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Successive applications of gibberellic acid in alternate bearing mandarins

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Abstract - Gibberellins can promote the inhibition of floral induction in citrus in order to reduce alternate bearing. However, not much is known about the responsiveness of *Citrus deliciosa* to exogenous applications of gibberellic acid. Furthermore, the successive application of gibberellins is barely studied. The main objective of this work was to evaluate the effect of successive applications of gibberellic acid (GA_3) on the reduction of spring flowering subsequent to low fruit load periods (off-year) in 'Montenegrina' and 'Rainha' mandarin trees, in southern Brazil. The treatments consisted of one to four successive applications of GA_3 (40 mg L^{-1}) with 21-day interval in off-year trees. Applications began in May and ended in July. There was a significant reduction in sprouting and flowering of 'Montenegrina' mandarin with more than two GA_3 successive applications compared to the control. To 'Rainha' mandarin, only two GA_3 applications reduced the sprouting and solely one GA_3 application was enough to reduce sprouting in relation to the control. GA_3 use reduced sprouting and flowering of Montenegrina and Rainha cultivars. The orchard age may be related to its level of alternate bearing and, therefore, to the greater or lesser sensitivity of gibberellin applications.

Index terms: plant growth regulators, citrus, *Citrus deliciosa* Tenore, flowering.

Aplicações sucessivas de ácido giberélico em tangerineiras em alternância de produção

Resumo - As giberelinas podem promover a inibição da indução floral em citros com intuito de reduzir a alternância de produção. Contudo, pouco se sabe sobre a responsividade de *Citrus deliciosa* às aplicações exógenas de ácido giberélico. Além disso, a aplicação sucessiva de giberelinas é pouco estudada. Desta forma, objetivou-se avaliar o efeito de aplicações sucessivas de ácido giberélico (AG_3) sobre a redução do florescimento primaveril subsequente a safras de baixa carga de frutos (ano off) em tangerineiras 'Montenegrina' e 'Rainha', no Sul do Brasil. Os tratamentos

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consistiram em uma a quatro aplicações sucessivas de 40 mg L^{-1} de AG_3, com intervalos de 21 dias, em plantas em ano off. O início das aplicações foi em maio, e o término, em julho. Houve redução significativa de brotação e de florescimento com mais de duas aplicações sucessivas de AG_3 para a tangerineira 'Montenegrina' em relação ao controle. Para a tangerineira 'Rainha', apenas com duas aplicações de AG_3, o florescimento foi reduzido, enquanto apenas uma aplicação já foi suficiente para reduzir a brotação em relação ao controle. O uso de AG_3 reduz brotação e florescimento das cultivares Montenegrina e Rainha. A idade do pomar pode estar relacionada ao seu nível de alternância produtiva e, desta forma, à maior ou à menor sensibilidade a aplicações de giberelinas.

Termos para indexação: Fitorreguladores, citros, *Citrus deliciosa* Tenore, florescimento.

Gibberellins use in citrus is highly widespread, which improves fruit production and quality (GARMENDIA et al., 2019). As a plant growth regulator, gibberellins are applied in citrus floral induction with the aim of inhibiting flowering and to reduce the alternate bearing. Alternate bearing is a complex problem, present in several fruit species, including some citrus species. This procedure is performed in low fruit load periods (off-year), inhibiting the excessive flowering that would occur in the subsequent cycle. Gibberellic acid (GA_3) as an inhibitor of floral induction in citrus is widely documented (MONSELISE; HALEVY, 1964; GOLDSCHMIDT; MONSELISE, 1972; GUARDIOLA et al., 1982; DAVENPORT, 1990; KOSHITA et al., 1999; MUÑOZ-FAMBUENA et al., 2012).

The timing of gibberellin application is a decisive factor to obtain the expected result. The best results are achieved at the end of November and mid-December for the northern hemisphere (GUARDIOLA et al., 1982; AGUSTÍ, 2003). The use of gibberellins can reduce flowering even after the start of differentiation (AGUSTÍ, 2003). The differentiation involves anatomical and morphological transition from the vegetative meristem to the floral meristem (DAVENPORT, 1990; ALBRIGO; GALÁN-SAÚCO, 2004).

