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Use of Plant Regulators in the Off-Season Flower Induction of 'Tahiti' Acid Lime and 'Ponkan' Mandarin

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Abstract

Brazil is one of the world's largest citrus producers. To maintain the uniformity of annual production during seasonality, there is a need to apply new technologies in agriculture to enhance plant productivity. Applications of plant regulators in fruit trees are part of methods to standardize production. Thus, the aim of this study was to evaluate the use of plant growth regulators Paclobutrazol (PBZ) and Ethephon, associated with potassium sulfate and calcium nitrate foliar fertilizers, in the off-season flower induction in 'Tahiti' acid lime and 'Ponkan' mandarin crops, for off-season production. The experiment was carried out in properties located in the municipality of Aparecida do Taboado/MS, with randomized blocks design, containing 2 treatments (without/with application of plant growth regulators), 4 blocks with 50 replicates, with each plant evaluated being considered an experimental unit. At 150 days after the application of products, number of flowers per square meter of plant canopy and fruit set in both crops were evaluated, in addition to their diameter. 'Tahiti' acid lime plants treated with PBZ obtained average of 77.24 fruits per m²/plant canopy, while controls had 16.08 fruits m²/plant canopy. In 'Ponkan' mandarin, treated plants obtained 87.49 fruits per m²/plant canopy, while controls had 30.94 fruits m²/ plant canopy. The execution schedule and products used as plant regulators, especially Paclobutrazol, applied to 'Tahiti' acid lime and 'Ponkan' mandarin plants allowed significant increase in the amounts of flowers and fruits, making it possible to plan the staggering of flowering induction and production of these cultivars.

Keywords: *Citrus latifólia*; *Citrus reticulada* blanco; Floral induction.

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1. Introduction

Brazil is one of the world's largest citrus producers, being the largest producer of oranges and the second largest in the production of 'Tahiti' acid lime [1,2]. Other species, such as 'Ponkan' mandarin, have also been gaining prominence because Brazil is one of the important consumers of this table fruit [3,4]. Irregular production and seasonality are factors that directly interfere in the development of these crops, leading to price fluctuation peaks from July, culminating in the months of September to December, with drop to normal prices from January for both crops [5]. Therefore, management technologies that manipulate the production season can bring benefits to the entire production chain of these crops, allowing better crop planning according to market demands [6,7]. In this sense, according to the authors in [8], one option is the use of plant regulators, which act on uniformity, productivity and amount of flowers produced by plants, among which Paclobutrazol (PBZ) stands out, which has been used as a tool to promote flowering by promoting the cease of the vegetative growth due to the inhibition of the biosynthesis of gibberellins [9]. In this context, the aim of this study was to evaluate the use of plant growth regulators Paclobutrazol (PBZ) and Ethephon, associated with potassium sulfate and calcium nitrate foliar fertilizers in the off-season flower induction in 'Tahiti' acid lime and 'Ponkan' mandarin crops, for off-season production.

2. Materials and Methods

The experiment was carried out at Pedra Negra and Conquista Farms, in the municipality of Aparecida do Taboado, Mato Grosso do Sul, whose geographic coordinates are 20°2' 58. 98" S and 51°3' 41. 27" W and 330 meters a.s.l. According to the Koppen's classification, the local climate is tropical hot and humid (WA), with rainy season in the summer and dry season in the winter. The average temperature during the experimental period was 25ºC and the average precipitation was 140 mm (Figure 1).

