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Influence of prohexadione-calcium on temperate fruit crops – A Review

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ABSTRACT

Plant growth retardants have great potential to maintain a balance between vegetative and reproductive growth of the plants. Prohexadione-calcium is reported as a novel plant growth retardant that inhibits late stages of gibberellins (GAs) biosynthesis in plants. It is emerged as one of an important management tool that an orchardist has available to control vegetative growth in plants and can enhance flowering and fruit quality. Currently, prohexadione-calcium is considered as a reduced risk compound, since it exhibits negligible toxicological effects and has been reported to degrade in higher plants with a halflife of a few weeks and in soil with a halflife of less than a week. The available literature on the effect of prohexadione-calcium on vegetative growth, fruit set, fruit yield, fruit quality and return bloom have been reviewed in this paper.

Key words: *Prohexadione-calcium, Vegetative growth, Flowering, Fruit yield, Quality and Return bloom*

Introduction

It is very essential to maintain a balance between vegetative and reproductive growth to accelerate flowering and to prevent shading affect in productive fruit plants. An uncontrolled increase in vegetative growth negatively affects productivity, fruit quality, pests and diseases control as well as significantly increase the costs of pruning (Lal *et al*., 2020 and (Forshey and Elifving, 1989). Such competition between the vegetative and reproductive growth may reduce the number of fruit cells, thereby, limiting the fruits from reaching their potential size and adversely affecting quality yield. Several plant growth regulators are evaluated for their potential to reduce vegetative growth of fruits tree, thereby reducing pruning costs and improving fruit quality. Growth retardants such as adenile benzyl amine (used to reduce the physiological loss in weight in fruits), GA_{12} aldehyde (used to reduce the acidity in fruits) (Wani *et al*., 2007), chloroqemutat (causes reduction in shoot length) and ethephon (that requires high dose for shoot reduction but sometimes it leads to substantial thinning) (Greene *et al*., 2003). However, it has been reported that prohexadione-calcium significantly reduces these problems when applied at appropriate time and in proper quantity (Yoder *et al*., 1999; Rademacher and Kober, 2003).

The gibberellin biosynthesis inhibitor prohexadione-calcium (3-oxido-4propionyl-5-oxo-3-cyclohexene-carboxylate) is now marketed by BASF as Apogee. It is currently approved for use in apples to control excessive vegetative growth (Byers and Yoder, 1999; Owens and Stover, 1999). The total re-

sult is a decrease in immobile, biologically active GA_1 levels and an increase in mobile, but inactive GA_{20} levels (Evans *et al.,* 1997). Furthermore, pro-ca has a number of advantages over other plant growth inhibitors, including low toxicity and a short persistence period in plants and soil. It inhibits the manufacturing of phenol, which delays senescence by lowering ethylene production in plants and improves resilience to numerous diseases and insects. Now-a-day's prohexadione-calcium is emerged as a new strategy that produces shortest shoot length and did not have any negative effect on yield and return bloom (Shehaj *et al.,* 2015). In a study, Sabatini *et al.* (2003) observed that prohexadione-calcium spray to various apple and pear cultivars showed increased in chlorophyll content in leaves of the current season shoot growth on a fresh mass basis. Similar reports were also given by Prive *et al.* (2004) in apple, Mandemaker *et al.*, (2005) in avocado, Elifving *et al.,* (2003) in pear, and Yoder *et al.* (1999) in apple and pear. There are also a few reports on prohexadione-calcium effect on flowering or alternate bearing in fruit plants (Cline *et al.*, 2007; Byers *et al*., 2004). So, prohexadione-calcium is only a solution for reducing the vegetative growth in fruit plants without affecting yield and fruit quality.

Chemistry and manufacture

Active constituent

Ihara Chemical Industry Co., Ltd, 1800 Nakanoko, Fujikawa-cho, Ihara-gun, Shizuoka, Japan, manufactures the active ingredient prohexadione-calcium (Approval Number: 59700).