Several factors affect the flowering management of citrus cultivars with gibberellins including such as varietal characteristics, orchard management, harvest load, tree development stage and climatic conditions. The Montenegrina and Rainha (*Citrus deliciosa* Tenore) cultivars are late maturing. Even with seeds, they have a flavor and color that ensures the Brazilian market sales, providing higher prices than the other mandarins that have earlier harvest: 'Caí' and 'Pareci'. However, *C. deliciosa* late maturing cultivars show a great tendency to alternate bearing. Besides that, cultural practices to overcome this problem are aimed at the year-on of production, such as pruning and thinning (RODRIGUES et al., 1998; SARTORI et al., 2007; GONZATTO et al., 2016). Nonetheless, these procedures are not enough to completely overcome the problem.

Hence, experiments directed to specific genotypes and with edaphoclimatic conditions of interest become essential to achieve reproducible results in field conditions. Thereby, the objective of this study was to evaluate the effect of successive applications of gibberellic acid (GA_3) on the reduction of spring flowering subsequent to low fruit load periods (off-year) in 'Montenegrina' and 'Rainha' mandarin trees, in southern Brazil.

This work consisted of two experiments, both conducted in 2018. The first experiment was performed with 'Montenegrina' mandarin in an orchard located at 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. The orchard was 30-year-old, and the trees underwent rejuvenation pruning in 2015. The soil is classified as *Argissolo Vermelho distrófico típico* (SANTOS et al., 2018). Diversely, the second

experiment was accomplished with 'Rainha' mandarin, in a 5-year-old orchard, located in the municipality of Montenegro - RS (29º 37' 47" S and 51º 28' 34" W, at 23 m altitude). The soil of the orchard is classified as an *Argissolo Vermelho distrófico espessarênico* (SANTOS et al., 2018). In both experiments the plants were grafted on *Poncirus trifoliata*. According to the Köppen-Geiger's classification, the climate of the region is Cfa type, with anual mean temperature below 18°C in the coldest month and greater than 22°C in the warmest month. Moreover, a well distributed rainfall, between 100 and 170 mm is also a feature of Cfa type (ALVARES et al., 2013; VALÉRIO et al., 2018).

The selected trees were in the year-off, (i.e. with zero or almost zero fruit load) Gibberelic acid was employed in a concentration of 40 mg L^{-1} (GA_3, ProGibb 400, 40% m/m, Sumitomo Chemical do Brasil Representações LTDA., São Paulo, SP, Brazil, manufactured in the U.S.A.) (GRAVINA, 2007). One to four successive applications were performed, with intervals of 21 days. Applications began in May and ended in July or early August. The applications in 'Montenegrina', occur on: 05/24/2018, 06/15/2018, 07/10/2018 and 08/03/2018. On the other hand, to 'Rainha',the applications occurred in the following dates: 05/21/2018, 06/09/2018, 06/30/2018 and 07/21/2018. Control plants did not receive GA_3 applications. Randomized blocks experimental design, with five treatments, four replications and one plant per experimental unit was accomplished The treatments were described as the following: control (without GA_3 application); only 1 application in May; 2 sequential applications (in May and June), 3 sequential applications (in May, June and July for 'Montenegrina'; and in May, beginning of June and end of June for 'Rainha'; and 4 sequential applications (in May, June, July and August for 'Montenegrina'; and in May, beginning of June, end of June and July for 'Rainha'). A spray mixture provided plant coverage until the point of runoff. In addition, a non-ionic 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.). Additionaly, a pH reducer (phosphoric acid, 30% P_2O_5, Quimifol P30W, Tietê, SP Brazil) was also employed to adjust spray water pH to pH 4.5 prior to the GA_3 dilution.

