



Semina: Ciências Agrárias

ISSN: 1676-546X

ISSN: 1679-0359

Universidade Estadual de Londrina

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Vighi; Becker, Tais Barbosa; Vignolo, Gerson Kleinick; Antunes, Luis Eduardo Correa
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Semina: Ciências Agrárias, vol. 39, no. 5, 2018, September-October, pp. 1937-1944
Universidade Estadual de Londrina

DOI: <https://doi.org/10.5433/1679-0359.2018v39n5p1937>

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Concentration and periods of application of prohexadione calcium in the growth of strawberry seedlings

Concentrações e épocas de aplicação do proexadione cálcio no crescimento de mudas de morangueiro

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Abstract

For strawberry cultivation in Brazil, producers are dependent on imported seedlings. An alternative strategy to reduce this dependence is the use of seedlings obtained from nursery plants grown in a protected environment. However, as these seedlings are produced in the summer and planted at the end of this season or the spring of the following year, it is necessary to control growth to reduce the energy costs of the plants. The objective of this study was to evaluate the effect of different concentrations and periods of application of proexadione calcium (ProCa) on growth control of strawberry seedlings. The experiment was carried out in a greenhouse with seedlings of the cultivars 'Aromas' and 'Camarosa', produced by rooting stolons and kept in polystyrene trays of 72 cells in a substrate of carbonized rice husk. The experimental design was completely randomized, with a 4×2 factorial scheme (4 concentrations of ProCa: 0, 100, 200, and 400 mg L⁻¹ \times 2 periods of application: at 20 and 30 days after the planting period of rooting stolon). Plant survival, crown diameter, petiole length, total leaf area, specific leaf area, chlorophyll concentration, and dry mass of the crown and shoot were evaluated. The application of ProCa at 20 days after the planting period of the rooting stolon at the concentrations of 200 and 400 mg L⁻¹ favored the reduction of petiole length in plants of 'Aromas' strawberry and total leaf aerial in 'Camarosa' strawberry. The application of ProCa from the concentration of 100 mg L⁻¹ reduced the vegetative growth of 'Aromas' and 'Camarosa' strawberry seedlings cultivated in substrate.

Key-words: *Fragaria* \times *ananassa*. Inhibitor of gibberellin. Plant regulators.

Resumo

Para o cultivo do morangueiro no Brasil existe a dependência dos produtores em utilizar mudas importadas. Com intuito de reduzir essa dependência, uma das alternativas é o uso de mudas obtidas de plantas matrizes cultivadas em ambiente protegido. Porém, como essas mudas são produzidas no verão e plantadas no final desta estação ou no outono do ano seguinte, é necessário controlar o crescimento para diminuir os gastos energéticos das plantas. Objetivou-se com o trabalho avaliar o efeito de diferentes concentrações e épocas de aplicação de proexadione cálcio (ProCa) no controle de

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crescimento de mudas de morangueiro com torrão. O experimento foi realizado em casa de vegetação com mudas das cultivares Aromas e Camarosa, produzidas por enraizamento de estolões e mantidas em bandejas de poliestireno de 72 células em substrato casca de arroz carbonizada. O delineamento experimental utilizado foi inteiramente casualizado, com esquema fatorial 4x2 (4 concentrações de ProCa: 0, 100, 200 e 400 mg L⁻¹ x 2 épocas de aplicação: aos 20 e aos 30 dias após o período de plantio do estolão para enraizamento). Avaliaram-se nas mudas: sobrevivência de plantas, diâmetro de coroa, comprimento de pecíolo, área foliar total, área foliar específica, concentração de clorofila e massa seca da coroa e da parte aérea. A aplicação de ProCa aos 20 dias após o período de plantio do estolão para enraizamento nas concentrações de 200 e 400 mg L⁻¹ favorece a redução do comprimento do pecíolo em morangueiro 'Aromas' e a área foliar total em morangueiro 'Camarosa'. A aplicação de ProCa a partir da concentração de 100 mg L⁻¹ reduz o crescimento vegetativo de mudas de morangueiro 'Aromas' e 'Camarosa' cultivadas em substrato.

