Doi: https://doi.org/10.17584/rcch.2021v15i3.12601

Application of prohexadione calcium in strawberry seedlings cv. Pircinque

Aplicación de prohexadiona de calcio en plántulas de fresa cv. Pircinque

ZULMA PAOLA MOLANO[1, 3]
LEO RUFATO[1]
DIEGO MIRANDA[2]
ANTONIO FELIPPE FAGUERAZZI[1]

Seedling and fruits of strawberry cv. Pircinque.

Photos: Z.P. Molano

ABSTRACT

In Brazil, strawberry production depends on the importation of seedlings from different countries, with variable levels of adaptation, different cold requirements, and high costs. The national production of seedlings with good genetic, physiological and phytosanitary quality is a viable alternative for Brazilian strawberry producers. The objective was to evaluate the effect of applications with different concentrations and timing using calcium prohexadione (ProCa) (growth retardant in various cultivated species) on magnitude changes in propagation structures, growth and development of strawberry seedlings cv. Pircinque, productive behavior, and fruit quality. This experiment was carried out in the Agricultural Sciences Center of the Santa Catarina State University (CAV-UDESC) in Lages / SC (Brazil), with a 5 x 2 factorial design, five concentrations of prohexadione calcium (0, 100, 200, 300 and 400 mg L^{-1}), and two application schedules (20 and 30 days after planting the rooting stolon). In the seedlings, the following were evaluated: number of leaves, crown diameter, seedling height, and leaf area. The parameters evaluated in the strawberry plants were: number of fruits per plant, average fruit weight, total fruit weight, and dry weight of leaves and crowns during the 2019/2020 cycle. Additionally, firmness, total soluble solids (TSS), titratable acidity (TA), and ratio were evaluated. Applying ProCa significantly reduced vegetative growth and increased physiological responses in the 'Pircinque' strawberry seedlings, such as leaf area and height for both application periods. On the other hand, the ProCa concentrations did not influence the number of fruits, fruit mass per plant, or fruit quality parameters, with the exception of firmness, which was favored by the 400 mg L^{-1} concentration. The results were related to the application timing, where, at 20 DAT, the highest values of the morphological variables in the seedlings and plants were observed. On the contrary, applications at 30 DAT decreased the number of commercial fruits per plant.

Additional keywords: *Fragaria* × *ananassa*; plant growth bio-regulators; propagation; fruit quality.

[1] Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Lages (Brazil). ORCID Molano, Z.P.: 0000-0002-3558-0610; ORCID Rufato, L.: 0000-0001-9545-7035; ORCID Faguerazzi, A.F.: 0000-0002-8731-2618

[2] Universidad Nacional de Colombia, Faculty of Agricultural Sciences, Bogota (Colombia). ORCID Miranda, D.: 0000-0002-3232-0901

[3] Corresponding author. zpmolanoa@unal.edu.co

RESUMEN

En Brasil, la producción de fresas está condicionada por la importación de plántulas de diferentes países, con niveles de adaptación variables, diferentes requerimientos de frío y altos costos del material introducido. La producción nacional de plántulas de buena calidad genética, fisiológica y fitosanitaria, se constituye en una alternativa viable para los productores brasileros dedicados a esta actividad. El objetivo de este trabajo fue evaluar el efecto de la aplicación de diferentes concentraciones y épocas de aplicación de prohexadiona de Calcio (Pro-Ca) (retardante del crecimiento en varias especies cultivadas), sobre los cambios de magnitud de las estructuras de propagación, el crecimiento y desarrollo de plántulas, el comportamiento productivo y la calidad del fruto de fresa cv. Pircinque. El experimento se llevó a cabo en el Centro de Ciencias Agrarias de la Universidad Estatal de Santa Catarina (CAV-UDESC) en Lages / SC (Brasil), con un diseño factorial 5 x 2, cinco concentraciones de prohexadiona de calcio (0, 100, 200, 300 y 400 mg L^{-1}) y dos períodos de aplicación (20 y 30 días después de transplante del estolón). En las plántulas se evaluó: número de hojas, diámetro de corona, altura de plántula y área foliar. Los parámetros evaluados en las plantas de fresa fueron: número de frutos por planta, peso medio del fruto, peso total de frutos por planta, peso seco de hojas y coronas, durante el ciclo 2019/2020. Además, se evaluó la firmeza, los sólidos solubles totales (TSS), la acidez titulable (AT) y la relación de madurez. La aplicación de Pro-Ca redujo significativamente el crecimiento vegetativo e indujo un aumento de las respuestas fisiológicas en plántulas de fresa cv. Pircinque, como el área foliar y la altura para ambas épocas de aplicación. Por otro lado, las concentraciones de Pro-Ca no influyeron en el número de frutos y la masa de frutos por planta, ni en los parámetros de calidad del fruto, con excepción de la firmeza, donde fue favorecida por la concentración de 400 mg L^{-1}. Los resultados se relacionaron con el factor época de aplicación, donde a los 20 DDT se presentaron los valores más altos de las variables morfológicas de las plántulas y plantas; por el contrario, aplicaciones a los 30 DDT disminuyen el número de frutos comerciales por planta.

Palabras clave adicionales: *Fragaria ananassa*; biorreguladores de crecimiento vegetal; propagación; calidad de la fruta.

Received: 16-03-2021 Accepted: 12-07-2021 Published: 02-11-2021

INTRODUCTION

Global strawberry production (*Fragaria* × *ananassa* Duch.) has been growing in absolute numbers, going from 7,879,108 t (2013) to 12,106,585 t (2019), a 46% increase in recent years. In Brazil, according to data from Embrapa, Incaper-ES, Emater (DF, MG, PR, RS), APTA and Epagri-SC, the cultivated area of this small fruit has grown in recent years. South America stands out every year in increases in strawberry production. According to the latest data provided by the FAO, production grew from 8,242,262 t ha^{-1} in 2017 to 8,885,028 t ha^{-1} in 2019, a significant increase of 642,766 t ha^{-1}, which represents an 8% increase. Brazilian producers cultivated about 4,500 ha of strawberry in the 2019 harvest, around 165,000 t (Antunes *et al*., 2020).

In fruit and vegetable crops, the quality of seedlings and other propagules is very important for the quality and quantity of subsequent production. For this reason, the quality of strawberry (*Fragaria* × *ananassa* Duch.) propagules has a direct influence on the yield and quality of fruit after transplanting; propagule quality is estimated to account for 80% of the crop quality (Jun *et al*., 2014).