Mode of action

The mode of action of prohexadione-calcium differs from other gibberellin biosynthesis inhibitors currently in use in commercial horticulture. Many of these growth regulators, including the quaternary ammonium compounds, substituted pyrimidines, norbornenodiazetine derivatives, and triazole derivatives function by interrupting the synthesis of gibberellin early in the biosynthetic pathway, specifically at the synthesis of ent-kaurene. Prohexadione-Ca is known to interfere with the 3-ß hydroxylation of GA_{20} to GA_1 (Lal *et al*., 2020 and Lal *et al*., 2018). The net effect is a reduction in immobile, biologically active GA_1 and an increase in the levels of mobile, but inactive GA_{20} (Evans *et al*., 1997; Graebe, 1987).

Effect of prohexadione-calcium on different plant characteristics

Vegetative growth characteristics

Prohexadione-calcium, a gibberellin biosynthesis inhibitor, was used successfully on temperate fruits plants to control the excessive vegetative growth (Solar *et al*., 2012 and Greene, 1999).

The plants sprayed twice with prohexadione-calcium @ 200 ppm recorded minimum annual shoot extension growth of 16.92 cm and leaf area (22.20 cm^2) along with increase in fruit yield (51.24 kg) (Lal *et al*., 2020). Kim *et al.* (2019) revealed that application of prohexadione-calcium resulted in minimum petiole length (16.4 cm), crown diameter (16.5 cm) as well as leaf area (1449.3 cm^2/plant). Carra *et al*. (2016) also noted greatest reduction in shoot length at the rates of 400 and 300 mg/l prohexadione-calcium, where the reduction was 58 percent and 54 percent, respectively, relative to control in Smith pear plants. Cetinbas *et al.* (2015) study the effect of prohexadione-calcium on apple cultivar Starcrimson delicious planted on a MM111 rootstock and noted 10 percent reduction of annual growth and shoot length by 31 percent. They also reported that the number of nodes and average internode length were significantly reduced for Pro-Ca-treated shoots, conferring a higher node density relative to control shoots. Prohexadione-calcium (250 mg/l) spray at 30 days after full bloom showed reduction in shoot length (209.70 cm) as compared to control (258.10 cm). Cares *et al.* (2014) reported that prohexadione-calcium (250 mg/l) application on Lappins and Sweet Heart cultivars of sweet cherry showed reduction in elongation of terminal shoots up to 13-15 cm. They also reported that pro-ca treatment reduced both length and number of internodes, resulted in a reduction in total leaf area. Reduction in vigor of d Anjou pear about 35 percent was noticed with prohexadione-calcium (250 mg/l) either in single application or double application to dormant headed ($1/3^{rd}$ pruned) and unpruned shoots (Pasa *et al.*, 2014). Hawerroth *et al*. (2012) noted that the use of prohexadione-calcium was effective in controlling the vegetative growth of Hosui pears and decreased winter pruning, by reducing total weight and number of pruned shoots. Jacyna and Lipa (2011) also noted reduction in shoot length (37.2 cm) and intermodal length (17 mm) in cherries with 125 and 250 mg/l, respectively prohexadione-

