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Successive applications of gibberellic acid to reduce flowering of 'Montenegrina' mandarin in alternate bearing

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Abstract – The objective of this work was to evaluate the number of successive applications of gibberellic acid (GA_3), during the autumn-winter period, for its effect on the reduction of flowering, in the subsequent spring after periods of low fruit load (off-year), in 'Montenegrina' mandarin trees (*Citrus deliciosa*). Sequential applications at 40 mg L^{-1} GA_3 were tested from one to four times, at 21-day intervals, beginning in the end of May. The experiment was carried out in a randomized complete block design, with four treatments and a control, four replicates, and one plant per experimental unit. Flowering, sprouting, types of shoots, fruit set, and fruit diameter were evaluated. The fruit set increases exponentially with the reduction of flowering intensity. The use of GA_3 in two, three, or four sequential applications, from May to June, May to July, and from May to August, at 21-day intervals, reduces the intensity of flowering and sprouting of alternate bearing plants in the subsequent spring, increasing mixed shoots and reducing floral shoots. The use of four sequential applications of 40 mg L^{-1} GA_3 promotes a great increase in the frequency of single flower terminal leafy shoots and favors the increase of fruit fixation and size.

Index terms: *Citrus deliciosa*, floral induction, gibberellin, plant growth regulators.

Aplicações sucessivas de ácido giberélico para redução de florescimento de tangerineira 'Montenegrina' em alternância de produção

Resumo – O objetivo deste trabalho foi avaliar o número de aplicações sucessivas de ácido giberélico (GA_3), no período de outono-inverno, quanto a seu efeito na redução do florescimento, na primavera subsequente após períodos de baixa carga de frutos (ano “off”), em tangerineiras 'Montenegrina' (*Citrus deliciosa*). As aplicações sequenciais a 40 mg L^{-1} de GA_3 foram testadas de uma a quatro vezes, com intervalos de 21 dias, com início no final de maio. O experimento foi realizado em delineamento de blocos ao acaso, com quatro tratamentos e controle, quatro repetições e uma planta por unidade experimental. Floração, brotação, tipos de brotos, frutificação e diâmetro dos frutos foram avaliados. A frutificação diminui exponencialmente com o aumento da intensidade de floração. O uso de GA_3 em duas, três ou quatro aplicações sequenciais, de maio a junho, maio a julho e maio a agosto, reduz a intensidade de floração e brotação em plantas com produção alternada na primavera subsequente, o que aumenta os brotos mistos e reduz os brotos florais. O uso de quatro aplicações sequenciais de 40 mg L^{-1} de GA_3 promove grande incremento na frequência de brotos campaneiros e favorece o aumento da fixação e do tamanho dos frutos.

Termos para indexação: *Citrus deliciosa*, indução floral, giberelina, reguladores de crescimento vegetal.

Introduction

Brazil is the second largest citrus producer in the world, with more than 19 million tonnes in 2019. The cultivation of mandarin trees in the country (although less expressive than that of orange trees) has a great socioeconomic importance, with production of approximately one million tonnes. Other largest mandarin producers are China (19.71 million tonnes), Spain (1.83 million tonnes), and Turkey (1.40 million tonnes) (FAO, 2021).

The 'Montenegrina' mandarin tree (*Citrus deliciosa* Tenore) is a cultivar selected in the municipality of Montenegro, state of Rio Grande do Sul (RS), Brazil. This cultivar stands out as the main one among the mandarin trees in RS (Brugnara et al., 2012). The state ranks third in the production of mandarins in the country, with 90,852 tonnes of citrus fruit annually, behind the states of Minas Gerais (138,604 tonnes) and São Paulo (96,325 tonnes) (IBGE, 2020).

