



## Parthenocarpy induction and reduction of seeds in fruits of ‘Nadorcott’ mandarin

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### ABSTRACT

Mandarins commercialization at international market requires seedless fruit production in addition to its ideal organoleptic and visual quality. The objective of the work was to evaluate the efficiency of applications of gibberellic acid and of copper sulfate during anthesis in reducing the number of seeds and in the productive performance of the ‘Nadorcott’ mandarin in the conditions of the southern Brazil. The experiment was performed in an orchard of ‘Nadorcott’ mandarin trees grafted on *Poncirus trifoliata*. Two applications were conducted at anthesis with different levels and combinations of gibberellic acid and copper sulfate. Also, a net was used as one of the treatments to prevent pollination. The net treatment provided a high frequency of seedless fruits, with greater skin color, however with less production. Gibberellic acid applications increased linearly the frequency of seedless fruits and with a maximum of two seeds. Copper sulphate applications did not affect any evaluated characteristics. Sprays of gibberellic acid of 100 mg L<sup>-1</sup> concentration reduce the number of seeds per fruit and increase parthenocarpy, without effect on fruit size and peel color.

**Keywords:** *Citrus* sp.; gibberellic acid; copper sulfate; net; pollination.

### INTRODUCTION

‘Nadorcott’ mandarin (*Citrus* spp. × (*Citrus sinensis* Osbeck × *Citrus reticulata* Blanco)), also known as ‘Afourer’ or ‘W Murcott’ (Gambetta *et al.*, 2013), is highly demanded in the international market (Otero & Rivas, 2017). The fruits have excellent organoleptic and aesthetic characteristics, mainly due to its flavor and intense coloration of the juice and peel. This variety is an interesting diversification option for Brazilian citrus, which has a profile of mandarin cultivars that is not very attractive to the international market, concentrated in varieties with seeds, such as ‘Ponkan’ (*Citrus reticulata*) (Barry *et al.*, 2020). As a result, Brazil exported only 440 tons of mandarins in 2019, representing less than 0.05% of its production which was approximately 984.9 thousand tons (FAO, 2021).

At first, ‘Nadorcott’ was described as self-incompatible, not producing seeds when self-pollinated (Chao, 2005). However, in South American crop regions, there is a large presence of seeds in fruits, even in isolated orchards (Gravina *et al.*, 2016). Hence, technologies for inducing parthenocarpy and reducing the occurrence of seeds in ‘Nadorcott’ mandarin are essential for its commercialization in the international market (Otero & Rivas, 2017) as a consequence of the demand for seedless fruits. Depending on the commercialization route, seedless fruits with maximum of one seed (Gravina *et al.*, 2011) or two seeds (Oliveira *et al.*, 2011) can be accepted.

Among the main options for the production of seedless citrus fruits are the isolated planting of self-incompatible cultivars (Chao *et al.*, 2005), pollination restriction with covering net (Gravina *et al.*, 2016) and application of insect repellent (Garmendia *et al.*, 2019).

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In addition, there is the feasibility of using gibberellic acid (GA<sub>3</sub>) and copper sulfate (CuSO<sub>4</sub>) sprays during anthesis. As evidenced previously, the GA<sub>3</sub> application on flowering accelerates the ovule development or may reduce the growth of the pollen tube (Mesejo *et al.*, 2008). Furthermore, spraying CuSO<sub>4</sub> on flowering reduced pollen germination and pollen tube growth (Mesejo *et al.*, 2006). In addition, applications of indole acetic acid (IAA) in non-pollinated ovaries of orange ‘Pineapple’ increased fruit set at the same levels as that obtained for pollinated ovaries. This effect is due to activation of biosynthesis pathways and to inhibition of gibberellin catabolism (Bermejo *et al.*, 2017).

Despite the possibility of using these compounds, there are no reports of this technology in the edaphoclimatic conditions of southern Brazil. Moreover, there also no reports on the use of higher concentration than 50 mg L<sup>-1</sup> of GA<sub>3</sub> and 125 mg L<sup>-1</sup> of CuSO<sub>4</sub> in Nadorcott cultivar. Therefore, this work aimed to assess the reduction of seeds number, induction to parthenocarpy and the productive behavior of ‘Nadorcott’ mandarin tree using net coverage or gibberellic acid and/or copper sulphate applications, during anthesis, in the conditions of southern Brazil.