calcium applications. Prohexadione-calcium (250 or 500 mg/l) spray to entire canopy after topping resulted in reduction of lemon shoot growth by 30 percent for 5 weeks (Garner *et al*., 2010). Prohexadione-calcium (0, 75 and 125 mg/l) with 0.5 (volume/volume) LI 700 surfactant on apple cultivars Empire and Royal Court showed significant reduction in shoot length (Cline *et al.,* 2007). Basak (2007) reported that prohexadione-calcium spray on Jonagold apple trees during two consecutive years with different application rate (75, 150, 75+75 mg dm^{-3}) and (125, 175, 200 mg dm^{-3}) resulted in shorter shoot length and was proved to be effective in induction of feathering in apple plants. Medjdoub *et al.* (2005) noted that prohexadione-calcium is an effective plant bio-regulator in reduction of shoot length without any harmful effect on apple plant with higher rates and right timing. Paulson *et al.* (2005) also observed that pro-ca spray on apple and pear trees inhibits the gibberellins synthesis and resulted in reduction in number of *C. rosaccana* insect along with the reduction in shoot length. Prohexadione-calcium application rate 1.4 percent active ingredient decreased shoot growth of Hass avocado plants by 10 percent to 20 percent. Glenn and Miller (2005) study the effect of prohexadione-calcium with multiple application rates on shoot growth of young bearing apple trees cultivar Spur delicious and noted that prohexadione at 45 g/100 l resulted in shorter shoot growth. Prohexadione-calcium (150-250 mg/l) caused shorter shoot length and internodal distance in apple plants (Norelli and Miller, 2004). Elifving *et al.* (2003) reported that prohexadione-calcium spray resulted in reduction of shoot length up to 60 percent in Bartlett pear. The application of prohexadione-calcium on McIntosh, Northern Spy, Vista Billa and Red Spur apple cultivars at 125 mg/l resulted in significant reduction in shoot length as well as number of nodes (Prive *et al.,* 2004). The relative decrease in vigor was also noticed by Basak and Rademacher (2000) in stone fruits and reported that prohexadione-calcium reduces shoot elongation in fruit trees due to inhibition in the biosynthesis of gibberellic acid as it stops the formation of GA_1 (active form) from GA_{20}. Air blast application of prohexadione-calcium reduced average shoot length and internodal length of Stayman/seedling apple trees (92.2 cm and 2.3 cm respectively) as compared to control (Byers and Yoder, 1999). Similar results were also results were also reported by Byers *et al*. (2004) in apple, Medjdoub *et al.* (2005) in apple and Costa *et al*. (2001) in pear.

Phenotypic fruit characteristics

Prohexadione spray at 125 mg dm^{-3} on Golden delicious apple caused increase in fruit weight (212.4 g), fruit diameter (78.9 mm) and fruit L/D ratio (0.92) (Atay and Koyuncu, 2017). Double spray of prohexadione-calcium (200 ppm) resulted in higher fruit weight (82.85 g), fruit length (6.81 cm) and fruit diameter (6.44 cm) as compared to control plants (Lal *et al*., 2018). Pasa and Einhorn (2017) also noted good result in terms of fruit weight (205.78 g) with pro-ca spray at 250 mg/l as compared to untreated Starkrimson pear trees. An averaged fruit weight (230.96 g) in Passe Crassane pear plants was observed greater in 150-ppm prohexadione-calcium spray (Shehaj *et al*., 2015). Highest fruit width (80.71 and 79.34 mm), fruit length (72.04 mm in the second year) and fruit weight (222.68 g in the second year) were observed with 250 g/100 l pro-ca dose in Starcrimson delicious apple plants (Çetinbas *et al*., 2015). In a study, conducted by Guak (2013) on the apple cv. Golden delicious to determine the efficacy of prohexadione-calcium, ethephon both at 250 mg/l and transient water, resulted in good higher fruit weight. Chitu *et al.* (2013) also reported that prohexadione-calcium increases storage life and firmness of fruits in Triumph persimmon plants. Prohexadione calcium at 10 SL resulted in increase in fruit weight and fruit size (Sabajeviene *et al.*, 2008). The higher fruit mean weight by 18-44 percent in apple cultivars Empire and Royal Court was noticed by prohexadione spray (Cline *et al*., 2007). In a study, Jacyna and Lipa (2011) reported that fruit diameter was reduced by 125 mg/l pro-ca (A-trees), but fruit shape (L/D ratio) and mass were reduced by 250 mg/l pro-ca (B-trees), and such reduction was also exhibited by C-trees treated with 250 mg/l prohexadione-calcium. Prohexadione-calcium at 1.0 percent and 1.5 percent significantly increased mean fruit weight compared to untreated plants in Hass avocado plants (Mandemaker *et al*., 2005). Smit *et al.* (2005) noted that prohexadione-calcium use in pear plants increases fruit firmness by inhibiting the ethylene synthesis and action. Giudice *et al.* (2004) reported that an average cluster weight (36.11 g) and berry weight (0.81 g) in the subsequent season were all decreased by applications of 250 mg/l prohexadione-calcium as compared to control with in terms of cluster weight (127.03 g) and berry

weight (1.38 g). On contradictory, Elifving *et al.* (2003) reported that Bartlett fruit size was decreased when high concentrations of prohexadione-calcium were applied during the cell-division phase of fruit development. While, Coasta *et al.* (2001) noted that single use of prohexadione-calcium (250 mg/l) increases fruit weight and fruit size of Abbe Fetel pear plants. Prohexadione-calcium at 250 mg/l inhibits growth of lateral shoots and increases the fruit size and fruit weight of apple and pear plant (Yoder *et al*., 1999). Similar reports were also given by Itai (2009) in pear, Vercammen *et al*., (2005) in pear, Meintjes *et al*. (2005) in pear and Lombard and Richardson (1982) in pear.