Although the strawberry is physiologically a perennial species, in the conventional production system, it is renewed after a production cycle because of phytosanitary problems that limit production (Verdial *et al*., 2009). This creates a domestic demand of 175 million seedlings per year (Antunes and Peres, 2013), which mostly come from Argentina, Chile, and Spain (Barreto *et al*., 2018). The development of seedling production systems that, in addition to the sanitary condition, have physiological conditions for the accumulation of reserves and flower differentiation are needed to compete in terms of precocity, fruit caliber and sweet taste, with productivity similar to imported, seedlings that have economic viability (Antunes *et al*., 2020).

In addition, the production of domestic strawberry seedlings with sanitary and physiological quality throughout commercial planting (March-July) is a growing demand in different producing regions to reduce the dependence of Brazilian producers on the acquisition of imported seedlings. To ensure this, is necessary to reduce the excessive vegetative growth of the aerial part of seedlings to accumulate more reserves in the crown and root and, thus, develop better quality seedlings (Cocco *et al*., 2015).

There is excessive vegetative growth of the aerial part of seedlings, decreasing the accumulation of reserves in the crown and roots during the early summer as a result of the favorable environmental conditions for development (Cocco *et al*., 2015; Pereira *et al*., 2016). This is why it is necessary to reduce vegetative growth in seedlings (Carra *et al*., 2017), which can be carried out with the use of plant growth bioregulators, including calcium prohexadione (Pro-Ca).

One alternative to guarantee the productivity of crops and improve fruit quality is the use of plant growth regulators (Hawerroth and Petri, 2014), including prohexadione calcium (calcium 3-oxide-4-propionyl-5-oxo-3-cyclohexane carboxylate), which delays the growth of strawberry seedlings (Black, 2004; Reekie *et al*., 2005). This plant bioregulator belongs to the acyl-cyclohexanediones class, compounds that regulate plant height but also play an important role in other physiological phenomena, such as floral initiation and fruit fixation (Taiz *et al*., 2017).

Recent studies have highlighted the application of growth retardants in different fruit crops, such as apple (Duyvelshoff and Cline, 2013), pear (Einhorn *et al*., 2014), sweet cherry (Jacyna and Lipa, 2010), avocado (Mandemaker *et al*., 2005), and raspberry (Poledica *et al*., 2012). In strawberry, Black (2004) explained that, although the application of Pro-Ca in the fall increased the formation of 'Chandler' crowns, it did not affect the number of inflorescences during the following year. Reekie *et al*. (2005) showed that treatments with Pro-Ca applied in the nursery improved the establishment of plants in the production field, with positive effects on the precocity of the culture and the yield of marketable fruits.

Min Kim *et al*. (2019) with strawberry cultivar Maehyang showed that the FW (fresh weight) and DW (dry weight) of the first runner plant were not significantly different in response to different concentrations of Pro-Ca treatments but the DW of the second runner plant and the FW and DW of the third runner plant were greatest in the 50 mg L^{-1} Pro-Ca drench treatment. These results suggested that the growth and production of Maehyang strawberry runner plants were greatest under the 50 mg L^{-1} Pro-Ca drench treatment. Thus, the objective was to evaluate the effect of different concentrations and application schedules of Pro-Ca on controlling strawberry cv. Pircinque seedling growth.

MATERIALS AND METHODS

This experiment was carried out in an experimental area belonging to the Center for Agricultural Sciences of the Universidade do Estado de Santa Catarina (CAV/UDESC), in Lages, SC, Brazil (27°47' S, 50°18' W, altitude 922 m) in 2019 and 2020. Strawberry cultivar Pircinque seedlings with clods (roots surrounded by substrate) were used. The local climate is classified as humid subtropical Cfb mesothermal according to the Köppen classification. The propagules of the stolons were obtained from the Pasa nursery, Farroupilha/RS on March 21, 2019, with a pair of leaves and placed in 50-cell polystyrene trays (internal volume of 124 mL), containing commercial substrate based on Pindstrup and Carolina Soil® (1:1).

The seedlings were kept for 10 d in a misting chamber with an irrigation frequency of 10 s every 10 min for the initial rooting period. Afterwards, they were transferred to the acclimatization chamber, where they were maintained for 20 d under controlled irrigation with an irrigation frequency of 30 s four times a day and a flow rate of 30 L h^{-1}. At 20 and 30 d after planting, the plant growth regulator Pro-Ca was applied (Prohexadione Calcium) (Viviful® with 27.5% a.i.) to the seedlings. The application was done with a manual sprayer (1 L capacity).

After 15 d of vernalization in the cold chamber at 2.5-3°C, the seedlings were planted on Jun 28, 2019 in the conventional system. The seedlings were established in beds raised 30 cm from the ground, 9 m long and 0.9 m wide, covered with black polyethylene with 50 μm (mulching). The spacing between the plants was 0.3 m, with three lines per bed in cuts made in the plastic.

The irrigation was done with three dip tapes per bed and emitters every 0.15 m to obtain the uniform wetting required by the culture. Fertigation was performed every two weeks with the aid of a venturi system using the nutrient solution proposed by Yamazaki, which includes N (NO_3: 5; NH_4: 0.5); P :1.5; K: 3; Ca: 2; Mg: 1; S: 1; Fe: 3; B: 0.5; Mn: 0.5; Zn: 0.05; Cu: 0.02; Mo: 0.01 in meq/L. Weed control was done with manual weeding between the beds and the plantings. Phytosanitary management was carried out when necessary with products registered by the Agricultural Ministry for the crop.

The climatic conditions were monitored with data collection using the Automatic Meteorological Station of Lages A865-SC, (27.8 S, 50.3 W, 953 m of altitude). The data were collected from the database of the National Institute of Meteorology - INMET. The climatic parameters were air temperature (°C) and average rainfall (mm) (Fig. 1).

At 60 d after the beginning of development period, the following were measured: plant height (mm) taken from the crown base to the fully expanded new leaf and crown diameter (mm), using a digital caliper (CD-20CPX, Mitutoyo Co. Ltd., Kawasaki, Japan); number of leaves and leaf area (cm^2), estimated using the methodology proposed by Zeist *et al*. (2014), with the sum of the product of the two dimensions of the leaf (LF), length (L) and width (W), in each leaf, according to Equation (1):

$$LA\ (cm^2) = [LF1\ (L \times W) + LF2\ (L \times W) + LF3\ (L \times W)] \quad (1)$$

At the end of the experiment, all plants were divided into each part (root, crown, and leaves), and the fresh mass and dry mass were measured with an electronic balance; a drying oven (Venticell-220, MMM Medcenter Einrichtungen GmbH, Planegg, Germany) at 65°C for 72 h, until constant mass was obtained, was used to prepare the plants for the dry mass measurement.