## MATERIAL AND METHODS

The experiment was performed in a ‘Nadorcott’ mandarin orchard grafted onto *Poncirus trifoliata* (L.) Raf, at the municipality of Eldorado do Sul, Rio Grande do Sul, Brazil (30° 06’ 47” S and 51° 39’ 53” W, 38 m altitude). The orchard was implanted in 2013 at a spacing of 6 m × 3 m (555.5 trees ha<sup>-1</sup>). The soil in the experimental area was classified as a Rhodic Ultisol (Santos *et al.*, 2018). The experimental area had an average annual temperature of 18.8 °C and rainfall of 1,455 mm per year (Bergamaschi *et al.*, 2013). The ‘Nadorcott’ orchard was located between an ‘Okitsu’ tangerine orchard in the north and ‘Murcott’ tangor orchard in the south. Orchard management followed regional recommendations regarding fertilization (SBCS, 2016) and cultural practices (Efrom & Souza, 2018) under rainfed conditions. In order to not interfere with the effect of treatments, pruning and fruit thinning were not performed. The experiment was designed as randomized blocks, with five repetitions and 10 treatments. Each tree represented one experimental unit. The treatments were arranged in a factorial scheme with an additional treatment: 3<sup>2</sup> + 1. Three concentrations of gibberellic acid (GA<sub>3</sub>) and of copper sulfate (CuSO<sub>4</sub>) were applied. Gibberellic acid was used in concentrations of 0, 50 and 100 mg L<sup>-1</sup> while CuSO<sub>4</sub> was used in concentrations of 0, 125 and 250 mg L<sup>-1</sup>. These concentrations were determined based on previous works (Gambetta *et al.*, 2013; Otero & Rivas, 2017). An additional treatment was also used in which the trees were individually and completely covered with 6 x 6

m white polyethylene net (50% of radiation reduction). Covering net was put from before the opening of flowers until the end of the petals fall, aiming to avoid pollination by insects. The applications of GA<sub>3</sub> and CuSO<sub>4</sub> occurred on two dates during flowering: on 09/08/2016, with 35-40% of flowers open; and on 09/15/2016, with 70-75% of flowers open. The applications were performed with a backpack sprayer to the point of run-off, generating a spray volume equivalent to 583 L ha<sup>-1</sup> (1.05 L tree<sup>-1</sup>). Trees which did not receive GA<sub>3</sub> and CuSO<sub>4</sub> treatments (0 mg L<sup>-1</sup>) was used as control.

In the fruit harvest, which occurred between 22nd (blocks one, two and three) and 23rd (blocks four and five) of May 2017, the fruit production in mass (FM) and in number (FN) per tree were quantified. A sample of 200 fruits from each experimental unit was taken. In this sample, the number of seeds of each fruit was quantified. From these data, the average number of seeds per fruit (S), the frequency of fruits without seeds (FS) and the frequency of fruits with two seeds or less (F2S) were determined. In a subsample of 15 fruits per experimental unit, the transverse diameter (d) was measured using a caliper and the mass (mf) of each fruit, using a digital scale. Moreover, in this subsample, the color of the fruits was measured with the Konica/Minolta CR 400® colorimeter, obtaining the brightness coordinates (L\*), a\* and b\*, of the CIE L\* a\* b\* color space (Mcguire, 1992). Through these parameters, the chromaticity (C), the color angle (h°) (Gonzatto *et al.* 2016), and the citrus color index (IC) (Jimenez-Cuesta *et al.*, 1981) were calculated.

The analysis of variances was performed via mixed models, assuming the “blocks” factor as random, using the PROC MIXED routine of the SAS 9.4® software. Each analysis of variance was performed in two moments (Yassin *et al.*, 2002): joint analysis and analysis of factorial structure. In the joint analysis, the source of variation “Treatments” was unfolded in the orthogonal contrast “Net coverage vs Factorial structure”, aiming to separate the additional treatment (Net coverage) from the factorial structure. Factorial structure analysis referred to the applications of compounds GA<sub>3</sub> and CuSO<sub>4</sub>, organized in the causes of variation “GA<sub>3</sub>”, “CuSO<sub>4</sub>” and “GA<sub>3</sub> × CuSO<sub>4</sub>”. When the F test was significant, considering the minimum level of significance  $\alpha = 0.05$ , the effects were complemented by means of polynomial regression. For treatments GA<sub>3</sub> (100 mg L<sup>-1</sup>), GA<sub>3</sub> + CuSO<sub>4</sub> (100 mg L<sup>-1</sup> + 125 mg L<sup>-1</sup>), Control and Net coverage, frequency distribution curves were adjusted by means of non-linear regression using the Sigmaplot 14.0® software. In addition, Pearson’s correlation analysis (r) of the residuals of the evaluated variables was achieved by SAS 9.4® software.