At stage 65 of the phenological scale BBCH (AGUSTÍ et al.,1995) (09/23/2018 in both experiments), sprouting frequencies and the intensity of flowering (flowers per 100 nodes) were evaluated in four branches, one per quadrant, with at least 150 nodes. In total, at least, 600 nodes per tree were evaluated. The shoots were classified as: single-flowering leafless shoots (SL) (0 leaf and 1 flower); multiple-flowering leafless shoots (ML) (0 leaf and >1 flower); single-flowering leafy shoot (SLY) (≥ 1 leaf and 1 flower); multiple-flowering leafy shoots (MLY) (≥ 1 leaf and > 1 flower); and vegetative shoots (VS) (≥1 leaf and 0 flower). In addition, single flower terminal leafy shoot (STLY) a subgroup of SLY, which has ≥3 leaves and 1 flower, were also counted.

Besides, in the 65 phenological stage, ten shoots of SL, STLY and MLY were demarcated in each experimental unit, in the 'Rainha' experiment and ten shoots of SL, STLY, MLY and SLM (unifloral shoots without leaves that occur in pairs or trios at the same node) in the 'Montenegrina' experiment. The fruits of the shoots were counted 71 days after full flowering (DAF) and 72 DAF, in 'Rainha' and 'Montenegrina', respectively, to determine the fruit set in each shoot type.

Furthermore, the fruit set was evaluated at 32 DAF (10/25/2018), 71 DAF (12/03/2018), 90 DAF (12/22/2018) and 105 DAF (01/06/2019) in 'Rainha' experiment. In the experiment with 'Montenegrina', fruit set were evaluated at 30 DAF (10/23/2018), 72 DAF (04/12/2018) and 107 DAF (01/08/2019). The evaluations of fruit set performed in October were related to phase 1 of fruit growth (before June drop) while the evaluations fullfiled in the beginning of December represent phase 2 of fruit growth (after June drop). Fruit set was calculated by the ratio between the number

of fruits and the number of flowers and expressed as a percentage.

All data were analyzed using the Rstudio® program. Data were submitted to analysis of variance and, previously, to the normality test of Shapiro-Wilk residues. To attend the assumption of normality, the variables SLY and MLY of the Rainha cultivar and VS of the Montenegrina cultivar were transformed to $\hat{y}^{1/2}$. The variables VS of 'Montenegrina' and MLY of 'Rainha', with non-normal residuals, were submitted to the Friedman test (package Stats). The test for means was performed using Tukey's test (HSD, $p < 0.05$) (Agricolae package).

There was a significant reduction in sprouting and flowering of 'Montenegrina' mandarin in which more than two successive applications of GA_3 were performed regarding the control. The reduction in flowering observed in relation to the control was 37.5%, 46.6% and 57.8% for two, three and four applications of GA_3, respectively. Otherwise, the behavior of 'Rainha' was erratic, because one application was enough to reduce the sprouting in relation to the control. However, for flowering reduction, two GA_3 applications were required in order to achieve a reduction of 28% in comparison with control (Figure 1). The reduction in sprouting and flowering to one or two applications of GA_3 is according to Muñoz-Fambuena et al. (2012) in which 'Salustiana' orange trees (*Citrus sinensis* (L.) Osbeck) with one application of 40 mg L^{-1} of GA_3, during the floral induction period, reduced the flowering and sprouting. The inhibition of flowering by gibberellic acid is due to the repression of the *Citrus Flowering Locus T* (*CiFT*) gene expression (MUÑOZ-FAMBUENA et al., 2012).

Shoot types were also influenced by GA_3 applications. In 'Montenegrina', the SLY frequencies were increased from two successive applications of GA_3 regarding control, while in SL the opposite effect occurred. The frequency of MLY and ML were not affected by treatments. The frequencies of VS and STLY were increased from three successive applications of GA_3 in relation to the control. Moreover, the trees that received four successive applications of GA_3 had more than half of their shoots of the STLY type. To 'Rainha' mandarin submitted to one application, there was an increase in the frequencies of SLY and STLY, while there was a reduction in the frequencies of SL. ML and VS frequencies were not affected by treatments. In 'Rainha', the highest frequency of STLY (20.4%) was observed with one application of GA_3 (Table 1).