The harvest began on October 11, 2019 and lasted until February 8, 2020, for a total of 25, starting when the fruits had at least 75% red colored epidermis. The fruits were weighed on a digital scale and counted. The number and mass of fruits per plant were obtained from the sum of all harvests and divided by the number 5. The average fruit mass was calculated with the ratio between these two variables. The fruits were classified as commercial (weight ≥10 g) and non-commercial (weight ≤10 g).

Additionally, quality parameters were measured, such as pulp firmness, with a digital penetrometer with a 6 mm diameter tip, taking two readings on the opposite sides in the equatorial zone of the fruits; total soluble solids (TSS), determined with a digital bench refractometer with temperature correction, expressed as g/100 g of soluble sugars (° Brix); titratable acid (TA), with a digital bench titrator through titration with 0.1 N NaOH solution; and TSS/TA ratio, calculated using the ratio between these two variables.

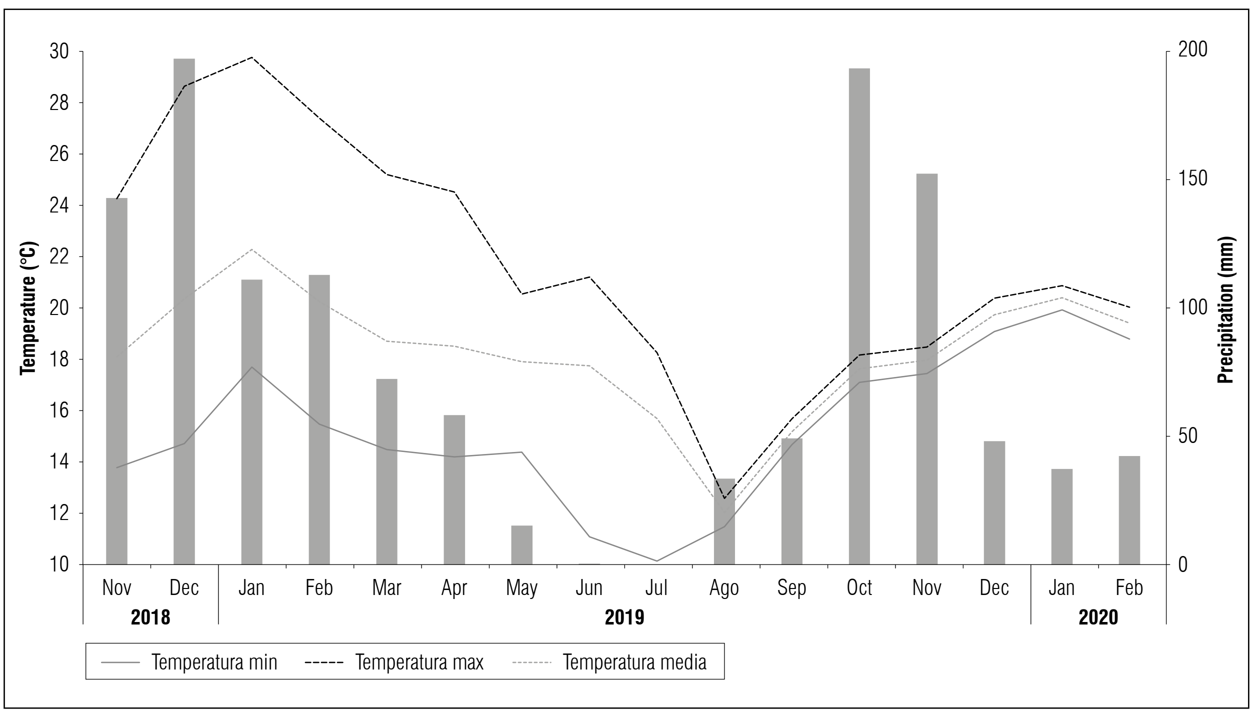

Figure 1. Monthly minimum, maximum and average temperature variations in the experiment period. Source: INMET (2020).

The experiment design was completely randomized, with a 5 × 2 factorial design, five concentrations of Pro-Ca (0, 100, 200, 300 and 400 mg L^{-1}), and two application schedules (20 and 30 d after planting or period of rooting stolon (DAP) with four replicates, each replicate composed of 10 plants). In the post-planting phase, the experiment design used randomized blocks (the criteria for blocking the treatments were the length of the bed and the slope) with the same factorial array used in the seedling phase, with four replicates, and the experiment unit consisted of four plants.

The statistical analyses were performed using SAS (SAS 9.4, SAS Institute Inc., Cary, NC, USA). The results were subjected to analysis of variance (ANOVA) and Tukey's tests. The variables with a significant effect for the quantitative factor were submitted to polynomial regression analysis (Silva and Azevedo, 2016).

RESULTS AND DISCUSSION

The height of the strawberry seedlings cv. Pircinque cultivated on substrate showed an interaction between the Pro-Ca concentrations and application schedules (Fig. 2A). It was observed that the height of the seedlings decreased with the increasing Pro-Ca concentrations under both application schedules, with an adjustment of the quadratic polynomial data (R^2 = 0.90 and 0.89 for 20 and 30 DAT, respectively; Fig. 2). The Pro-Ca applications at 20 DAT reduced the seedling height by 54.05% using 400 mg L^{-1} as compared to the control treatment. The petiole length of mother plants significantly decreased in the Pro-Ca treatments compared to control plants. At 30 DAT, there was a reduction of 45.63% in the petiole length using the same concentration, as compared to the absence of the phytoregulator (Fig. 2).

Prohexadione calcium (Pro-Ca) is known to be an inhibitor of new gibberellin biosynthesis (Mandemaker *et al*. 2005), which has been shown to be a useful tool for controlling excessive vegetative growth in some crops. In previous studies on strawberries, it has been shown that increasing the Pro-Ca concentration caused a reduction in vegetative growth in cultivar Camarosa (Pereira *et al*., 2016) and in cultivar Maehyang, where the petiole length was significantly inhibited with the 200 mg L^{-1} concentration treatment (Min Kim *et al*., 2019). In the present study, the same results were obtained in the Pro-Ca treatments. These results explained the fact that GA regulates cell elongation rather than cell division.

In previous studies, it has been shown that PGR treatments in rice, chrysanthemum, cucumber, apple, and tomato was associated with the suppression of plant stretchiness (Kim *et al*., 2007; Hawerroth and Petri, 2014; Kim *et al*., 2016). Ozbae and Ergun (2015) reported a 38% reduction in plant height in eggplant (*Solanum melongena*) with the concentrations of 150 mg L^{-1}. These results explained the fact that, when applied to plants, this phytoregulator inhibits cell elongation and growth by inducing a reduction in the levels of active gibberellins (Barreto *et al*., 2018).