Mandarin trees of the species *C. delicios*a show a tendency to alternate production, with irregular yield between successive harvests. It is a process that causes damage to the production system (Agustí et al., 2020). Several studies have already been carried out on the pruning, hand thinning, and chemical thinning of fruit (Rosa et al., 2012; Gonzatto et al., 2016) in 'Montenegrina' mandarin trees. These management techniques applied in on-year aim mainly to reduce alternate bearing and to increase fruit size. However, they are not always sufficient to reduce the problem. In on-years, later flowering can also be affected because of the presence of fruit (Muñoz-Fambuena et al., 2011; Nishikawa et al., 2012; Shalom et al., 2012). This is due to the repression of the *Citrus FLOWERING LOCUS T* (*CiFT2*), that is one of the main genes responsible for floral induction. This repression is caused by the expression of the gene CcMADS19 when in the presence of fruit (Agustí et al., 2020). According to Ramos-Hurtado et al. (2006), in experiments carried out in edaphoclimatic conditions similar to the ones of the present work, the estimated period of floral induction for 'Montenegrina' mandarin trees would be in July.

The alternate bearing is a complex process that needs to be tackled (Mesejo et al., 2019). In addition, there is a lack of information on the ecophysiological behavior of the flowering of 'Montenegrina' through successive applications of gibberellic acid (GA_3) in different periods. It is important that sequential applications are tested, since determining the period of floral induction is difficult. *C. deliciosa* is a little studied species regarding the use of gibberellins in flowering.

In a review of studies using GA_3 in citrus, Garmendia et al. (2019) found only one report on *C. deliciosa*, by which the reduction of this species flowering was not possible with this phytoregulator (Ramos-Hurtado et al., 2006).

Growth regulators in citrus have been used to alter flowering, improve fruit set, thinning, and abscission (Agustí & Primo-Millo, 2020). In general, these management strategies are focused on practices developed in the on-year, with few studies considering the off-year as a way to manage alternate bearing. Therefore, more studies are necessary for the manipulation in the autumn-winter period with low-yield crops (off-years) as a complementary strategy. This management strategy to balance production can be performed through the inhibition of flowering with the use of GA_3 (Muñoz-Fambuena et al., 2012).

The objective of this work was to evaluate the effect of the number of successive applications of gibberellic acid, in the autumn-winter period, on the reduction of spring flowering following the low fruit load harvest (off-year) in 'Montenegrina' mandarin trees.

Materials and Methods

The experiment was carried out in an experimental orchard managed with a conventional system, located in the Horticulture and Forestry Sector of the agricultural experimental station of the Universidade Federal do Rio Grande do Sul (EEA/UFRGS), in the municipality of Eldorado do Sul (30°05'32"S, 51°40'20"W, at 55 m altitude), in the state of Rio Grande do Sul, Brazil. According to the Köppen-Geiger's classification, the climate of the region is of the Cfa type, with annual mean temperature of the coldest month below 18°C and temperature of the warmest month greater than 22°C, and monthly, well distributed rainfall, between 100 and 170 mm (Alvares et al., 2013; Valério et al., 2018). The 30-year-old orchard consists of *Citrus deliciosa* 'Montenegrina' grafted on *Poncirus trifoliata* and subjected to rejuvenation pruning in 2015. This orchard shows an intense alternate bearing. The soil is classified as Argissolo Vermelho distrófico típico

(Santos et al., 2018), which corresponds to a Rhodic Ultisol (Soil Survey Staff, 2014). The trees are 4 m row apart on the line, and those selected for the application of the treatments had no fruit load (off-year).

A rate of 40 mg L^{-1} of gibberellic acid (GA_3, ProGibb 400, 40% m/m, Sumitomo Chemical do Brasil Representações LTDA., São Paulo, SP, Brazil, manufactured in the U.S.A.) were used, which is the most used concentration for this purpose (Gravina, 2007). Zero to four successive applications were performed at intervals of 21 days. The applications started in May and ended in August. The experiment was carried out in a randomized complete block design, with five treatments, four replicates, and one plant per plot. The five treatments were: a control without GA_3 application; one GA_3 application on 05/24/2018; two GA_3 applications, one on 05/24/2018 and other on 06/15/2018; three GA_3 applications, one on 05/24/2018, other on 06/15/2018 and the other on 07/10/2018; and four GA3 applications, one on 05/24/2018, and the others on 06/15/2018, 07/10/2018, and 08/03/2018.