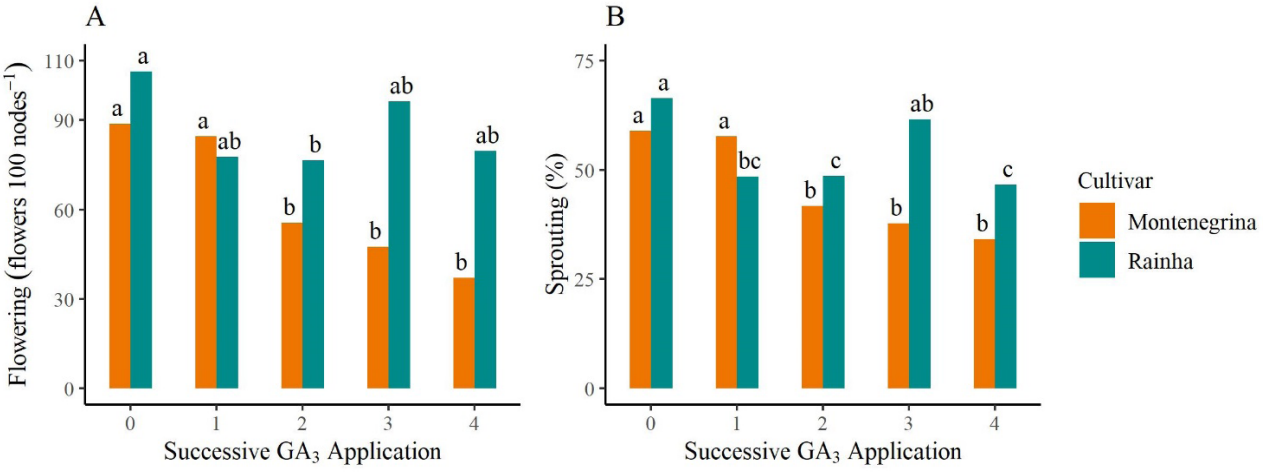

Figure 1. Flowering intensity (A) and sprouting frequency (B) of mandarin 'Montenegrina' and 'Rainha' submitted to successive applications of 40 mg L^{-1} of gibberellic acid (GA_3). For each cultivar means followed by the same letter do not differ by the HSD test ($p < 0.05$). 'Montenegrina' - flowering ($p < 0.0001$) and sprouting ($p < 0.0001$). 'Rainha' – flowering ($p = 0.0110$) and sprouting ($p < 0.0001$).

Table 1. Frequency (%) of single-flowering leafy shoot (SLY), single-flowering leafless shoots (SL), multiple-flowering leafy shoots (MLY), multiple-flowering leafless shoots (ML), vegetative shoots (VS) and single flower terminal leafy shoot (STLY) of mandarin 'Montenegrina' and 'Rainha' submitted to successive applications of 40 mg L^{-1} of gibberellic acid (GA_3). Means followed by the same letter, within each cultivar, do not differ by the HSD test ($p < 0.05$). Eldorado do Sul and Montenegro - RS, 2018.

Cultivar	GA_3 applications	SLY	SL	MLY	ML	VS	STLY
Montenegrina	0	14.6 d	81.0 a	0.2 ns	0.2 ns	4.0 c	5.6 c
	1	20.4 cd	77.0 a	1.0	0.3	1.3 c	6.0 c
	2	31.1 c	56.4 b	6.9	1.2	4.3 bc	14.6 bc
	3	47.7 b	32.8 c	9.8	1.0	8.6 ab	24.5 b
	4	67.3 a	16.7 d	4.0	0.3	11.6 a	54.3 a
	p	<0.0001	<0.0001	>0.05*	>0.05*	<0.0001	<0.0001
Rainha	0	17.2 b	74.8 a	6.6 b	0.8 ns	0.6 ns	4.0 b
	1	31.2 a	44.9 b	11.0 b	1.0	11.8	20.4 a
	2	26.0 a	58.3 ab	13.3 ab	0.8	1.5	12.0 ab
	3	15.8 b	68.6 a	9.6 b	2.1	3.9	5.5 b
	4	30.0 a	45.7 b	21.4 a	1.8	1.2	9.0 b
	p	0.0271	0.0001	0.00248	>0.05*	>0.05*	0.0012

*Friedman test

In report of Sartori et al. (2007), 'Montenegrina' experiment in southern Brazil, under similar edaphoclimatic conditions to this work, the application of 10 mg L^{-1} of GA_3 in the autumn period did not inhibit flowering. Ramos-Hurtado et al. (2006), in a similar study with 'Montenegrina', using four concentrations of GA_3 (0, 20, 40 and 60 mg L^{-1}), but with one single applications on three different dates (April, May and June), did not had flowering inhibition. In our report, a similar result for 'Montenegrina' was found, since a single application of GA_3 did not result in significant differences. On the other hand, to cultivar Rainha, results were similar Muñoz-Fambuena et al. (2012), as one application of 40 mg L^{-1} of GA_3 during the floral induction period in 'Salustiana' sweet orange reduced the frequency of SL.