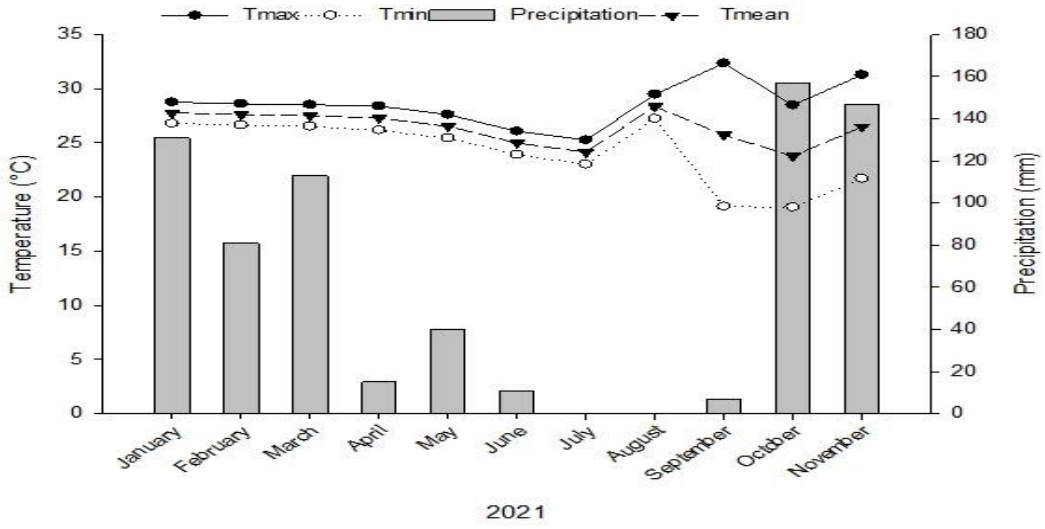

Figure 1: Monthly maximum, average and minimum temperatures (°C) and monthly precipitation totals (mm) of the microregion of Aparecida do Taboado. Aparecida do Taboado - MS, Brazil, 2021.

In experiment with 'Tahiti' acid lime (*Citrus latifólia* Tanaka), plants with canopy formed with 'Quebra Galho' cultivar grafted onto "Rangpur" lemon tree (*Citrus limonia* Osbeck), aged 5 years, cultivated in spacing of 8.0 meters between rows and 5.0 meters between plants were used, totaling 250 plants per hectare.

In experiment with mandarin (*Citrus reticulata* Blanco), plants with canopy formed with 'Ponkan' cultivar, also grafted onto "Rangpur" lemon tree, with 5 years of age, cultivated in spacing of 8.0 meters between rows and 2.5 meters between plants were used, totaling 500 plants per hectare.

Fertilization and pest and disease control were carried out throughout the experimental period, following specific recommendations for acid lime and mandarin crops.

The experimental design used in both cultures was randomized blocks, containing 4 blocks and 50 plants per block, totaling 200 plants per treatment, with 2 treatments, being T0 (control), without chemical flowering management; and T1, with chemical flowering management, according to protocol described below:

The active ingredient of Paclobutrazol (PBZ), whose trade name is CULTAR® marketed by company SYNGENTA, was used at dose of 1 gram of active ingredient per linear meter of canopy, with 5 grams for 'Tahiti' acid lime and 2 grams for 'Ponkan' mandarin, applied via soil, in the second half of January, after the second vegetative flow and the main harvest, maintaining drip irrigation with average daily volume of 60 liters per plant.

Twenty days after PBZ application, to contain the emission of vegetative branches, participating in the maturation process of branches [10], four Potassium Sulfate foliar applications were carried out, at concentration of 2.5%, in 20-day intervals. After the end of K_2SO_4 applications, together with irrigation interruption, 100 days after PBZ application, two foliar applications with Ethrel (300 PPM) were performed, with 7-day interval between applications, aiming at plant stress and complete maturation of branches [11]. Then, foliar Calcium Nitrate applications $(CaNO_3)_2$ were started, with 7-day interval, at concentration of 2.5% of the tank volume to stimulate the floral differentiation of buds [12].

At 150 days after PBZ application, in both treatments and crops, flowering quantification analyses were performed through the use of 1m² template to count flowers emerged on both sides of plants. After 30 days of flower quantification, fruit set evaluation was performed by counting "two-millimeters length" type fruits using the same template described in the previous analysis.

At 280 days after PBZ application, also in both treatments and crops, when fruits were in phenological stage 6 – "marble" fruit [13], fruit diameter was evaluated with the aid of digital caliper in mm, using the same template described in the previous analyses.

Data obtained were analyzed using the SISVAR software [14]. The normality hypothesis was tested using the Shapiro-Wilk test, then, analysis of variance was performed using the F test at 5% probability.

3. Results

From the application of Paclobutrazol (PBZ) until the beginning of the appearance of the first flowers in both 'Tahiti' acid lime and 'Ponkan' mandarin crops, all plants flowered at 150 days, showing that the chemical management used did not promote early flowering, corroborating data found by the authors in [15], who evaluated different PBZ concentrations for flower induction in mango and observed that there was no positive effect in anticipating flowering, since all treatments started panicle emergence in practically the same week.

The authors in [16] evaluated the interaction of PBZ with fulvic acids and free amino acids in the flowering of "Keitt" mango cultivar and observed that all treatments had the same flowering percentage at 126 days after application, with no significant effect, which reveals that there was no flowering delay or anticipation.

Regarding flowering quantification, fruit setting and fruit diameter of 'Tahiti' acid lime, it can be seen in Table 1 that there was significant difference between treatments for all analyzed variables.