Palavras-chave: *Fragaria x ananassa*. Inibidor de giberelina. Reguladores vegetais.

Introduction

Strawberry cultivation plays an important socioeconomic role in South and Southeast Brazil, with production destined for *in natura* consumption and for the food industry (MAZARO et al., 2013). In spite of this potential, the culture presents some limiting factors, particularly in obtaining seedlings, because the greatest part of the seedlings used in Brazil are imported from Patagonia (Argentina and Chile), due to the excellent conditions for cultivation in that region (ANTUNES; PERES, 2013; GONÇALVES et al., 2016). In addition to the growing dependence of producers on imported seedlings, the planting time of the crops needs to be matched to the time of arrival of the seedlings, as there are constant delays in their delivery (DAL PÍCIO et al., 2013), which causes difficulties in planning and conducting cultivation.

The production of seedlings in Brazil thus stands in need of strategies to reduce the dependence of producers on imported seedlings. One alternative proposed is the use of seedlings obtained from mother plants grown in protected environments during spring and summer (MENZEL; SMITH, 2012). This type of seedling has advantages, including the possibility of staggering production and planting seedlings at the recommended time in other words, anticipating the planting, which allows early production with greater profitability (COCCO et al., 2011).

However, while seedling production initiatives in the national territory have been undertaken in the summer, the seedlings were planted only at the end of the season or the following spring. During this period, excessive development of the aerial part occurs, which necessitates control of plant growth (PEREIRA et al., 2016). These seedlings, when produced under favorable environmental conditions, may also accumulate fewer reserves in the crown and roots, which consequently decreases the quality of the seedling (COCCO et al., 2015).

One of the alternatives to reduce vegetative growth of fruit is the use of growth regulators (HAWERROTH; PETRI, 2014; REZAZADEH; HARKESS, 2015; PASA; EINHORNET, 2017). The growth regulators used in fruit cultivation include prohexadione-calcium (ProCa) (calcium 3-oxide-4-propionyl-5-oxo-3-cyclohexane carboxylate), which is a phytohormone that inhibits the final steps of gibberellin (GA) biosynthesis (HAWERROTH; PETRI, 2014). ProCa reduces the activity of GA₂₀ 3β-hydroxylase, an enzyme that acts on the conversion of GA₂₀ (inactive gibberellins) to GA₁ (active gibberellins), thus controlling vegetative growth (KANG et al., 2010; HAWERROTH; PETRI, 2014). ProCa is effective in controlling vegetative growth and thus can be used for this purpose in strawberry seedlings (REEKIE et al., 2005; PEREIRA et al., 2016), as the domestic seedlings exhibit greater leaf growth and reduced reserve accumulation.

In this context, in order to reduce the dependence of Brazilian producers on the acquisition of imported strawberry seedlings, studies should be carried out to improve the quality of the domestic seedlings. However, for this it is necessary to control the growth of the aerial part of these seedlings to accumulate more reserves in the crown and root and, thus, develop seedlings of better quality. Therefore, the objective of this study was to evaluate the effect of different concentrations and times of application of ProCa on the growth control of strawberry seedlings.

Material and Methods

The experiment was carried out in a greenhouse at the EMBRAPA Temperate Climate Research Center, located in the municipality of Pelotas, Rio Grande do Sul (latitude: 31°40'S and longitude: 52°26'W, altitude 60 m) during the months of March to May 2017. The strawberry cultivars 'Camarosa' (short day) and 'Aromas' (neutral day) were used. These seedlings were produced by rooting stolons removed from parent plants that were planted in November 2016 and maintained on roof tiles covered with black and white double-sided polyethylene. The culture channels were filled with carbonized rice husk. Stolons were set to rooting in March 2017 in 72 cell polystyrene trays with Carolina Soil Padrão® substrate.