The GA_3 applications were performed with a manual backpack sprayer (Guarany, Itu, SP, Brazil). The calibration of the time and volume of application was performed according to the flow rate of the sprayer nozzle, considering the volume of the canopy of the plants. The basis for calculating the volume was 4.0 L per tree, providing coverage to the plants until the point of runoff. The spray pressure was 0.5 MPa. In addition, a nonionic spreader-sticker was used (polyether copolymer and silicone 1,000 g L^{-1}, Silwet L-77 Ag, Momentive Performance Materials Indústria de Silicones LTDA., Waterford, U.S.A.), pH reducer (phosphoric acid, 30% P_2O_5, Quimifol P30W, Tietê, SP Brazil), with the spray adjusted to pH 4.5 prior to the GA_3 dilution.

The BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) scale for citrus (Agustí et al., 1995) was used to monitor phenology and spring sprouting. For this purpose, four branches were marked, one per quadrant, with at least 150 nodes, totaling at least 600 nodes per tree. The beginning of flowering occurred in September 18, 2018 and ended in October 3, 2018. At stage 65 of the phenological scale (in September 23, 2018), sprouting frequencies and the intensity of flowering (flowers 100 per nodes) were evaluated in the same branches marked to monitor phenology.

The different types of shoots were grouped into three categories, similarly to those was performed by Muñoz-Fambuena et al. (2012): vegetative shoots (Sv) (≥1 leaf and 0 flower); floral shoots (Sf) (0 leaf and ≥1 flower); and vegetative and floral shoots (Sm) (≥1 leaf and ≥1 flower). In addition, single flower terminal leafy shoots (Smt), which are those with ≥3 leaves and 1 flower, a subgroup of Sm, were also counted. The frequency of occurrence of the different types of shoots was presented in relation to their total at the phenological stage 65. In addition, the frequency of nodes with multiple (two or more) shoots (Nm), in relation to the number of sprouted buds, was evaluated.

Fruit counts were performed at 30, 72, 86, and 107 days after anthesis (DAA), according to the BBCH scale for citrus, calculating fruit set (Fs) on each date, by means of the ratio between the number of fruit and the number of flowers, expressed as a percentage. Fruit diameter was measured at 107 days after full flowering. Eight fruit were measured in each experimental unit (two fruit per marked branch).

The data were subjected to the analysis of variance via mixed models, using the SAS 9.4 PROC MIXED routine (SAS Institute Inc., Cary, NC, USA), and complemented by the Tukey's test, at 5% probability. Pearson's linear correlation analysis of part of the studied variables was also performed, using the software RStudio (RStudio Team, 2018).

Results and Discussion

Sprouting and flowering were reduced by 29% and 37%, respectively, after two applications of gibberellic acid (GA_3), in comparison with the control treatment (Figure 1). However, treatments with more than two applications of the phytoregulator did not show differences for both variables. The significant reduction of sprouting and flowering after two applications of GA_3, in comparison with the control treatment, is similar to the results found by Muñoz-Fambuena et al. (2012), who evaluated 'Salustiana' orange trees (*Citrus sinensis* (L.) Osbeck) with an application of 40 mg L^{-1} of GA_3 during the floral induction period. In addition, after two applications, no significant differences were observed for sprouting and flowering. This behavior may be linked to the period of floral induction, since with two applications there was a significant reduction of the variables mentioned in relation to the control treatment. According to same authors the inhibition of flowering by gibberellic acid is due to the repression of the expression of the *Citrus FLOWERING LOCUS T* (*CiFT*) gene.

Sartori et al. (2007) also worked with 'Montenegrina' in the same region, in Southern Brazil, and concluded that the application of 10 mg L^{-1} GA_3 in the fall, did not inhibit flowering. Ramos-Hurtado et al. (2006), in a similar study with 'Montenegrina', using four concentrations of GA3 (0, 20, 40, and 60 mg L^{-1}), but with single applications in three different dates (April, May, and June), attained no flowering inhibition results. The same fact occurred in the present study with the single application of GA_3, and no significant differences were observed. One possible explanation is the distribution of GA_3 applications, that is, performing sequential applications of gibberellin would allow of a greater chance of success in the reduction of flowering. This is because the time of induction and floral differentiation is not quite known for this cultivar (Ramos-Hurtado et al., 2006).

The application of GA_3 to decrease flowering is recommended when floral induction occurs, which is generally in the fall and early winter (Gravina, 2007; Mesejo et al., 2019). However, determining the period of floral induction is difficult, since the morphological differences between vegetative and reproductive buds are observable only during floral differentiation (Iglesias et al., 2007). In an experiment carried out in the same region with 'Montenegrina', Ramos-Hurtado et al. (2006) concluded that, for this cultivar, floral induction occurs in July.