The increase in SLY (especially in STLY) as well as a higher occurrence of VS, improved the quality of sprouting, as flower buds with the presence of leaves are more likely to fruit set (JAHN, 1973; IGLESIAS et al., 2007). In a study with 'Ortanique' tango tree (*C. sinensis* × *C. reticulata*), single flower terminal leafy shoot fruit set almost 40% of the fruits, while mixed buds (SLY + MLY) fruit set approximately 17% of the fruits. The leafless shoots were the ones that least caused fruit set in this tangor, lesser than 10% (CUNHA BARROS; GRAVINA, 2006).

The fruit set (Figure 2) had a significant difference at 107 DAF for 'Montenegrina' and at 32 DAF for 'Rainha'. In 'Montenegrina', the use of four successive applications promoted greater fruit set in relation to the other treatments. In 'Rainha', one application of GA_3 resulted in greater fruit set. Fruit set has a negative correlation with flowering, the lower the energy expenditure with flowering, the greater the fruit tends to be set (GRIEBELER et al.. 2021).

As for the types of shoots and their fruit set, STLY and SL set more fruit in 'Rainha', and STLY had a greater fruit set in 'Montenegrina'. On the other hand, GA_3 applications did not interfere in fruit set frequency of the different types of shoots analyzed individually (Table 2).

Two to four successive applications of 40 mg L^{-1} of GA_3 promoted a significant reduction in sprouting and flowering, as well as an improvement in the quality of sprouting in 'Montenegrina'. Nevertheless, only four