Likewise, the total leaf area of 'Pircinque' strawberry seedlings was influenced by Pro-Ca concentrations, following a quadratic model, where the lowest values were observed with the higher applications of 300 and 400 mg L^{-1}, which decreased the leaf area by 36.9 and 44.2%, respectively, in relation to the 0 mg L^{-1} concentration of Pro-Ca (Fig. 2B). The effect of reducing the leaf area in the seedlings caused by the Pro-Ca concentrations may have been due to the reduction of the leaf size. Previous studies (Barreto *et al*., 2018) have reported that concentrations of 200 and 400 mg L^{-1} of Pro-Ca markedly reduced vegetative growth indicators such as petiole length and leaf area in strawberry cultivars Camarosa and Aromas, where this response is related to the cultivar.

In the transplant phase in the field, the plants with a smaller size and foliar area suffered less injury and presented better performance than the large plants. Studies conducted by Reekie *et al*. (2005) showed that minimizing transplant stress and good root development seedlings are ideal conditions to obtain good performance in the field and, consequently, maximum fruit yield.

This reduction in leaf area was observed by Pereira *et al*. (2016), who found a decrease of 32, 26 and 17% of the total leaf area in 'Camarosa' strawberry seedlings in response to the concentrations 200, 400 and 800 mg L^{-1} of Pro-Ca, respectively, which possibly occurred because of the hormonal regulation exercised by Pro-Ca. This means that, when this phytoregulator is applied, it reduces in GA levels that inhibit cell elongation and leaf growth (Petri *et al*., 2016). Therefore, it is likely that the effective concentration of Pro-Ca for vegetative growth inhibition will be different for each strawberry cultivar.

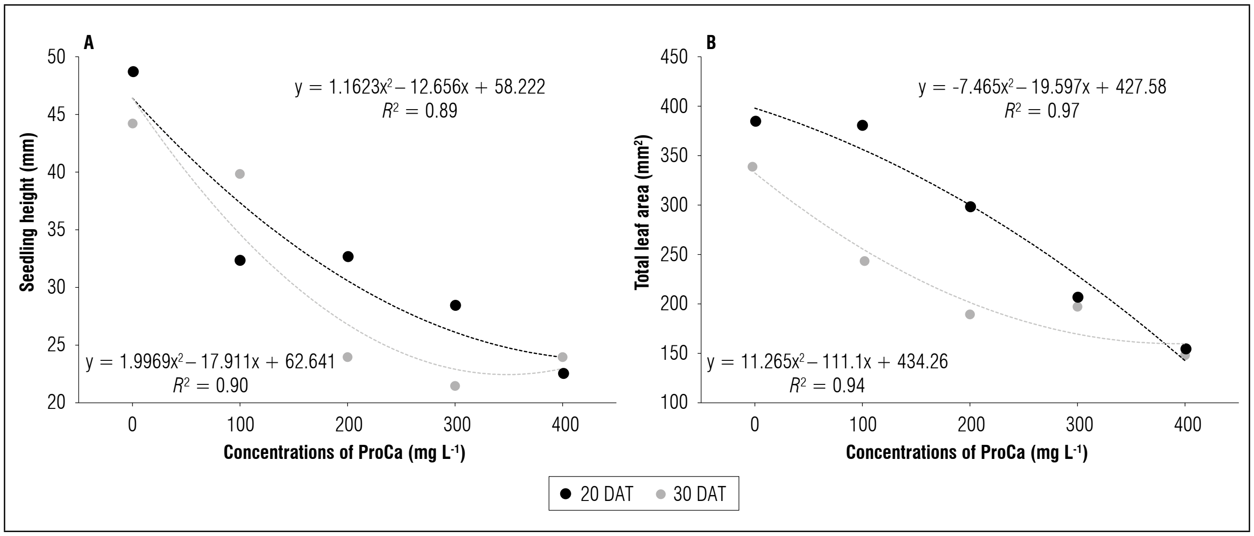

Figure 2. Morphological variables of strawberry seedlings cv. Pircinque. A, seedling height; B, total leaf area as function of Pro-Ca concentration applied at 20 and 30 DAT.

Leaves have important functions and are one of the main organs for which plants normally compete for sunlight, absorbing it and influencing photosynthetic rates and growth (Taiz *et al*., 2017). The number of leaves can be attributed to the genetic characteristics of plants; however, eventually, applications of plant regulators may interfere with the expressiveness of genes and, thus, influence this variable (Min Kim *et al*., 2019).

At the time of transplanting to the field, there were significant differences in the number of leaves emitted per plant (2.07 for control plants), as compared to the plants that received 300 mg L^{-1} of Pro-Ca at 20 DAP, which had 3.93 leaves per plant. Thus, the number of leaves of the treated seedlings was adjusted to a quadratic model (Fig. 3A), with an increase in the number of leaves when compared to the control when concentrations between 100 and 300 mg L^{-1} were used. The greatest reduction occurred when Pro-Ca was applied at 30 d after the planting period of the rooting stolon with 400 mg L^{-1} of Pro-Ca. Likewise, Reekie *et al*. (2005) observed that 62.5 mg L of Pro-Ca applied via leaf sprinkling on strawberry cultivars Sweet Charlie and Camarosa did not affect the number of leaves.

Some of the principal parameters to determine the strawberry seedling quality at the time of transplantation are related to the crown diameter since a positive relationship has been established between its diameter, its mass, and the productive potential of the plants. Accordingly, it was hypothesized that bigger transplants would result in greater strawberry production (Cocco *et al*., 2011; Torres-Quezada *et al*., 2015). The crown diameter of cv. Pircinque seedlings submitted to concentrations of Pro-Ca had an increasing trend in the different treatments; the curves were adjusted to a quadratic model for both application schedules, at 20 DAT and 30 DAT (Fig. 3).

The behavior of each application timing was different. At 20 DAT, the seedlings that received the highest concentration, 400 mg L^{-1}, had an increase of 9% in diameter in relation to the control plants. At 30 DAT, the increase was 3%, which was not significant. However, the average crown diameter (8.95 mm) achieved as a result of the application of Pro-Ca was above 8.0 mm, which, according to Cocco *et al*. (2011), is recommended for bare root strawberry seedlings as an appropriate crown diameter. Barreto *et al*. (2018) used Pro-Ca concentrations between 0 and 400 mg L^{-1} applied at 20 or 30 DAT on strawberry cultivars Camarosa and Aromas and found that the diameter of the crown decreased by up to 15.6% with concentrations 400 mg L^{-1}. Pereira *et al*. (2016) worked with the cultivar Camarosa and obtained a crown diameter greater than 8 mm only with the application of 200 mg L^{-1} of prohexadione calcium.