The seedlings were kept for 10 days in a nebulization chamber with an irrigation frequency of 10 seconds every 30 minutes for the initial period of rooting. Afterwards, the seedlings were transferred to the acclimatization chamber, where they were kept for 20 days, under controlled irrigation (during the development period), with an irrigation frequency of thirty seconds, four times a day and a flow rate of 30 L per hour. After the end of the development period, the seedlings were submitted to the growth control treatments, with the application of the plant growth regulator ProCa (Viviful® with 27.5% i.a.). The application of the product was carried out via

hand spray (1 liter capacity), using a volume of 3 mL per plant.

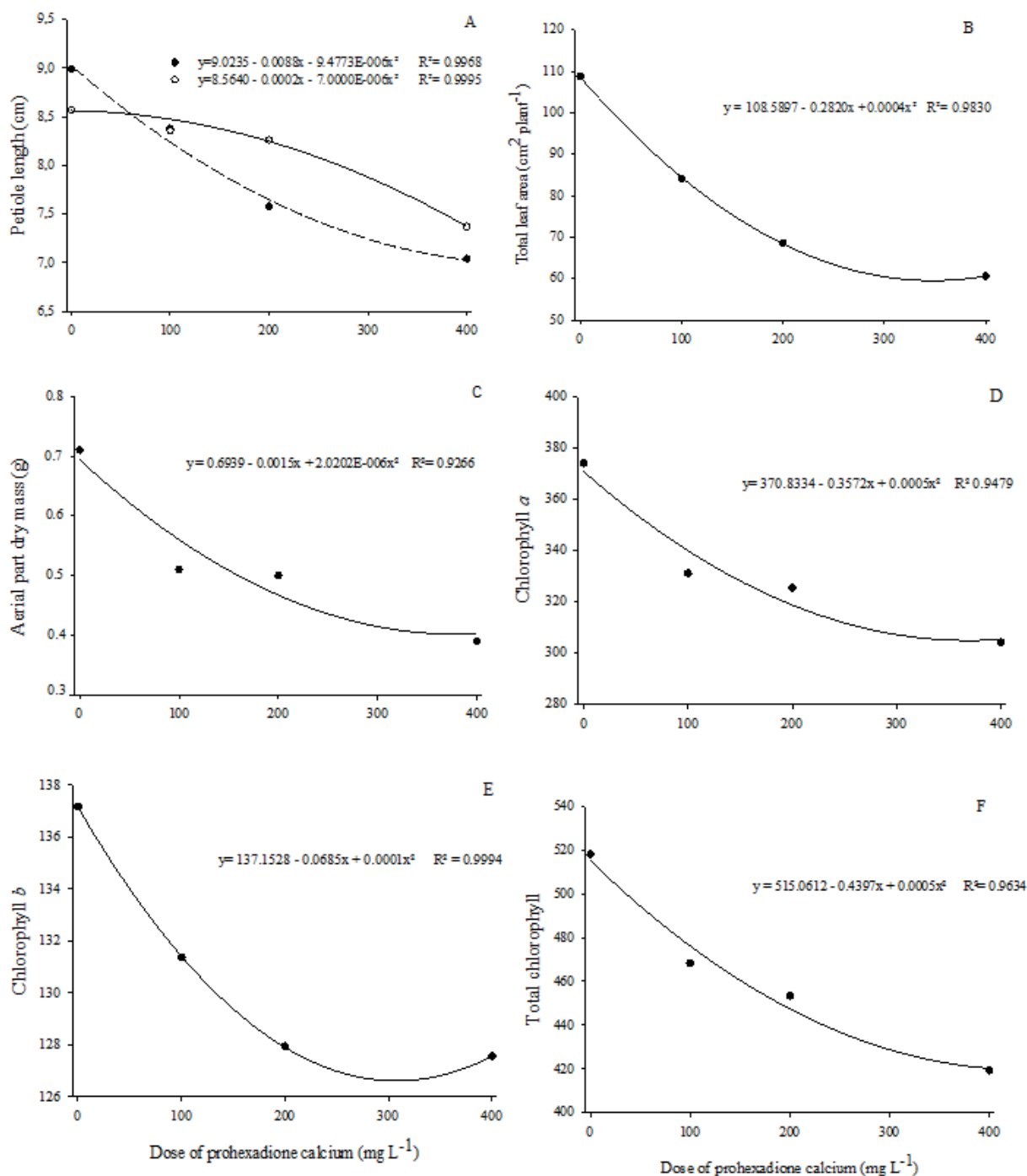
The experimental design was completely randomized, with a 4×2 factorial scheme (4 concentrations of prohexadione calcium: 0, 100, 200, and 400 mg L⁻¹ \times 2 periods of application: at 20 and 30 days after the planting period of rooting stolon) with 4 replicates, each replicate composed of 12 plants. At 60 days after the beginning of the development period, the following evaluations were performed: plant survival, obtained by counting the number of live plants; crown diameter (mm), measured with the aid of a digital caliper; petiole length (cm), made using a graduated ruler; total leaf area (cm² plant⁻¹), measured using the LI-3100C Area Meter equipment (Li-Cor Inc., Lincoln, NE, USA); specific leaf area, obtained by the relation between total leaf area and number of leaves; concentration of chlorophyll *a*, *b* and total, evaluated with a chlorophyll meter (ClorofiLOG - Falker Automação Agrícola Ltda., Porto Alegre, RS); and dry mass of the crown (g) and aerial part (g), obtained by drying the different parts of all the seedlings in an oven at 65 °C until constant weight.