Table 1: Quantification of flowers, "two-millimeters length" fruit set and "marble" fruit diameter of 'Tahiti' acid lime plants, submitted or not to the application of plant growth regulators Paclobutrazol (PBZ) and Ethephon, associated with potassium sulfate and calcium nitrate foliar fertilizers.

Source of Variation	**Flowering**	**Fruit setting**	**Fruit diameter**
	'Tahiti' acid lime	**'Tahiti' acid lime**	**'Tahiti' acid lime (mm)**
Treatments	Mean Square		
	601826.850625*	374023.980625*	7748.400625*
1	22.55	16.08	37.41
2	100.13	77.24	28.61
CV%	**4.50**	**5.73**	**13.91**
Average	**61.34**	**46.66**	**33.01**

* Significant by the F test at 5% probability.

As for the number of flowers, it was observed that the control group had average of 22.55 flowers per m^2/ plant canopy, while the group treated with plant growth regulators, particularly PBZ, had average of 100.13 flowers per m^2/ plant canopy, which means a 444% difference between treatments. The authors in [17] evaluated the flowering of 'Tahiti' acid lime submitted to water stress and treated with Paclobutrazol observed that the PBZ concentrations applied increased the number of flowers in irrigated and water stressed plants by 137% and 371%, respectively, when compared to the number of flowers emitted by control plants, corroborating the data of the present work.

According to the authors in [18], flower setting of 'Tahiti' acid lime in periods from May and June had average

of 10.94 flowers/m² plant canopy, the same flowering period of the present work. Authors in [19] evaluated the effect of gibberellic acid on the flower setting in this crop and obtained average, in the control treatment, of 173.53 flowers/m² plant canopy. This difference in the number of flowers is probably due to the time of evaluation through the time of flowering season due to the amount of carbohydrate reserves in plants, much greater in spring after a winter dormancy period than in summer [20].

Several studies have demonstrated the importance of low temperature condition to induce flowering in citrus [17]. This temperature condition has been shown to have an effect on the seasonal flowering periodicity; in short, for flowering to occur in citrus, two events are needed: plants must reach maturity and they must be exposed to the correct environmental signals [21].

Regarding fruit set quantification of 'Tahiti' acid lime plants, positive response of treated plants in relation to control plants was observed, with average of 77.24 fruits per m²/plant canopy (386.2 fruits per plant) for the first and 16.08 fruits per m²/ plant canopy (80 fruits per plant) for the second, indicating a 480% increase between treatments.

The application of plant growth inhibitors, such as PBZ, not only inhibit plant overgrowth [22], but also proves to be a good practice to increase the amount of carbohydrates in storage organs [23], which are used in the formation and development of flowers and fruits of citrus trees [24], justifying the higher values found in plants treated with growth regulators, as can be seen in Figure 2.

Figure 2: From left to right, vegetative development, flowering and fruiting of 'Tahiti' acid lime plants treated with 5 g PBZ (1) and control (2).

Regarding the percentage of fruits in relation to the amount of flowers, it was observed that, for both treatments, it was possible to obtain more than 70% flower setting, being 77.14% and 71.3% for plants treated with PBZ

and control plants, respectively. The authors in [25] evaluated the fruit setting percentage of 'Tahiti' acid lime "Quebra Galho" cultivar grafted onto "Rangpur" lemon and obtained means ranging from 70.8% to 84.7% of fruit setting per plant at 23 days after anthesis, corroborating results found in the present study.

According to the authors in [26], the number of fruits per plant is a considerable characteristic with regard to the quality of clones and rootstocks used; however, it is noteworthy that fruits must have standard size, since very small or very large fruits have no preference for marketing.

Regarding the diameter of 'Tahiti' acid lime fruits, it was verified in Table 1 that this parameter was inversely proportional to the quantification of flowers and fruits. In this case, fruits from the control treatment had average of 3.7 cm in diameter, while fruits from plants treated with PBZ had average of 2.8 cm in diameter, which results were expected, because the greater the number of fruits, the greater the competition for metabolites and the smaller their size and mass, making fruit mass inversely proportional to the pending load [27].

Regarding flowering quantification, setting and diameter of 'Ponkan' mandarin fruits, it was observed in Table 2 that there was significant difference between treatments for all analyzed variables.

Table 2: Flower quantification, "two-millimeters length" fruit set and "marble" fruit diameter of 'Ponkan' mandarin plants submitted or not to the application of plant growth regulators Paclobutrazol (PBZ) and ethephon, associated with potassium sulfate and calcium nitrate foliar fertilizers.