The results of dry leaf matter and crown dry matter were influenced by the timing and concentration of Pro-Ca (Fig. 4A, 4B). The plants, when subjected to applications of the regulator, produced a greater

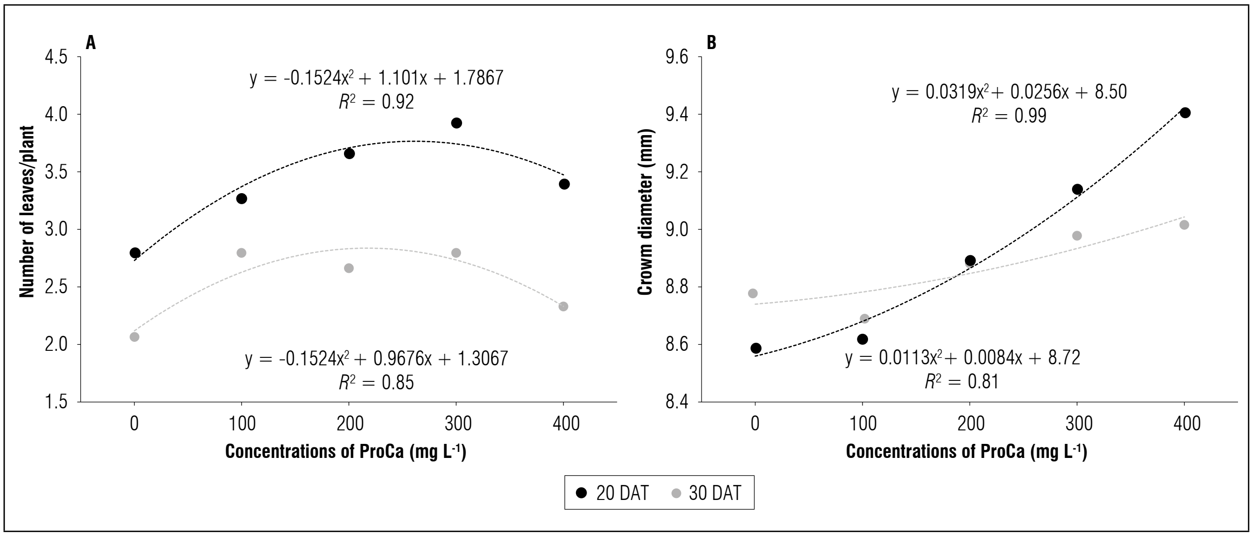

Figure 3. Morphological variables of strawberry seedlings cv. Pircinque. A, number of leaves per plant; B, crown diameter as function of Pro-Ca concentration applied at 20 and 30 DAT.

crown mass than those without applications. However, this increase in concentration may end up decreasing the crown production with the cultivar Pircinque. The concentration of 100 mg L^{-1} of Pro-Ca helped in the production of 4.11 g of dry mass of crowns, and 400 mg L^{-1} of Pro-Ca at 20 DAT decreased production to 2.23 g of crown dry mass (Fig. 4A). This indicates that, if the concentration increased beyond 200 mg L^{-1} of Pro-Ca, the plants may produce less crowns or even stop growth, failing to recover after planting. Therefore, it is vital that the ideal concentration of Pro-Ca be applied during the seedling phase.

The leaf dry mass also showed a significant negative quadratic response to the Pro-Ca concentrations (Fig. 4B). The 200 mg L^{-1} concentration induced a 35% decrease in the leaf mass of the strawberry, as compared to the control treatment. These data agree with Hawerroth and Petri (2014), who, in apple trees, found a 54.7% reduction in the mass of pruned branches (330

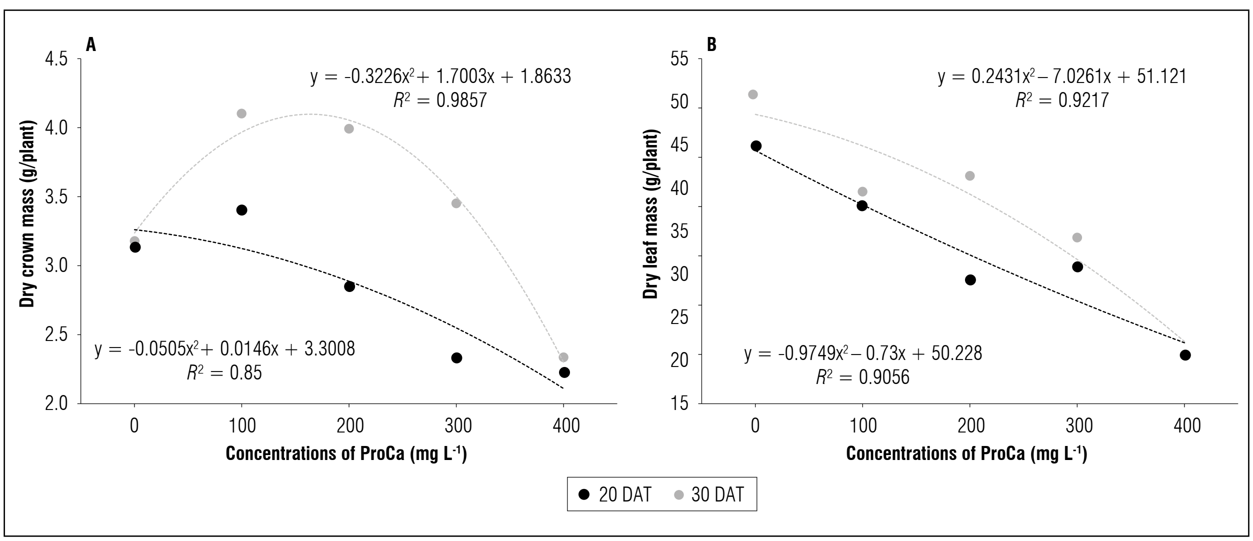

Figure 4. Dry mass of crown (A) and leaves of strawberry plants cv. Pircinque (B) as function of the concentration of Ca prohexadione applied at 20 and 30 DAT.

mg L^{-1}) in relation to plants not treated with Pro-Ca. In the present study, the reduction in leaf mass was probably due to the reduction of the total leaf area.

The productivity and quality of strawberry are determined according to the fruit number produced per plant, which is influenced by the climatological conditions and the management practices applied in the crop. In this study, the Pro-Ca concentrations did not influence the number of fruits per plant, average fruit mass or fruit mass per plant (Tab. 1). However, the applications of the growth regulator reduced the marketable harvested fruit number (fruits≥10 g) inversely to the increase in concentrations of Pro-Ca used for the applications at 30 DAT. In this way, the control plants with 21.78 commercial fruits per plant were 27% higher than the plants treated with the maximum concentration (400 mg L^{-1}).