The application of GA_3 increased the frequency of Sm in relation to the control treatment (Figure 2 A), and the frequency of this type of sprouting was maximized when more than three applications of GA_3 were used, reaching 57.5 and 71.3% of frequency, for three and four applications, respectively.

The Sf had their frequency decreased after two applications of GA_3 (Figure 2 B). This decrease continued progressively up to four applications, when the occurrence of this type of sprout was 17%. This is a significant reduction in comparison with the control treatment, which showed 81% of occurrence. The Smt (Figure 2 C) showed a 2.6-fold increase after two applications of GA_3. However, the use of four applications provided an increase of 9.6 times in relation to the control treatment. The Smt then accounted for 54.3% of the shoots.

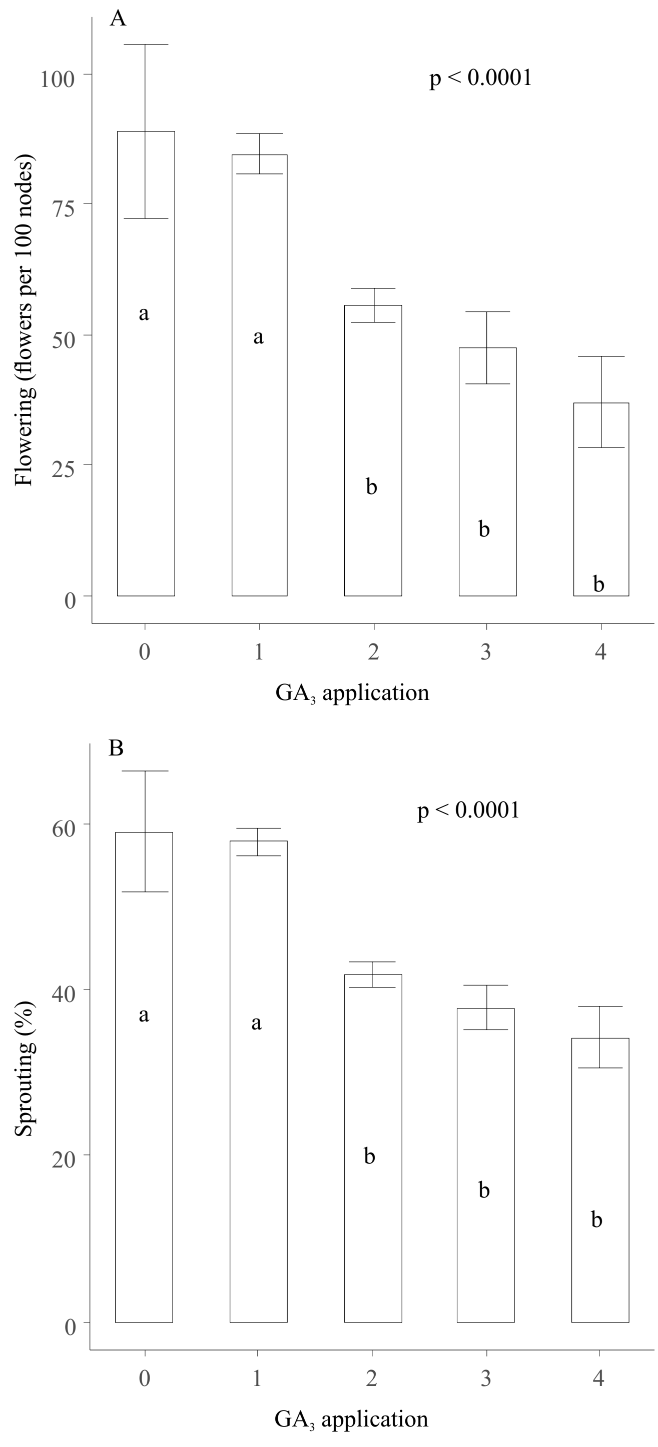

Figure 1. Flowering frequency (A) and sprouting (B) of 'Montenegrina' mandarin (*Citrus deliciosa*) trees subjected to 40 mg L^{-1} gibberellic acid (GA_3), from zero (control) to four applications in the autumn-winter period, at 21-day intervals. Each value is the average ± standard error of 4 trees and 4 branches per tree. Means followed by equal letters, in the bars, do not differ by the Tukey's test, at 5% probability.