Source of Variation	**Flowering** **'Ponkan' mandarin**	**Fruit setting** **'Ponkan' mandarin**	**Fruit diameter** **'Ponkan' mandarin (mm)**
Treatments	Mean Square		
	1358448.52*	319846.80*	18779.27*
1	46.52	30.94	49.16
2	163.07	87.49	35.45
CV%	**17.43**	**8.33**	**9.33**
Average	**104.79**	**59.22**	**42.31**

* Significant by the F test at 5% probability.

Regarding the number of flowers, it appears that the control group had average of 46.52 flowers per m² / plant canopy, while the group treated with plant growth regulators, particularly PBZ, had average of 163.07 flowers m² / plant canopy, which means a 350% difference between treatments.

The authors in [28] evaluated the flowering of 'Ponkan' mandarin fruits submitted to the application of gibberellic acid and obtained result of 55.1 flowers per plant in the first harvest and 85.3 flowers per plant in the following year for control plants, values lower than those found in the present work, which were 93.04 flowers

per plant in the control treatment and 326.14 flowers per plants treated with plant regulators.

The starch availability in reserve tissues in the season prior to flowering is one of the factors that determines the number of flowers emitted and fruit set [29,30,31], and the cease of the vegetative growth is the major factor responsible for starch accumulation, since the assimilate that should be used for vegetative growth is diverted to flowering intensification [32], with the cease of the vegetative growth being the main function of Pablobutrazol.

Regarding the fruit set of 'Ponkan' mandarin plants, it was observed that there was also positive response of treated plants compared to control plants, with average of 87.49 fruits per m²/plant canopy (174.98 fruits per plant) for the first and 30.94 fruits per m²/ plant canopy (61.88 fruits per plant) for the second, indicating a 282% increase between treatments.

The authors in [33] studied the effect of spacing on the quality of Citrus reticulata Blanco fruits and observed maximum of 26.98 fruits per plant in spacing of 3.0 m between plants and 7.0 m between rows, totaling approximately 470 plants per hectare, which density is lower than that used in the present work, but with lower fruit set values. This discrepancy in results is due, among other factors such as different cultural treatments, to climate differences between regions, since, for that work, which was carried out in Faisalabad, Pakistan, low rainfall levels, with annual accumulated of approximately 340 mm, without mentioning the use of irrigation.

High temperatures combined with low precipitation increase abiotic stress in citrus crops [34], causing fruit to fall in extreme weather conditions and directly affecting the amount of fruit on the tree and the production habit of the subsequent season [35].

Regarding the percentage of fruits in relation to the amount of flowers, it was observed that, for plants treated with PBZ, this index was 53.6%, while for untreated plants, it was 66.5%. The authors in [28] evaluated the setting of 'Ponkan' mandarin fruits submitted to the application of gibberellic acid and obtained average result of 57.6% for this variable, corroborating results found in the present work. According to the authors in [36], the fruit set index in 'Ponkan' mandarin fruits ranges from 10 to 15% of the total number of flowers that emerged at every 100 knots emitted by plants.

According to the authors in [37], Paclobutrazol as plant growth inhibitor, can substantially suppress shoot growth on many fruit trees, while increasing flowering and fruiting due to the greater photosynthetic capacity of leaves and accumulation of reserves.

Thus, it was observed that the application of PBZ provided a favorable effect on the flowering and setting of 'Ponkan' mandarin fruits, indicating that the application of PBZ before flowering can increase the number of flowers and consequently the amount of fruit on plants, as can be observed in Figure 3.

Figure 3: From left to right, flowering, vegetative development and fruiting of 'Ponkan' mandarin plants treated with 2 g of PBZ (1) and control (2).

Finally, regarding the diameter of 'Ponkan' mandarin and 'Tahiti' acid lime fruits, an inversely proportional effect was observed between this variable and the quantification of flowers and fruits. In this case, fruits from the control treatment had average of 4.9 cm in diameter, while fruits from plants treated with PBZ had average of 3.5 cm in diameter. However, according to the authors in [13], these fruits are in phenological stage 7 – "ping-pong ball", showing shorter period between flowering and fruiting.

4. Conclusion

The execution schedule and products used as plant regulators, especially Paclobutrazol, applied to 'Tahiti' acid lime and 'Ponkan' mandarin plants allowed significant increase in the amounts of flowers and fruits, making it possible to plan the staggering of flowering induction and production of these cultivars.

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