The low production obtained with the cv. Pircinque in the 2019/2020 cycle can be explained by the fact that the experiment was conducted in an open field, exposed to the rain, which favored the incidence of diseases such as anthracnose (*Colletotrichum acutatum*), which is responsible for direct damage to flowers and fruits, reducing the commercial fruits per plant. This variable was below that reported by Ferreira *et al*. (2019), who obtained averages between 31.8 and 50.5 fruits/plant for American strawberry cultivars. However, our results were close to those achieved by Zanin (2019), who obtained average values between 23.84 and 40.37 fruits/plant for the Pircinque cultivar. These differences were due to factors such as cultivar, planting date (Ariza *et al*., 2012), cultural practices (Araújo *et al*., 2016), fluctuations in temperature, irrigation and incidence of diseases (Costa *et al*., 2016).

Other studies have reported that Pro-Ca treatments reduced fruit number, with a better distribution of available photoassimilates (Jiang *et al*., 2011). The plant growth regulators had temporary action until the beginning of fruit production but no longer had an effect on these variables. For Reekie *et al*. (2005), the short duration of Pro-Ca does not compromise strawberry production but only delays the vegetative growth of seedlings.

There were no effects from the application of Pro-Ca in different periods on the organoleptic characteristics: soluble solids and ratio (Tab. 2). The more appreciated fruit quality parameters by consumers include pulp firmness (Figueroa-Cares *et al*., 2018), which is related to morphological characteristics and the agronomic management provided in pre- (adequate nutritional and water input, control of fungal and bacterial diseases) and postharvest (Casierra-Posada and Aguilar-Avendaño, 2008). Applying 400 mg L^{-1} of

Table 1. Production parameters of strawberry cv. Pircinque as function of Pro-Ca concentration applied at 20 and 30 DAT.

Period of application (DAT)	Pro-Ca concentration (mg L^{-1})	Total production (g/plant)	Commercial production (g/plant)	Total number of fruits per plant	Number of commercial fruits per plant	Average fruit mass (g/fruit)
20	0	270.80 NS	251.93 NS	23.30 NS	22.11 NS	12.65 NS
	100	280.03 NS	262.48 NS	23.83 NS	22.50 NS	13.01 NS
	200	265.57 NS	249.10 NS	23.67 NS	22.22 NS	12.04 NS
	300	213.13 NS	203.48 NS	20.30 NS	19.28 NS	10.73 NS
	400	190.93 NS	169.10 NS	19.77 NS	17.56 NS	9.27 NS
30	0	273.97 NS	251.60 NS	23.50 ns	21.78 a	12.84 NS
	100	241.20 NS	225.22 NS	21.00 NS	19.78 ab	12.84 NS
	200	235.13 NS	222.36 NS	19.93 NS	19.00 ab	12.96 NS
	300	199.37 NS	174.11 NS	17.87 NS	17.39 ab	12.56 NS
	400	187.33 NS	177.86 NS	18.10 NS	15.89 b	10.60 NS
Concentration		0.0026	0.0023	0.0029	0.0021	0.0584
Application timing		0.2157	0.1884	0.0102	0.0153	0.2309
Concentration x Period		0.8267	0.7295	0.6246	0.4662	0.31

Means followed by the same letter in the columns do not differ statistically from each other according to the Tukey test at 5% probability of error. NS not significant.

the PGR at 30 DAT resulted in the highest firmness value (3.47 N), as compared to the control, which did not have Pro-Ca applications (Tab. 2).

In general, the pulp firmness obtained in this study was different from the values obtained by Zanin (2019), 5.52 N for the cultivar Pircinque, in the same locality, Planalto Sul Catarinense in the 2017/2018 harvest, and by Dantas *et al*. (2017) for Albion and San Andreas cultivars, with 10.6 and 13.0 N, respectively, for the commercial maturation stage (80% red) in Bahia. These results can be explained by the fact that firmness is related to genetic and environmental factors (Camargo *et al*., 2011), such as a high temperature and relative humidity in the summer, which result in less firmness (Filgueira, 2008).

According to Asín and Vilardell (2006), result efficiency with plant regulators is dependent on climate, plant vigor, season, phenology of the plant, concentration and interval in which the sprayings are made. Basak and Krzewinska (2006) found that, in the second year of applications, the fruits of plants treated with Pro-Ca presented lower firmness indexes than control fruits. In addition, Carra *et al*. (2017) found that the application of prohexadione calcium did not affect the firmness behavior of 'Smith' pear fruits in cold storage. Ramírez *et al*. (2008) reported similar results in tomato fruits 'saladete' and by Greene (1999) in apples before storage.

Titratable acidity, in turn, is one of the main components of fruit flavor. At high levels, the acidity needs to be balanced with the sugar content, so as not to compromise the fruit acceptance by consumers (Carpenedo *et al*., 2016). The acid with the highest concentration in strawberries is citric acid although there are also considerable amounts of malic acid and, to a lesser extent, isocitric, succinic, oxalacetic, glycolic and glycolic acid (Azevedo, 2007).

For this parameter, a quadratic model was obtained, and an increasing response was obtained for the concentrations of Pro-Ca (Tab. 2). The increase in the phytohormone concentrations, up to a concentration of 200 mg L^{-1}, resulted in an increase of 16.3%, as compared to the control plants. According to our results, the mean titratable acidity, 0.75, was in the range reported by several authors (0.48 to 1.12%) for this parameter (Lemiska *et al*., 2014). For other fruit trees, the titratable acidity in 'Kent' mango fruits treated with Pro-Ca was between 0.83 and 0.91% (variation of 10%) at harvest, possibly related to the effect of this regulator on the inhibition of ethylene synthesis.

CONCLUSION

The application of Pro-Ca significantly reduced vegetative growth and increased physiological responses

Table 2. Quality parameters of strawberry cv. Pircinque as a function of Pro-Ca concentrations applied at 20 and 30 DAT.

