2004). MEDJDOUB and BLANCO (2004) stated that Pro-Ca applications decreased the shoot development significantly in the Golden Delicious/M9 tree, but they could not detect any negative effects on the fruit quality and yield.

In the study, the phytotoxic effect of the Pro-Ca applications on the shoots, leaves, and fruits, could not be observed. In many studies it was determined that Pro-Ca has no toxic effect (EVANS et al., 1997; WINKLER, 1997; GUAK et al., 2001), and moreover COSTA et al. (2004), stated that Pro-Ca, which prevents the activity of the epoxigenase enzyme that catalyses the final stage of the gibberellic acid metabolism, deactivates some pathogen infections in the apple and pear (such as black spot and fire blight diseases). Apart from many studies proving this, (COSTA et al., 2002; BAZI et al., 2003; RADEMACHER and KABER, 2003; BASTAS and MADEN, 2004) according to RADEMACHER (2004), Pro-Ca positively affects the formation of luteophorols in particular (3-deoxyflavonoids) acting like phytoalexin, and increasing the resistance of the offshoots to pathogen, which is a secondary effect.

As a result, it was concluded that controlling vegetative development by chemical methods can be useful in terms of decreasing medication cost and pruning labor without any loss in fruit quality or toxic effects in orchards in which semi vigor rootstocks such as MM 111 are used. The fact that the Pro-Ca effect period is short, that it does not harm the plant, its rapid composition in nature, that it is

an environmental friendly chemical, and that it does not negatively affect the fruit quality, makes it usable instead of other chemicals. Besides, controlling the vegetative development in young trees is considered to be important in terms of the early fruit laying of trees and obtaining high quality and an abundance of products. In this study, regarding the effects of the reduction of the apple vegetative shoot growth, and with prohexadione calcium application, does not decrease fruit quality as the 125 g/100 L Pro-Ca dosages for 'cv. Starcrimson Delicious/MM111 apple varieties can be recommended.

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