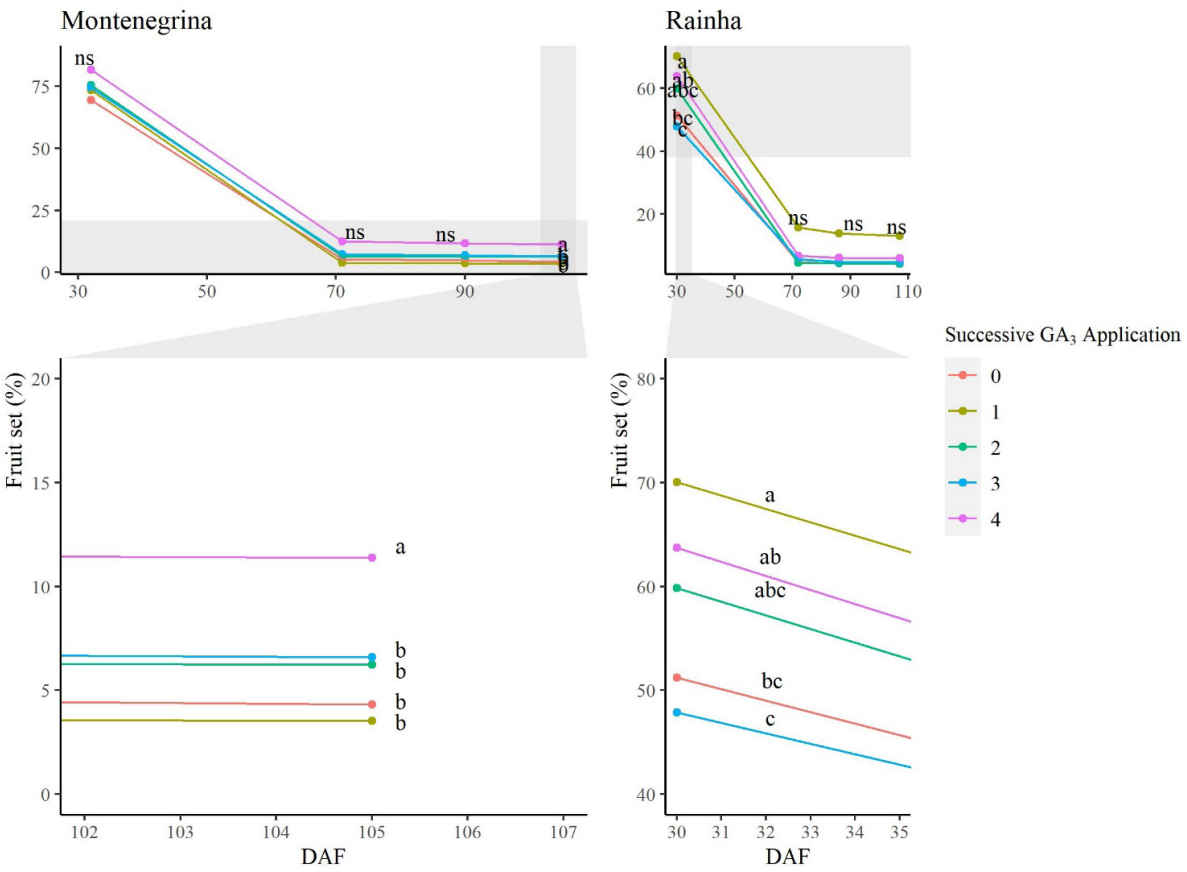

Figure 2. Frequency of fruit set over the days after full flowering (DAF) of 'Montenegrina' and 'Rainha' mandarin trees submitted to successive applications of 40 mg L^{-1} of gibberellic acid (GA_3). Means followed by the same letter, in each DAF, do not differ significantly by the HSD test ($p < 0.05$). 'Rainha' 32 DAF (p=0.0032) and 'Montenegrina' 107 DAF (p=0.0342). Eldorado do Sul and Montenegro - RS, 2018.

Table 2. Frequency of fruit set on shoots: multiple-flowering leafy shoots (MLY), single-flowering leafless shoots (SL), single flower terminal leafy shoot (STLY), unifloral shoots without leaves that occur in pairs or trios at the same node (SLM) of mandarin 'Montenegrina' and 'Rainha' submitted to successive applications of 40 mg L^{-1} of gibberellic acid (GA_3). Means followed by the same upper-case letter in the row, and lowercase in the column, do not differ from each other by the HSD test ($p < 0.05$). Fruit set at 71 DAF and 72 DAF for 'Rainha' and 'Montenegrina', respectively. 'Rainha'- GA_3 applications (p=0.2331) and shoot type (p=0.0009). 'Montenegrina'- GA_3 applications (p=0.0321) and shoot type (p=0.0004). Eldorado do Sul and Montenegro - RS, 2018.

Cultivar	GA_3 applications	MLY	SL	STLY	SLM	Mean
Montenegrina	0	7.3	10.0	35.0	36.8	22.3 ns
	1	2.3	2.5	10.0	2.0	4.2
	2	7.4	12.5	25.0	3.5	12.1
	3	8.4	12.5	52.5	8.8	20.5
	4	3.7	15.0	20.0	0.5	9.8
	Mean	**5.8 B**	**10.5 B**	**28.5 A**	**10.3 B**	
Rainha	0	4.8	15.0	15.0		11.6 ns
	1	8.2	25.0	20.0		17.7
	2	7.6	20.0	7.5		11.7
	3	10.6	7.5	30.0		16.0
	4	6.4	27.5	25.0		19.6
	Mean	**7.5 B**	**19.0 A**	**19.50 A**		

successive applications of GA_3 increased fruit set in 'Montenegrina'. Two successive applications of 40 mg L^{-1} of GA_3 reduced sprouting and flowering in 'Rainha' mandarin, while only one application of GA_3 increased sprouting quality. The age of the orchard may be related to its level of alternate bearing and to the greater or lesser sensitivity to gibberellin applications in order to reduce flowering. GA_3 application did not affect the fruit set rate of specific shoots. Single flower terminal leafy shoot set more fruit compared to the other types of shoots evaluated.

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