Application timing (DAT)	Pro-Ca concentration (mg L^{-1})	Firmness (N)	Soluble solids (°Brix)	Titratable acidity (%)	Ratio (SS/AT)
20	0	2.80 NS	10.20 NS	7.7 ab	14.09 NS
	100	2.47 NS	10.43 NS	8.5 ab	12.34 NS
	200	2.30 NS	10.83 NS	9.20 b	11.77 NS
	300	2.44 NS	10.87 NS	8.4 ab	12.93 NS
	400	3.06 NS	10.47 NS	6.00 a	17.60 NS
30	0	2.30 a	9.80 NS	7.53 NS	13.28 NS
	100	2.02 a	10.10 NS	8.27 NS	12.78 NS
	200	2.52 ab	10.47 NS	8.20 NS	12.78 NS
	300	2.67 ab	10.17 NS	7.50 NS	13.58 NS
	400	3.47 b	10.20 NS	7.33 NS	13.90 NS
Concentration		0.0049	0.9386	0.0148	0.2453
Period		0.9059	0.4244	0.5956	0.6384
Concentration x period		0.2297	0.9990	0.2555	0.5820

Means followed by the same letter in the columns do not differ statistically from each other according to the Tukey test at 5% probability of error. ns: not significant.

in 'Pircinque' strawberry seedlings, such as leaf area and height for both application schedules. On the other hand, the Pro-Ca concentrations did not influence the number of fruits, the mass of fruits per plant, or the quality parameters of the fruits, with the exception of firmness, which was stimulated by the concentration of 400 mg L^{-1}. The results were related to the application timing, where, at 20 DAT, the highest values of the morphological variables of the seedlings and plants were observed, and, on the contrary, applications at 30 DAT decreased the number of commercial fruits per plant.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

BIBLIOGRAPHIC REFERENCES

Antunes, L.E.C., S. Bonow, and C. Reisser Júnior. 2020. Morango: crescimento constante em área e produção. Anuário Campo & Negocio HF 37, 88-92.

Antunes, L.E.C. and N.A. Peres. 2013. Strawberry production in Brazil and South America. Int. J. Fruit Sci. 13(1-2), 156-161. Doi: 10.1080/15538362.2012.698147

Araújo, V., G. Vignolo, E. Perin, C. Piana, C. Silveira, and C. Medeiros. 2016. Foliar fertilization with gradual release of shale-based nutrients in strawberry and its effect on yield and compounds with functional potential. Científica 44(3), 338-345. Doi: 10.15361/1984-5529.2016v44n3p338-345

Ariza, M.T., C. Soria, J.J. Medina-Mínguez, and E. Martínez-Ferri. 2012. Cultivar, planting date, pollination, and low temperatures affect incidence of misshapen fruits in strawberry plants grown under tunnels. HortScience 47(11), 1569-1573. Doi: 10.21273/HORTSCI.47.11.1569

Asín, L. and P. Vilardell. 2006. Effect of paclobutrazol and prohexadione-calcium on shoot growth rate and growth control in "Blanquilla" and "Conference" Pear. Acta Hortic. 727, 133-138. Doi: 10.17660/ActaHortic.2006.727.14

Azevedo, S.M.C. 2007. Estudo de taxas de respiração e de factores de qualidade na conservação de morango fresco (*Fragaria x ananassa* Duch.) 225f. MSc thesis. Portal da Universidade Aberta, Lisboa.

Barreto, C.F.L.V., S.I. Ferreira, A.V. Costa, T.B. Schiavon, G.K. Becker, and L.E.C. Vignolo. 2018. Concentration and periods of application of prohexadione calcium in the growth of strawberry seedlings. Semina: Ciênc. Agrár. 39, 1937-1944. Doi: 10.5433/1679-0359.2018v39n5p1937

Basak, A. and D. Krzewinska. 2006. Effect of Prohexadione-Ca (Regalis®) on the effectiveness of NAA and BA used for fruitlet thinning in apple trees. Acta Hort. 727, 139-144. Doi: 10.17660/ActaHortic.2006.727.15

Black, B. 2004. Prohexadione-calcium decreases fall runners and advances branch crowns of 'Chandler' strawberry in a cold-climate annual production system. J. Am. Soc. Hort. Sci. 129, 479-485. Doi: 10.21273/JASHS.129.4.0479

Camargo, L.K.P., J.T.V. Resende, T.T. Tominaga, S.M. Kurchaidt, C.K. Camargo, and A.S.T. Figueiredo. 2011. Postharvest quality of strawberry fruits produced in organic and conventional systems. Hortic. Bras. 29(4), 577-583. Doi: 10.1590/S0102-05362011000400022

Carpenedo, S., L.E.C. Antunes, and R.O. Treptow. 2016. Caracterização sensorial de morangos cultivados na região de Pelotas. Hortic. Bras. 34(4), 565-570. Doi: 10.1590/s0102-053620160417

Carra, B., J.C. Fachinello, E.S. Abreu, M.S. Pasa, D. Spagnol, M.A. Giovanaz, and C.P. Silva. 2017. Prohexadione calcium reduces vegetative growth and increases fruit set of 'Smith' pear trees, in Southern Brazil. Pes. Agrop. Bras. 52, 177-185. Doi: 10.1590/1678-4499.298

Casierra-Posada, F. and E. Aguilar-Avendaño. 2008. Calidad en frutos de tomate (*Solanum lycopersicum* L.) cosechados en diferentes estados de madurez. Agron. Colomb. 26(2), 300-307.

Cocco, C., J.L. Andriolo, F.L. Cardoso, L. Erpen, and O.J. Schmitt. 2011. Crown size and transplant type on the strawberry yield. Sci. Agric. 68(4), 489-493. Doi: 10.1590/S0103-90162011000400015

Cocco, C., M.A. Gonçalves, L. Picolotto, L.V. Ferreira, and L.E.C. Antunes. 2015. Crescimento, desenvolvimento e produção de morangueiro a partir de mudas com diferentes volumes de torrão. Rev. Bras. Frutic. 34(4), 919-927. Doi: 10.1590/0100-2945-250/14

Costa, A.F., P.E. Teodoro, L.L. Bhering, N.R. Leal, F.D. Tardin, and R.F. Daher. 2016. Biplot analysis of strawberry genotypes recommended for the state of Espírito Santo. Genet. Mol. Res. 15(3), 15038919. Doi: 10.4238/gmr.15038919

Dantas, E., T. Rebouças, J. Luz, and C. Amaral. 2017. Frutos de cultivares de morangueiros submetidos ao L-MCP em temperatura refrigerada. Sci. Plena, 13. Doi: 10.14808/sci.plena.2017.090202

Duyvelshoff, C. and J.A. Cline. 2013. Ethephon and prohexadione-calcium influence the flowering, early yield, and vegetative growth of young 'Northern Spy' apple trees. Sci. Hort. 151, 128-134. Doi: 10.1016/j.scienta.2012.12.002

Einhorn, T.C., M.S. Pasa, and J. Turner. 2014. 'D'Anjou' pear shoot growth and return bloom, but not fruit size, are reduced by prohexadione-calcium. HortScience 49, 180-187. Doi: 10.21273/HORTSCI.49.2.180

Ferreira, J.F.S. 2019. Fruit yield and survival of five commercial strawberry cultivars underfield cultivation and salinity stress. Sci. Hortic. 243, 401-410. Doi: 10.1016/j.scienta.2018.07.016

Figueroa-Cares, I.E., O. Cruz-Álvarez, M.T. Martínez-Damián, J.E. Rodríguez-Pérez, M.T. Colinas-León, and S. Valle-Guadarrama. 2018. Calidad nutricional y capacidad antioxidante en variedades y genotipos nativos de tomate (*Solanum Lycopersicum* L.). Rev. Fac. Agron. (Luz) 35(1), 63-84.