The data were submitted to analysis of variance by the F-test and, when the effect was significant, the Tukey's test was performed and the variables with a significant effect for the quantitative factor were submitted to polynomial regression analysis using the statistical software ASSISTAT (SILVA; AZEVEDO, 2016).

Results and Discussion

The petiole length of 'Aromas' strawberry seedlings cultivated on substrate showed an interaction between ProCa concentrations and periods of application (Figure 1A). It was observed that the petiole length of the seedlings of this cultivar decreased with increasing ProCa concentrations at both periods of application.

Figure 1. Petiole length (A), total leaf area (B), aerial part dry mass (C), chlorophyll *a* (D), chlorophyll *b* (E), and total chlorophyll (F) in response to application of concentrations and period of application of calcium prohexadione in 'Aromas' strawberry seedlings cultivated in substrate. Embrapa Climate Temperate, RS, 2017.



Regarding the periods of application, the reduction in petiole length was greater when ProCa was applied at 20 days after the planting period of the rooting stolon under the application of 200

and 400 mg L⁻¹. his effect is probably due to ProCa acting in the inhibition of gibberellin synthesis. This reduces the levels of gibberellin, which functions in cell division, cell elongation, and growth (TAIZ

et al., 2017). Reduction in the length of apple (HAWERROTH; PETRI, 2014) and pear (PASA; EINHORNET, 2014, PASA; EINHORNET, 2017) branches has also been observed, as well as reduction of the petiole of strawberry seedlings (PEREIRA et al., 2016) following the application of ProCa.

The concentration of ProCa had a significant effect on total leaf area, aerial part dry matter, and chlorophyll *a*, *b*, and *total* content. However, none of these variables presented differences between the periods of application of ProCa in the strawberry cultivar 'Aromas'. The total leaf area of 'Aromas' strawberry seedlings was influenced by ProCa concentrations and the lowest values were observed with the applications of 200 and 400 mg L⁻¹, which decreased leaf area by 36.9% and 44.2% respectively in relation to the 0 mg L⁻¹ dose of ProCa (Figure 1B). In this way, the effect of reducing the leaf area of the seedlings caused by the ProCa concentrations may be due to the reduction of leaf size.