## RESULTS AND DISCUSSION

The physical restriction of pollination by the net coverage reduced the number of seeds per fruit (S), increased the frequency of seedless fruits (FS) and frequency of fruits with a maximum of two seeds (F2S) regarding to the treatments without net coverage. The fruits obtained from net coverage treatment had 1.7 seeds fruit<sup>-1</sup>. The frequency of fruits without seeds (FS) and with a maximum of two seeds (F2S) was 73.1% and 82.2%, respectively. The mass (FM) and the number (FN) of fruits grown on 'Nadorcott' plants submitted to the use of net was 15.9 kg and 176 fruits per tree, which was reduced in relation to other treatments. Nevertheless, the diameter and the average mass of the fruits were not affected by the treatments (Table 1).

The presence of some seeds at the net coverage treatment may be due to the flowers that rest on the net as well as to small defects in the net and its placement (Gravina *et al.*, 2011). Besides, the existence of pollen sources near the orchard may also explain this effect. The reduction of fruit production on trees treated with covering has been observed in previous studies, which is associated with the decrease in fruit set caused by this treatment (Otero & Rivas, 2017).

Despite the lower fruit production with the net coverage, the harvested fruits presented a higher peel luminosity (L\*) indicating a greater brightness in relation to the others treatments. Furthermore, the fruits had a higher chromaticity (C) indicating a higher purity of color. The fruits were more colorful and with more orange shades which is due to the higher CI values and lower  $h^\circ$  values (Table 1). The more colorful fruits observed in the net coverage treatment are probably explained by the lower number of fruits per plant. This causes less arching of branches and a greater exposure of the fruits to radiation. Furthermore, there are an excess of fruits in treatments without net coverage in flowering because there was no thinning of fruits in the experiment. A similar effect in fruit color was previously observed to mandarin trees submitted to chemical or manual fruit thinning in relation to non-thinning treatments (Gonzatto *et al.*, 2016). Negative correlation between the fruit color index (CI) and the average number of seeds fruit<sup>-1</sup> (S) was also observed ( $r = -0.46, p = 0.0045$ ).

When evaluating the spray treatments at anthesis, only the applications of gibberellic acid (GA<sub>3</sub>) had an effect on the frequency of seedless (FS) or on the frequency of a maximum of two seeds (F2S) (Table 1). This effect increased linearly for both variables in the evaluated concentrations (Table 1). The following equations were adjusted: FS =  $1.1 + 0.08GA_3$  ( $R^2 = 0.25, p = 0.0005$ ) and F2S =  $17.2 + 0.28GA_3$  ( $R^2 = 0.11, p = 0.0245$ ).

In previous reports, applications of GA<sub>3</sub> with concentrations ranging from 10 to 50 mg L<sup>-1</sup> in this cultivar had an effect in reducing the number of seeds per fruit (Mesejo *et al.*, 2008; Gambetta *et al.*, 2013; Otero & Rivas, 2017). Additionally, this effect was improved when combinations of CuSO<sub>4</sub> in concentration from 25 mg L<sup>-1</sup> (Otero & Rivas, 2017) to 125 mg L<sup>-1</sup> were employed (Gambetta *et al.*, 2013).

There was no effect of spraying with CuSO<sub>4</sub> at anthesis on any characteristic, even at concentrations of 250 mg L<sup>-1</sup> (Table 1). In previous study under cross-pollination conditions in Spain, Mesejo *et al.* (2006) observed a reduction in pollen germination, in the number of seeds per fruit, increasing the frequency of parthenocarpic fruits in 'Afourer' tangor with one application of 25 mg L<sup>-1</sup> of CuSO<sub>4</sub> in full bloom. In Uruguay, six successive applications were made of CuSO<sub>4</sub> during flowering, and the effective concentration was 50 mg L<sup>-1</sup> (Otero & Rivas, 2017). In Gambetta *et al.* (2013), successive applications of CuSO<sub>4</sub> (125 mg L<sup>-1</sup>, two to three times) neither reduce the number of seeds per fruit nor increased the frequency of seedless fruits neither, although there was a reduction in pollen germination. Therefore, the effect of CuSO<sub>4</sub> applications appears to be dependent on a high frequency and concentrations of spraying during flowering, especially in subtropical climates. This present experiment was sprayed only twice, explaining the non-effect.

Regarding to the sprays, the treatments with of GA<sub>3</sub> 100 mg L<sup>-1</sup> and GA<sub>3</sub> 100 mg L<sup>-1</sup> + CuSO<sub>4</sub> 125 mg L<sup>-1</sup> applications were the most effective in reducing the number of seeds, with FS > 10% (10.2 and 10.4%, respectively) and F2S > 30% (33.6 and 36.7%, respectively) (Table 1).