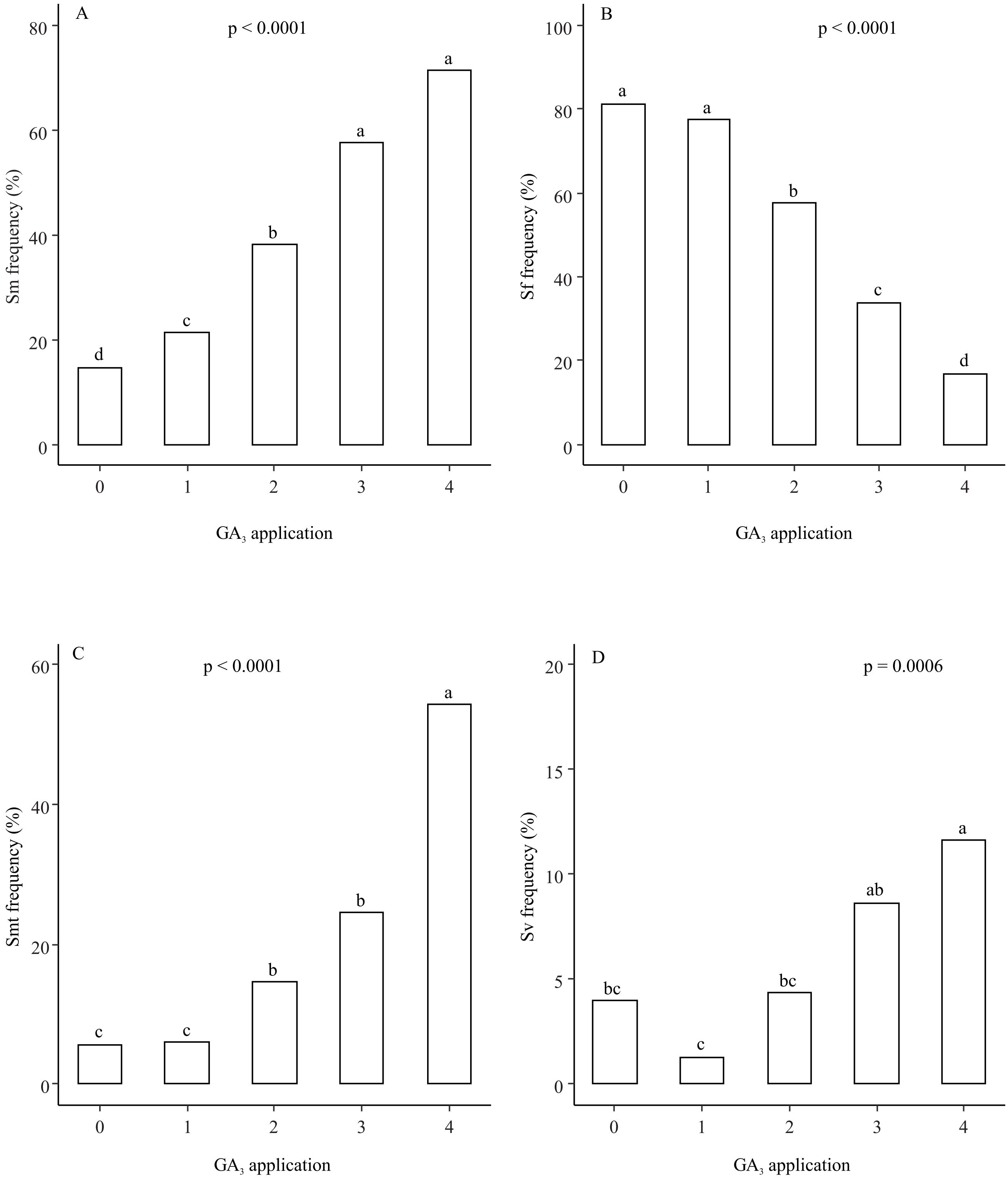

Figure 2. Frequency of vegetative and floral shoots (Sm) (A), floral shoots (Sf) (B), single flower terminal leafy shoots (Smt) (C), and vegetative shoots (Sv) (D) of 'Montenegrina' mandarin (*Citrus deliciosa*) trees subjected to 40 mg L^{-1} gibberellic acid (GA_3), from zero (control) to four applications in the autumn-winter period, at 21-day intervals. Means followed by equal letters, in the bars, do not differ by the Tukey's test, at 5% probability.