Bio-chemical characteristics

Impact of different concentrations of prohexadione-calcium on 0900 Ziraat sweet cherry plants was evaluated by Aglar (2018) who reported that prohexadione-calcium (250 mg/l + AMS) showed a decrease in level of acidity (0.60) as compared to other untreated 0900 Ziraat sweet cherry plants. Double spray of prohexadione-calcium at 200 ppm one at complete petal fall and second at four weeks after first spray resulted in increased percentage of total soluble solids (13.21) and total sugars (10.23) with decreased percentage of fruit acidity (0.43) as compared to untreated pear plants (Lal *et al*., 2018). Cares *et al*. (2014) reported an increase in total soluble solid content of about 17.4% and 19.1% in the sweet cherry cultivars Lappins and Sweetheart, respectively, with the application of 250 mg/l pro-ca Cetinbas *et al*. (2015) also reported a significant increase in total soluble solid content (14.15%) and a decrease in the level of acidity (0.13%) in Starcrimson apple with the use of (250 g/100 l) pro-ca. Inac *et al*. (2013) reported that application of prohexadione-calcium at pre blooming and cluster thinning at verasion resulted in increase in wine quality and phenolic composition of the treated wines of Lal Roja. In an experiment on effects of prohexadione-calcium on yield components and fruit composition of Cabernet Sauvignon in southern Brazil, it was recorded that application of paclobutrazol at 250 mg/l at flower separating stage increased total soluble content percentage (18.7%) as compared to other treatments. Guak., (2013) also recorded 15.9 percent of total soluble solids with prohexadione-calcium at 250 ppm in Golden delicious apple plants. Zahiha and Singh (2013) reported that prohexadione-calcium when applied at two times with 500 mg and 750 mg volume resulted in increased TSS and fruit quality of Cripps pink apple cultivar. In a study, Solar *et al*. (2012) reported that treated Franquette walnut fruits showed a higher total phenolic content (TPC), more hydroxycinnamic acids (HC), and flavanols, whilst the contents of flavonols and gallic acid had decreased due to Pro-Ca. Poledica *et al*. (2004) also recorded that SSC was slightly lower in the control treatment (10.5%) than in pro-ca or young removal treatment (R), whereas, a very high TA was obtained in the control treatment (2.14%) in Willamette raspberry plants. Mesa *et al.* (2012) also reported that prohexadione-calcium lead to more penetration of light into tree interior of canopy and hence, increased the total soluble solids in Castlebrite apricot fruits. In a study, Petkovsek *et al*. (2009) reported that prohexadione-calcium caused a significant decrease in the synthesis of catechin, epicatechin, rutin, quercitrin, phloretin and phloridzin in the treated leaves and fruits. They also reported that treated leaves, the content of total phenolic compounds increased by approximately 23 percent as compared to untreated leaves and in contrast, Pro-Ca caused a significantly lower content of total phenolics in the fruits which is also linked with a lower antioxidant activity. In a study, Sabajeviene *et al.* (2008) reported that prohexadione-calcium at 10 SL resulted in increase in TSS, total sugars, reducing sugars with

Table 1. Chemical characteristics of the active constituent

Common name	Prohexadione-calcium
Synonyms and Code Number	BX-112, KUH-883, KUM-883, LAB 285 342, BAS 9054 W, BAS 122 W, BAS 125 W
Chemical name (IUPAC)	Calcium 3-oxido-5-oxo-4-propionylcyclohex-3enecarboxylate
(CA)	Calcium 3-oxido-5-oxo-4-propionylcyclohex-3enecarboxylate
Chemical Abstracts Service (CAS)	
Registry Number	127277-53-6
Molecular formula	$C_{10}H_{10}CaO_5$
Molecular weight	250.26

decreased percentage of Jona apple. In an experiment, Giudice *et al*. (2004) noted that prohexadione-calcium at 250 mg/l applied to field-grown Cabernet franc, Cabernet Sauvignon, Chardonnay and Seyval showed increase in color intensity, total anthocyanin and total phenols compared to untreated plants. Owens and Stover (1999) applied prohexadione-calcium in the fall to young Golden delicious apple plants growing in the nursery and reported that application of prohexadione-calcium increased the nonstructural carbohydrates content in the shoots of the plants.