Filgueira, F. 2008. Novo manual de olericultura. 3nd ed. Ufv, Viçosa, Brazil.

Greene, D.W. 1999.Tree growth management and fruit quality of apple trees treated with prohexadione-calcium (Bas-125). Hortscience 34, 1209-1212. Doi: 10.21273/HORTSCI.34.7.1209

Hawerroth, F.J. and J.L. Petri. 2014. Crescimento vegetativo de macieiras 'Fuji Suprema' sob influência da época de aplicação de proexadione cálcio. Rev. Bras. Frutic. 36(2), 373-380. Doi: 10.1590/0100-2945-375/13

Jacyna, T. and T. Lipa. 2010. Direct and apparent residual effects of prohexadione-calcium applied to young cropping sweet cherry trees. Acta Agrobot. 63, 87-92. Doi: 10.5586/aa.2010.010

Jiang, T., P. Wang, X. Yin, B. Zhang, C. Xu, X. Li, and K. Chen. 2011. Ethylene biosynthesis and expression of related genes in loquat fruit at different developmental and ripening stages. Sci. Hortic. 130(2), 452-458. Doi: 10.1016/j.scient.2011.07.019

Jun, H.J., E.H. Jeon, S.I. Kang, and G.H. Bae. 2014. Optimum nutrient solution strength for Korean strawberry cultivar 'Daewang' during seedling period. Korean J. Hortic. Sci. Technol. 32, 812-818. Doi: 10.7235/hort.2014.14079

Kim, H.C., Y.H. Cho, Y.G. Ku, S.J. Hwang, and J.H. Bae. 2016. Growth characteristics of grafted tomato seedlings following treatment with various concentrations of diniconazole during the summer growth season. Korean J. Hortic. Sci. Technol. 34, 249-256. Doi: 10.12972/kjhst.20160026

Kim, H.Y., I.J. Lee, M. Hamayun, J.T. Kim, J.G. Won, I.C. Hwang, and K.U. Kim. 2007. Effect of prohexadione-calcium on growth components and endogenous gibberellins contents of rice (*Oryza sativa* L.). J. Agron. Crop Sci. 193, 445-451. Doi: 10.1111/j.1439-037X.2007.00280.x

Lemiska, A., V. Pauletti, F.L. Cuquel, and M.A.C. Zawadneak. 2014. Produção e qualidade da fruta do morangueiro sob influência da aplicação de boro. Ciênc. Rural 44(4), 622-628. Doi: 10.1590/S0103-84782014000400008

Mandemaker, A.J., J.G.M. Cutting, D.B. Smith, and J. Dixon. 2005. Effect of prohexadione -calcium on shoot growth, fruit set and retention in 'Hass' avocado in New Zealand. New Zealand Avocado Growers' Association Annual Research Report 5, 35-42.

Min Kim, H., H.R. Lee, H.J. Kang, and S.J. Hwang. 2019. Prohexadione-calcium application during vegetative growth affects growth of mother plants, runners, and runner plants of Maehyang strawberry. Agronomy 9, 155-166. Doi: 10.3390/agronomy9030155

Ozbae, N. and N. Ergun. 2015. Prohexadione calcium on the growth and qualite of eggplant seedlings. Pesq. Agrop. Bras. 50(10), 932-938. Doi: 10.1590/S0100-204X2015001000009

Pereira, I.D.S., M.A. Goncalves, L. Picolotto, G.K. Vignolo, and L.E.C. Antunes. 2016. Controle do crescimento de mudas de morangueiro 'Camarosa' cultivadas em substrato comercial pela aplicação de proexadione cálcio. Rev. Cienc. Agrar. 59, 93-98. Doi: 10.4322/rca.2126

Petri, J.L., F.J. Hawerroth, G.B. Leite, A.A. Sezerino, and M. Couto. 2016. Reguladores de crescimento para frutíferas de clima temperado. Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri). Florianópolis, Brazil.

Poledica, M., J. Milivojević, D. Radivojević, and J. Dragišić Maksimović. 2012. Prohexadione-Ca and young cane removal treatments control growth, productivity, and fruit quality of the 'Willamette' raspberry. Turk. J. Agric. For. 36, 680-687. Doi: 10.3906/tar-1201-18

Ramírez, H., B. Herrera, Y.H. Méndez, A. Benavides, J.A. De La Cruz, V. Álvarez, and J.A. Villareal. 2008. Prohexadiona de calcio disminuye el contenido de giberelinas endógenas en ápices de tomate saladette y chile pimiento. Rev. Chapingo Ser. Hortic. 14(2), 193-198. Doi: 10.5154/r.rchsh.2007.12.058

Reekie, J.Y., P.R. Hicklenton, and P.C. Struik. 2005. Prohexadione-calcium modifies growth and increases photosynthesis in strawberry nursery plants. Can. J. Plant Sci. 85, 671-677. Doi: 10.4141/P04-113

Taiz, L., E. Zeiger, I. Moller, and A. Murphy. 2017. Fisiologia e desenvolvimento vegetal. Art Med, Porto Alegre, Brazil.

Torres-Quezada, E.A., L. Zotarelli, V.M. Whitaker, B.M. Santos, and I. Hernandez Ochoa. 2015. Initial crown diameter of strawberry bare-root transplants affects early and total fruit yield. HortTechnology 25(2), 203-208. Doi: 10.21273/HORTTECH.25.2.203

Verdial, M.F., J. Tessarioli Neto, K. Minami, J.A. Scarpare Filho, P.J. Christoffoleti, F.V. Scarpare, J.F. Barela, J.S. Del Aguila, and R.A. Kluge. 2009. Fisiologia de mudas de morangueiro produzidas em sistema convencional e em vasos suspensos. Rev. Bras. Frutic. 31(2), 524-531. Doi: 10.1590/S0100-29452009000200029

Zanin, D.S. 2019. Divergência genética morfoagronômica e seleção de genótipos avançados de morangueiro. PhD thesis. Universidade do Estado de Santa Catarina, Lages, Brazil.

Zeist, A.R., J.R.F. Oliveira, R.B. Lima Filho, M.L.S. Silva, and J.T.V. Resende. 2014. Comparação de métodos de estimativa de área foliar em morangueiro. Pesq. Agrop. 20(1/2), 33-40.