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The increase of the number of GA_3 applications promoted an increase in Smt (Figure 2 C), as well as a higher occurrence of Sv (Figure 2 D). This represents an improvement of the sprouting quality with the use of two or more applications of GA_3, that is, Sm are more likely to fix fruit (Iglesias et al., 2007). It is important to note that in 'Ortanique' (*C. sinensis* × *C. reticulata*) tangor trees, the Smt fix almost 40% of fruit, while the Sm fix approximately 17%. The Sf are the ones that are less fixed in this tangor tree, fixing less than 10% (Cunha Barros & Gravina, 2006). In the present work, it was also possible to observe higher Fs resulting from the action of gibberellins, but sequential applications of GA_3 were necessary to promote greater fruiting.

The increase of Smt may be associated with a higher concentration of GA_3 that was promoted by four applications of the phytoregulator and also by the temporal distribution of the applications, reducing the differentiation of axillary flowers in the bud. This is a possibility because of terminal flowers differentiated earlier than axillary flowers in multiflowered shoots (Lord & Eckard, 1985).

The percentage of Nm decreased by 60% after two applications of GA_3, in comparison with the control treatment (Figure 3), allowing of a more balanced distribution of shoots per sprouted node. In addition, there was a positive correlation ($r = 0.90$ and 0.85 respectively) of Nm with flowering and sprouting (Figure 4).

The occurrence of multiple shoots (Nm) was identified in this cultivar as reaching more than 40% of the sprouted nodes. This value is much higher than those observed by Agustí et al. (1992) in 'Satsuma' mandarin trees and in 'Salustiana' orange trees, which showed an occurrence of double shoots close to 4% and 3%, respectively.

The Fs at 72, 86, and 107 DAA (Table 1) was significantly higher with four applications of GA_3. However, Fs at 30 DAA (before the physiological drop of fruit) was not affected by the number of applications. The largest fruit diameters were obtained with two and four applications, which differed from the control. This can be partially explained by the positive correlation ($r = 0.29$) with Sm (Figure 4).

According to Gravina (2007), the single GA_3 application of 40 mg L^{-1} on 'Montenegrina', in June, in Uruguay, provided a considerable decrease of flowering, an increase of Smt and Sv, and a decrease of Sf; an increase of Fs was also observed and it was justified by the improvement of sprouting quality. Similar results were obtained in this work regarding sprouting quality and Fs (at 72, 86, and 107 DAA). In addition, there was an increase of the fruit diameter, but more applications of GA_3 were necessary to obtain these results, which can be attributed the age of the orchard under study and its intense alternate bearing.

The increase of fruit diameter attained with the applications of GA_3 is an important result (Table 1). Applications of GA_3 (10 mg L^{-1}) during anthesis increased the number of cell layers in the pericarp and juice sacs in the ovary of 'Satsuma' mandarin trees (Mesejo et al., 2016). Other authors also reported that exogenous applications of gibberellic acid promote the activity of the enzyme Rubisco, which increases its carboxylate activity (Manjili et al., 2012); therefore, there may be a relationship with the increase of fruit set and fruit diameter. However, there is no information on the period of residual effect of exogenous application of gibberellic acid in the cultivar.