Similarly to the total leaf area, the dry mass of the aerial part of the 'Aromas' strawberry seedlings decreased with increased ProCa concentrations (Figure 1C). The greatest reduction was observed with the application of 400 mg L⁻¹ of ProCa, which decreased the aerial part dry mass by 45.07% in relation to the control seedlings. This reduction of the leaf aerial parts observed in the seedlings with ProCa application, and especially in the highest concentration tested in this experiment (400 mg L⁻¹), possibly occurred due to the hormonal regulation exerted by ProCa. That is, when this phyto regulator is applied, it induces a reduction of GA levels that inhibits cell elongation and leaf growth (PEREIRA et al., 2016).

Chlorophyll *a* (Figure 1D), chlorophyll *b* (Figure 1E), and total chlorophyll (Figure 1F) content decreased with increasing concentrations of ProCa. Thus, ProCa in this experiment altered the concentration of pigments involved in photosynthesis, since chlorophyll *a* is the pigment

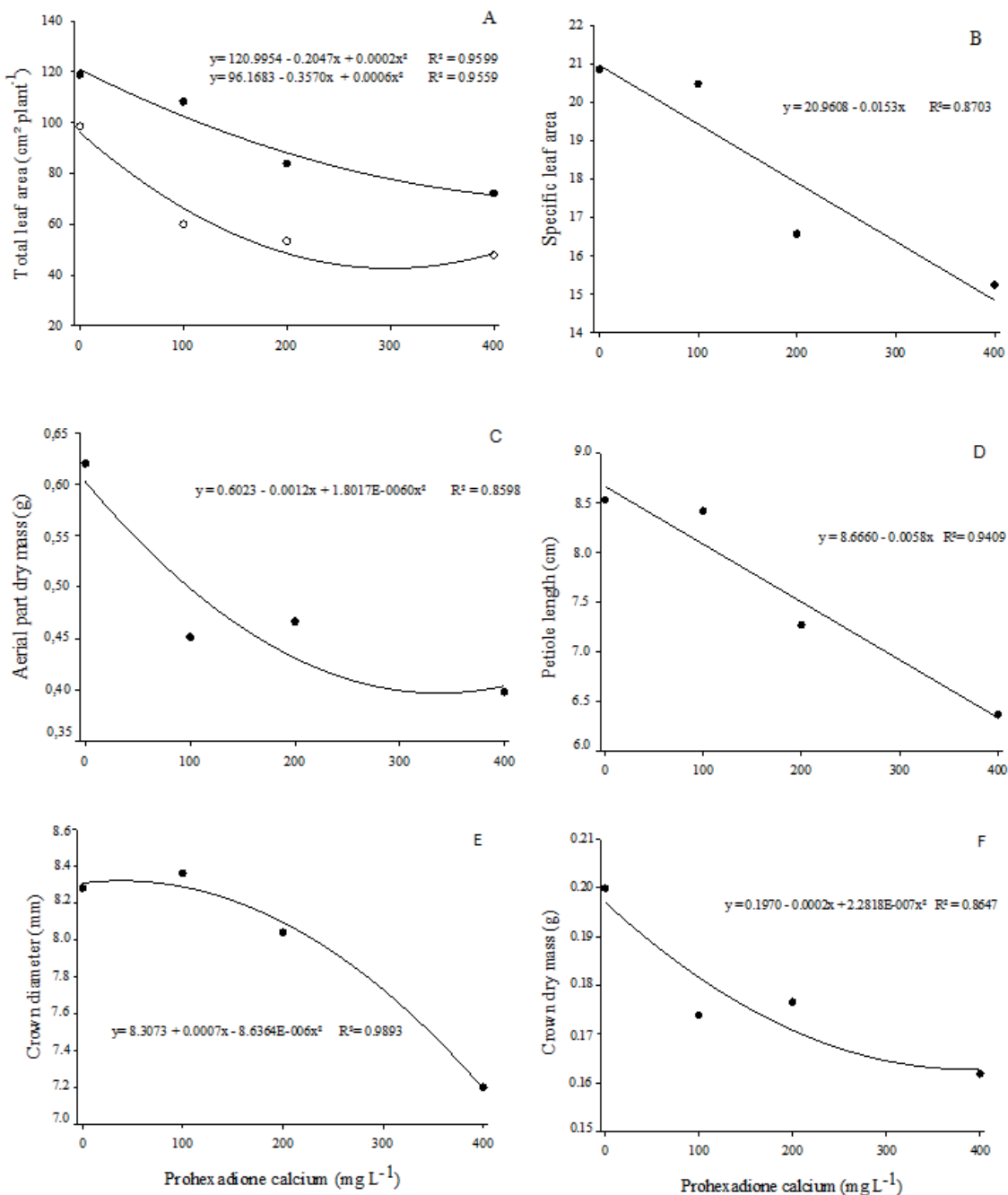
used to carry out the photochemical phase of photosynthesis and chlorophyll *b*, together with other pigments, aids in the absorption of light and the transfer of radiant energy to the reaction centers (TAIZ et al., 2017). In addition to the effect of ProCa on chlorophyll content, Pereira et al. (2016) verified that the use of this phyto regulator also causes physiological changes in strawberry seedlings, since it changes the photosynthetic activity of the leaves.