The frequency distribution of the number of seeds per fruit (S) is shown (Figure 1) for the treatments: GA<sub>3</sub> 100 mg L<sup>-1</sup>, GA<sub>3</sub> 100 mg L<sup>-1</sup> + CuSO<sub>4</sub> 125 mg L<sup>-1</sup>, control and net coverage Gaussian distributions for S were observed for the following treatments: applications of GA<sub>3</sub> and control, demonstrating a higher frequency of fruits with a lower average number of seeds as an effect of the use of the phytohormone. The control treatment exhibited a peak of fruit frequency of 4.8 seeds fruit<sup>-1</sup>, and a considerable frequency of fruits of about 10 seeds fruit<sup>-1</sup>. For the treatment with GA<sub>3</sub>, fruits without seed showed the highest frequency. Nevertheless, a high frequency of fruits with more than 10 seeds was also noticed. In relation to association of GA<sub>3</sub> 100 mg L<sup>-1</sup> and CuSO<sub>4</sub> 125 mg L<sup>-1</sup>, the maximum frequency of fruits was observed to be approximately 2.5 seeds fruit<sup>-1</sup>. There was an exponential decay behavior in the treatment with net coverage, showing the higher frequency of parthenocarpic fruits (Figure 1).

High concentrations of GA<sub>3</sub> (100 mg L<sup>-1</sup>) increased the parthenocarpy (Table 1). Nevertheless, it is still far from the obtained in this work when net coverage was used on the flowering period.

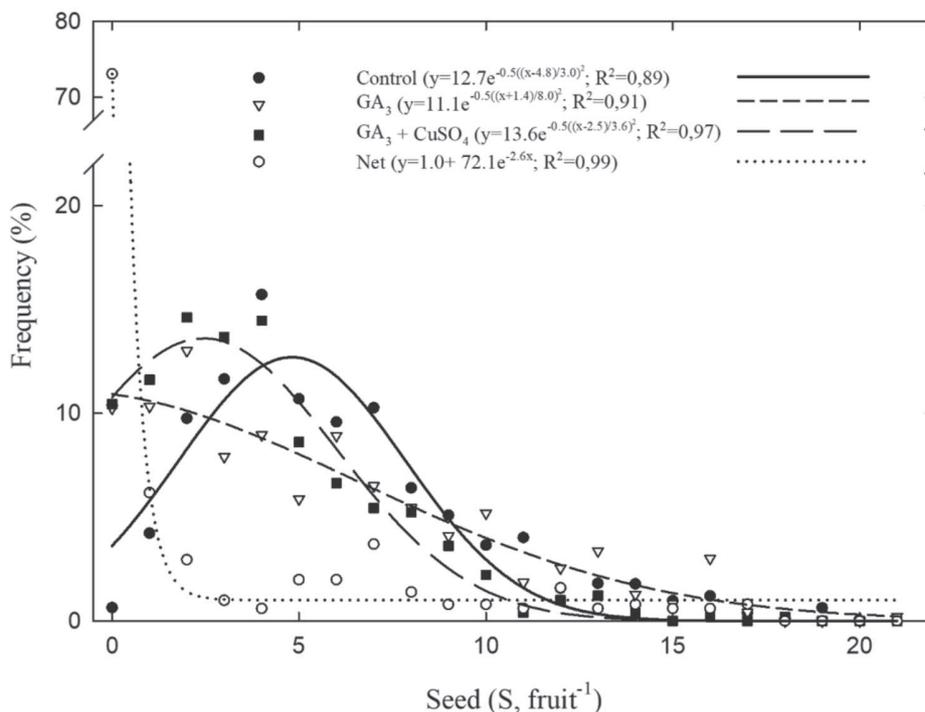
**Table 1:** Averages and probabilities of variances (ANOVA) for number of seeds per fruit (S), frequency of seedless fruits (FS) and frequency of fruits with two seeds or less (F2S), fruit production in mass (FM) and in number (FN) and in number (FN), transverse diameter (d) and average fruit mass ( $m_f$ ), luminosity (L\*), chromaticity (C), color angle ( $h^\circ$ ) and peel color index (CI) in 'Nadorcott' mandarin trees submitted to applications of gibberellic acid ( $GA_3$ ), copper sulfate ( $CuSO_4$ ) and net coverage use during flowering.