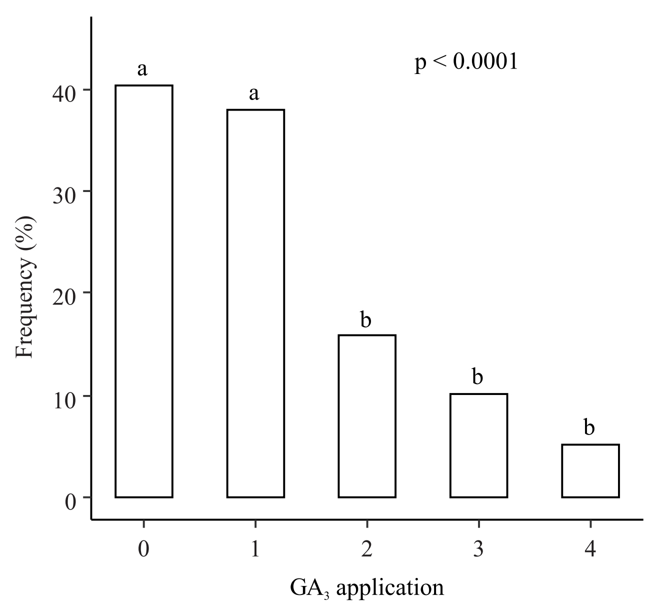

Figure 3. Frequency of nodes with multiple shoots, in relation to the sprouted nodes (Nm) of 'Montenegrina' mandarin (*Citrus deliciosa*) trees subjected to 40 mg L^{-1} gibberellic acid (GA_3), from zero (control) to four applications in the autumn-winter period, at 21-day intervals. Means followed by equal letters, in the bars, do not differ by the Tukey's test, at 5% probability.

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Fruit set showed a significant negative correlation (Figure 4) with sprouting, flowering, Nm, and Sf. That is, the greater the occurrence of sprouting, flowering, Nm, and Sf, the lower the Fs (at 107 DAA). The absence of leaves in Sf stands out as an important factor and a possible relation between the presence of leaves in the shoots and the nutrition of fruit, since the Sm showed a significant positive correlation with Fs (at 107 DAA). Flowering had a significant positive correlation with Nm.

The increase of the Smt occurrence was positively correlated with the increase of fruit set (Figure 4). The fruit diameter, however, showed no correlation with the other variables, but had a significant increase with two and four applications of the phytoregulator (Table 1).

When compared to fruit set data, flowering showed a hyperbolic decay behavior (Figure 5). In this way, the Fs decreased dramatically as flowering intensity increased up to 50 flowers per 100 nodes. At flowering intensities greater than 50 flowers per 100 nodes, the Fs reduced slightly and linearly, averaging 3.76±1.46%, remaining at low values. Above 100 flowers per 100 nodes, the Fs reduced to an average of 2.89±1.46%. A lower flowering frequency (40 flowers per 100 nodes) occurred when two, three, or four applications were performed, and the Fs increased exponentially (Figure 5).

Working with 'Nova' mandarin, Gravina (2007) showed similar results for flowering and Fs (Figure 5), reporting that this relationship can be divided into two phases. The transition values for the present study were around 50 flowers per 100 nodes, meaning that the decreasing flowering values, from this value, increase the Fs. Gravina (2007) mentions values between 80 and 90 flowers per 100 nodes, as a transient flowering frequency range between the two phases.

The divergences in the results regarding the number of applications and its efficiency in reducing flowering can be explained by the period in which it was performed. Early applications of GA_3 (May) do not have the expected effect on the subsequent spring flowering. Only treatments involving applications of GA_3 in the middle of June (treatment with two, three, and four successive applications of GA_3) were able to inhibit the sprouting and flowering of 'Montenegrina' mandarin trees (Figure 1). Therefore, differently from the period proposed by Ramos-Hurtado et al. (2006), the flowering-inducing time in 'Montenegrina' starts in June, as there is no reduction of flowering with single applications of GA_3 between April and early June.