The percentage of survival, crown diameter, crown dry mass, and specific leaf area in 'Aromas' strawberry seedlings grown on substrate were not influenced by ProCa concentrations or period of application. According to Privé et al. (2006), response to the use of ProCa depends on the seasonal pattern of aerial part growth, which is influenced by cultivar, plant management, and environmental factors.

In this study, average survival values of 96.34%, average crown diameter of 8.96 mm, crown dry mass of 0.23 g, and specific leaf area of 18.29 were found in 'Aromas' strawberry seedlings submitted to the application of ProCa. Although ProCa reduces the aerial part of the strawberry seedlings, this phyto regulator did not reduce the crown diameter or the crown dry mass, which are important parameters for evaluation of seedling quality, since they are positively correlated with productive potential (COCCO et al. al., 2015).

In the cultivar 'Camarosa', there were interactions between concentrations and period of application of ProCa for the variable total leaf area (Figure 2A). The more ProCa the plants received, the lower the values of total leaf area in both periods of application. However, the greatest reduction occurred when the ProCa was applied at 30 days after the planting period of the rooting stolon. Reductions in the total leaf area of 39.06, 45.86, and 51.45% concentrations of ProCa of 100, 200, and 400 mg L⁻¹ respectively, when compared to the plants that did not receive the phyto regulator.

Figure 2. Total leaf area (A), specific leaf area (B), aerial part dry mass (C), petiole length (D), crown diameter (E), and crown dry mass (F) in response to the application of prohexadione calcium concentrations in ‘Camarosa’ strawberry seedlings cultivated in substrate. Embrapa Temperate Climate, RS, 2017.



When ProCa was applied at 20 days after the planting period of the rooting stolon of ‘Camarosa’ strawberry seedlings, the greatest reductions in the total leaf area occurred in plants that received 200 and 400 mg L⁻¹ ProCa, which were 29.42% and 39.23%, respectively, when compared to

the controls seedlings. Pereira et al. (2016) also observed a reduction of 32%, 26%, and 17% in the total leaf area in the ‘Camarosa’ strawberry seedlings, in response to ProCa 200, 400, and 800 mg L⁻¹ concentrations respectively, when applied at 20 days after the planting period of rooting stolon.