Flowering

In a study, Aglar (2018) observed the effect of pro-ca on 0900 Ziraat sweet cherry plants and reported that 250 mg/l Pro-Ca + AMS resulted in significant increase in number of flowers (295.00), number of flowers per cm^2 (24.97), as compared to untreated plants. Passe Crassane pear plants treated with 150-ppm prohexadione-calcium showed reduction in abortion of flower lets (Shehaj *et al.*, 2015). Prohexadione-calcium application at 250 mg/l increased the number and size of flower buds and the number of floral primordia per bud (3.6) as well as advanced the development of floral buds primordia in sweet cherry (Cares *et al*., 2014). Poledica *et al*. (2004) reported that removal treatment once along with prohexadione-calcium spray during April-May on Willamette raspberry plants increased number of inflorescences per cane (45.3 ± 4.3) as compared to untreated plants (37.6 ± 2.0). Jacyna and Lipa (2011) indicated that 200 mg/l aqueous solution of prohexadione-calcium resulted in maximum number of flower clusters (5.26) per cm^2 of branch cross-sectional area.

Fruit set and fruit yield

The maximum fruit yield (51.24 kg/tree) was obtained from Clapp's Favorite variety of pear sprayed twice with 200 ppm of prohexadione-calcium (Lal *et al*., 2020). Kim *et al.* (2019) revealed that application of prohexadione-calcium at 50 mg/l resulted in maximum production of strawberry runners. Lal *et al.* (2018) carried out an experiment on response of prohexadione calcium and paclobutrazol on growth and physico-chemical characteristics of pear cv. Clapp's Favorite, it was noticed that maximum percentage of fruit set (18.34 %), maximum fruit yield (51.24 kg/tree) and yield efficiency (4.08) was recorded in plants treated with double application of 200 ppm prohexadione-calcium. Pasa and Einhorn (2017) also noted good result in terms of fruit number/tree (146.60), fruit and yield (29.27 kg) with pro-ca spray at 250 mg/l as compared to untreated Starkrimson pear trees. Atay and Koyuncu (2017) reported that double spray of prohexadione-calcium (75 and 50 mg dm^{-3}) at 3

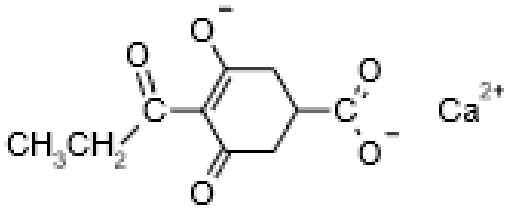
Table 2. Physical and chemical properties of the product

Distinguishing name	Regalis Plant Growth Regulator
Formulation name	Water dispersible granule
Active constituent concentration	Prohexadione-calcium (100 g/kg)
Mode of action	Plant growth regulator and retardant. Foliar applied and absorbed via green tissue; translocated basipetally, as well as acropetally, within plants
Physical state	Solid
Colour	Grey
Odour	Moderate spicy
Relative density	1.665
Bulk density	755 g/L (loose), 784 g/L (tapped)
Acidity, alkalinity or pH value	Not applicable
Viscosity	Not applicable
Surface tension (at 20°C)	46.5 mN/m at 0.1% , 43.1 mN/m at 1.0%
Flash point	Not applicable
Flammability	Not highly flammable
Auto flammability	Self-ignition at 371°C
Explosive properties	Not explosive
Corrosion characteristics	Not determined
Storage stability	Storage life 2 year (under polythene container)