Treatment	S (fruit <sup>-1</sup> )	FS (%)	F2S (%)	FM (kg tree <sup>-1</sup> )	FN (fruit tree <sup>-1</sup> )	d (mm)	$m_f$ (g fruit <sup>-1</sup> )	L*	C	$h^\circ$	CI
Control	6.0	0.6	14.6	40.4	517.4	59.4	95.5	54.0	53.4	76.8	4.3
$GA_3$ (50 mg L <sup>-1</sup> )	5.5	2.4	20.2	46.8	594.0	58.5	89.7	51.9	48.6	82.9	2.3
$GA_3$ (100 mg L <sup>-1</sup> )	5.4	10.2	33.6	37.2	475.4	59.0	92.1	51.5	48.0	85.1	1.4
$CuSO_4$ (125 mg L <sup>-1</sup> )	6.4	3.3	20.2	43.0	522.2	58.6	90.1	53.9	52.1	78.0	3.9
$CuSO_4$ (250 mg L <sup>-1</sup> )	5.9	2.2	22.9	34.7	496.2	57.9	88.7	54.8	54.4	75.0	4.9
$GA_3$ (50 mg L <sup>-1</sup> ) + $CuSO_4$ (125 mg L <sup>-1</sup> )	6.4	2.4	17.5	46.1	593.0	58.9	91.8	53.9	52.7	78.7	3.7
$GA_3$ (50 mg L <sup>-1</sup> ) + $CuSO_4$ (250 mg L <sup>-1</sup> )	5.7	5.1	20.8	42.6	556.2	59.8	95.1	52.1	49.8	79.9	3.3
$GA_3$ (100 mg L <sup>-1</sup> ) + $CuSO_4$ (125 mg L <sup>-1</sup> )	4.0	10.4	36.7	40.9	542.4	58.7	88.6	52.6	51.4	80.4	3.1
$GA_3$ (100 mg L <sup>-1</sup> ) + $CuSO_4$ (250 mg L <sup>-1</sup> )	5.4	9.2	25.9	37.2	462.8	59.8	96.8	53.4	52.3	80.4	3.1
Net	1.7	73.1	82.2	15.9	176.0	60.6	100.4	58.3	65.1	64.3	8.3
Joint Analysis <sup>(1)</sup>											
Treatments	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7716	0.5482	0.1214	0.0375	0.0282	0.0269
Net vs. Factorial structure <sup>(2)</sup>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1320	0.0666	0.0023	0.0002	0.0003	0.0003
Factorial structure analysis <sup>(3)</sup>											
$GA_3$	0.0548	0.0002	0.0014	0.0925	0.0514	0.7732	0.9435	0.3118	0.4695	0.2105	0.1842
$CuSO_4$	0.9860	0.8169	0.8502	0.2796	0.4316	0.8522	0.5614	0.6500	0.6547	0.5483	0.5232
$GA_3 \times CuSO_4$	0.2985	0.8440	0.3266	0.9210	0.9347	0.6456	0.4548	0.8677	0.8675	0.9306	0.9194
Linear adjustment - $GA_3$											
Quadratic Adjustment - $GA_3$		0.0001	0.0013								
		0.0968	0.0605								

<sup>(1)</sup> Joint ANOVA according to model:  $\hat{y}_{ijk} = \mu + \tau_i + \beta_j + \varepsilon_{ijk}$ .

<sup>(2)</sup> Orthogonal contrast between Net coverage treatment and the other treatments together (Factorial structure),

<sup>(3)</sup> Factorial ANOVA according to model:  $\hat{y}_{ijk} = \mu + \rho_i + \sigma_j + (\rho\sigma)_{ij} + \beta_k + \varepsilon_{(ij)k}$ .



**Figure 01:** Distribution frequency of the number of seeds per fruit in 'Nadorcott' mandarin trees submitted to applications of GA<sub>3</sub> (100 mg L<sup>-1</sup>) and GA<sub>3</sub> + CuSO<sub>4</sub> (100 mg L<sup>-1</sup> + 125 mg L<sup>-1</sup>), in relation to control treatments and net coverage use during flowering.

## CONCLUSIONS

The use of pollination restrictive net during flowering allows a high frequency of parthenocarpic fruits despite reducing the total fruit production.

In the edaphoclimatic conditions of the central depression of Rio Grande do Sul, sprays of 100 mg L<sup>-1</sup> of gibberellic acid during anthesis reduces the number of seeds and increases the induction of parthenocarpy in 'Nadorcott' mandarin fruits without reducing fruit production. In addition, applications of CuSO<sub>4</sub> did not reduce either the number of seeds or induces parthenocarpy.

For further studies, the investigation of the influence of a greater number of applications of GA<sub>3</sub> on flowering to reduce seeds in fruits and its association or not with other compounds are to be clarified, the use of net coverage and girdling should be tested together to verify the increase the fruit set and the frequency of fruits with few or no seeds.

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