The variables specific leaf area, aerial part dry mass, petiole length, crown diameter, and crown dry mass in the 'Camarosa' strawberry seedlings were influenced by ProCa concentrations. Specific leaf area of seedlings, which is the relationship between total leaf area and number of leaves, decreased linearly with increasing ProCa concentration and the smallest specific leaf area was observed in the 'Camarosa' seedlings that received 400 mg L⁻¹ of ProCa (Figure 2B).

The increase in ProCa concentration reduced the aerial part dry mass of the 'Camarosa' strawberry seedlings (Figure 2C). Regardless of the tested concentration, the dry mass of the aerial part of the strawberry seedlings that received the phytoregulator was considerably lower than that of the seedlings that did not.

The petiole length of the 'Camarosa' strawberry seedlings was reduced following the application of ProCa (Figure 2D). The concentration of 400 mg L⁻¹ of ProCa provided the smallest value of petiole length in the seedlings, with an average of 6.37 cm and a reduction of 25.05% in relation to the control treatment. Similar responses were found by Pereira et al. (2016), who obtained a reduction of 24 and 58% in the petiole length of 'Camarosa' strawberry seedlings with the application of 200 and 400 mg L⁻¹ of ProCa, respectively.

The crown diameter (Figure 2E) and crown dry mass (Figure 2F) decreased with increasing ProCa concentration. In both variables, the reduction was greater when the 400 mg L⁻¹ ProCa concentration was used. At this concentration, the crown diameter presented an average value of 7.2 mm, which was lower than that found in bare rooted seedlings, in which the diameter of the crown should be greater than 8 mm (COCCO et al., 2011). However, for seedlings produced in substrate, as is the case of this study, we do not yet have a recommendation of adequate diameter.

The period of application of ProCa in 'Camarosa' strawberry seedlings affected the crown diameter

and amount of chlorophyll *a* and total leaf area. Both variables had the highest values when the application was made at 20 days after the planting period of rooting stolon. In this study, average values of 8.53 mm for crown diameter, 362.1 for chlorophyll *a* content, and 501.84 for total chlorophyll content were observed following the application of ProCa at 20 days after the planting period of the rooting stolon of the seedlings of the cultivar 'Camarosa'.

The different ProCa concentrations did not influence the survival percentage of 'Camarosa' strawberry seedlings, nor did the chlorophyll *a*, *b*, or total content in the leaves. For this cultivar, the average survival values of the seedlings were 80.72%, after application of ProCa. The chlorophyll *a*, *b*, and total content in 'Camarosa' strawberry leaves submitted to ProCa application at 100, 200, and 400 mg L⁻¹ presented average values of 343.46, 139.61, and 473.51, respectively.

In both cultivars in this study, the application of ProCa did not cause statistical differences for the percentage of survival of the seedlings, indicating that the concentrations and the periods of application of ProCa had no phytotoxic effect. This result corroborates with those of Pereira et al. (2016), in which the application of ProCa in 'Camarosa' strawberry seedlings at concentrations of 0, 25, 50, 100, 200, 400, and 800 mg L⁻¹ had no effect on plant mortality.

Concentrations from 100 mg L⁻¹ of ProCa reduce the vegetative growth of 'Camarosa' and 'Aromas' strawberry seedlings, mainly the leaf area. The concentrations of 200 and 400 mg L⁻¹ of ProCa reduced the vegetative growth of the seedlings more markedly. However, this greater reduction of the leaf area may have an effect in reducing the photosynthetic rate, which may interfere with the development of this change in the field. In this sense, it is necessary to perform further studies that must be carried out after transplanting the seedlings to the field to investigate the possible impacts on aspects of production and fruit quality.

Conclusion

The application of prohexadione calcium at concentrations from 100 mg L⁻¹ reduces the vegetative growth of ‘Aromas’ and ‘Camarosa’ strawberry seedlings grown on substrate.

The period of application of ProCa influences the petiole length in plants of ‘Aromas’ strawberry and in the total leaf area in ‘Camarosa’ strawberry.

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