weeks interval resulted in higher fruit set percentage (32.8±6.4) in Golden Delicious/M9 apple trees. Pro-Ca at 300 mg/l resulted in significantly higher fruit set percentage (91.04 %), number of fruits per plant (346.6) and fruit yield (46.77 kg/plant) (Carra *et al*., 2016). Rufato and Brighenti (2014) also noted that prohexadione-calcium at 250 mg/l at flower separating stage, increased yield per vine (4.7 kg) in Cabernet Sauvigon grape. Guak (2013) obtained good fruit set percentage and fruit yield with prohexadione-calcium and ethephon at both 250 mg/l. Hawerroth *et al*. (2012) noted that control of vegetative growth, by prohexadione-calcium use, increased fruit production capacity of Hosui pears, mainly at 550 g/ha concentration. Poledica *et al*. (2004) also noted that removal treatment once along with prohexadione-calcium spray during April-May on Willamette Raspberry plants increased fruit number per cane (218 ± 23) and fruit yield per cane (864 ± 40 g). Greene (2008) carried out an experiment on effect of repeated annual applications of prohexadione-calcium on apple and reported that 125 mg/l spray of prohexadione-calcium increased number of fruits (3.4) per limb cross sectional area in McIntosh apple trees. Mandemaker *et al*. (2005) reported that prohexadione-calcium application @ 1.5 percent showed higher fruit set percentage in 2004 than in 2003 with the number of fruit per tree ranging from 1 to 18 in 2003 and from 2 to 71 in 2004. Meintjes *et al*. (2005) reported that prohexadione-calcium application to different pear cultivars *viz.* Rosemarie, Florella and Early Bon Chretien showed higher fruit set percentage. Application of pro-ca (250 mg/l) to single clusters of Cabernet Sauvignon and Chardonnay at bloom, or in the one-to-two-week pre bloom period decreased fruit set, whereas applications one to two weeks post bloom had no impact on fruit set (Giudice *et al*., 2004). Byers *et al*. (2004) reported that prohexadione-calcium [formulated as BAS-125 (10% Pro-Ca) or Apogee, (27.5% Pro-Ca + 56.1% $(NH4)_2SO_4$ + 16.4% other proprietary additives)] applied to Fuji/M9 trees in 3 applications at 250 mg/l (a.i. Pro-Ca), increased fruit set compared with the unsprayed control. Poledica *et al*. (2004) reported that removal treatment once along with prohexadione-calcium during April-May on Willamette Raspberry plants increased fruit number per cane (218 ± 23) and yield per cane (864 ± 40 g) as compared to untreated plants. Prohexadione-calcium at 250 mg/l inhibits growth of lateral shoots and increased number of fruit on apple and pear plants (Yoder *et al*., 1999).

Return bloom

The double spray of prohexadione-calcium @ 200 ppm, one at the complete petal fall stage and one at four weeks after the first spray, increased the return bloom percentage by about 24.04 in Clapp's Favorite pear plants. Pasa and Einhorn (2017) reported that prohexadione-calcium was found to be best for return bloom in stark crimson pear trees as it produces more number of flowering spurs (14.80%) and flowering shoots (8.60%) in second year of application. Shehaj *et al.* (2015) reported that the application of prohexadione-calcium (100 and 150 ppm) showed significant effect on return bloom of treated pear plants. On contrary to this, Carra *et al*. (2016) reported that return bloom was negatively affected by the use of pro-ca in 2013/2014 growing season in Smith pear trees, the same was noted in d Anjou pears (Pasa *et al*., 2014) and Mutsu apples (Greene, 2008). On contradictory, Sugar *et al*. (2004) reported that return bloom was not affected by pro-ca spray in Bartlett, d Anjou Blanquilla plants.



Chemical structure

Similar results were also reported by Asin *et al*., (2007) in apple, Medjdoub *et al*., (2005) apple and pear and Miller *et al*., (2002) in apple.

Conclusion

Gibberellic acids ameliorate the vegetative growth through cell division and cell elongation in fruit plants, which affects the reproductive growth resulting in lower quality fruit production. From the present comprehensive review, it can be concluded that the chemical prohexadione-calcium limits the biosynthesis of the active GA thereby inhibiting the excessive vegetative growth of fruit plants. Prohexadione thus can act as a potential plant bioregulator that controls the vegetative growth and improves fruit set and quality production.

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