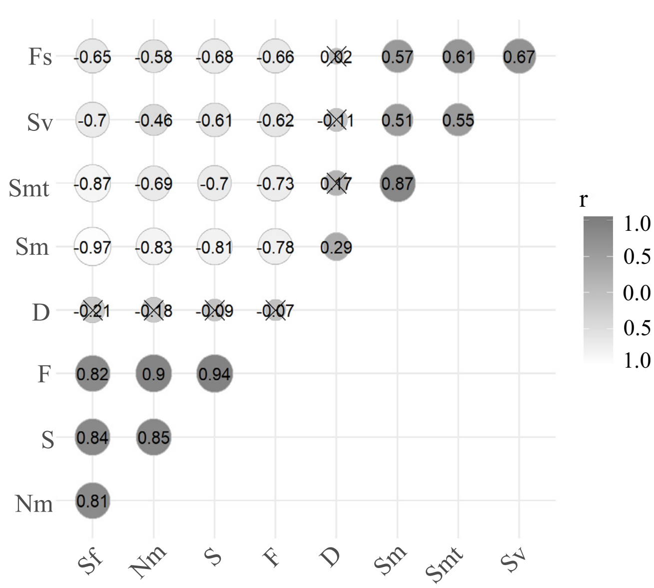

Figure 4. Pearson's linear correlation coefficients (r) between the main variables for sprouting (S), flowering (F), types of shoots (Sv, vegetative shoots; Sf, floral shoots; Sm, vegetative and floral shoots; Smt, single flower terminal leafy shoots), nodes with multiple shoots (Nm), and fruit set (Fs) of 'Montenegrina' mandarin (*Citrus deliciosa*) trees subjected to 40 mg L^{-1} gibberellic acid (GA_3), from zero (control) to four applications in the autumn-winter period, at 21-day intervals. Coefficients that have been hatched with × have p>0.01 and are considered nonsignificant.

Table 1. Fruit set (Fs) at 30, 72, 86, and 107 days after anthesis (DAA) and fruit diameter (D) of 'Montenegrina' mandarin (*Citrus deliciosa*) trees subjected to 40 mg L^{-1} gibberellic acid (GA_3), from zero (control) to four applications in the autumn-winter period, at 21-day intervals[1].

GA_3 application	Fs (%)				D (mm)
	30 DAA	72 DAA	86 DAA	107 DAA	
0	69.4	5.3b	4.9b	4.3b	25.6b
1	73.3	4.0b	3.7b	3.5b	26.1ab
2	75.3	6.6b	6.4b	6.2b	28.0a
3	74.3	7.4b	6.9b	6.6b	27.7ab
4	81.6	12.5a	11.7a	11.4a	28.1a
p	0.2905	0.0001	0.0001	<0.0001	0.0038

[1]Means followed by equal letters, in the columns, do not differ by the Tukey's test, at 5% probability. Eldorado do Sul, RS, 2018.

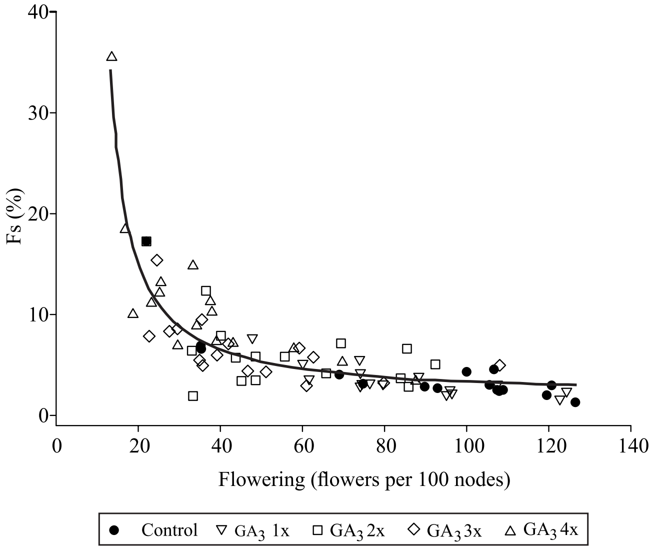

Figure 5. Relationship between flowering intensity (F) and fruit set (Fs) at 107 days after anthesis (DAA), in 'Montenegrina' mandarin (*Citrus deliciosa*) trees subjected to 40 mg L^{-1} gibberellic acid (GA_3), from zero (control) to four applications in the autumn-winter period, at 21-day intervals.

Conclusions

1. Two until four sequential applications of 40 mg L^{-1} of gibberellic acid (GA_3) from May to June, May to July, and May to August, at 21-day intervals are effective for reducing the intensity of flowering and sprouting in the subsequent spring alternate bearing 'Montenegrina' mandarin (*Citrus deliciosa*) trees; these applications increase vegetative and floral shoots and reduce flower shoots in the spring.
2. The use of four sequential applications of 40 mg L^{-1} of GA_3 from May to August, at 21-day intervals, promotes a great increase of the frequency of single flower terminal leafy shoots, increasing the fixation of fruit and their size.
3. The fruit set increases exponentially with the reduction of the flowering intensity.

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