Pre-harvest application of 2-(3-chlorophenoxy) propionic acid on pineapple plants

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Abstract

The application of plant regulators such as exogenous auxin may provide effects on the translocation of carbohydrates to plant sinks, which may result in larger parthenocarpic fruits. The objective of the study was to evaluate the effects of the application of 2-(3-chlorophenoxy) propionic acid on the growth and quality of pineapple fruits. The experiment was carried out in a randomized block design. After floral induction, 2-(3-chlorophenoxy) propionic acid (CPA) was applied directly on the inflorescences at the following doses: 0 mg L⁻¹; 40.0 mg L⁻¹; 80.0 mg L⁻¹; 120.0 mg L⁻¹ and 160.0 mg L⁻¹. Growth evaluations continued until 84 days after application, measuring fruit length (cm) with and without crown, crown length (cm), leaf D dry matter, fruit weight with and without crown (g), calculating if also, the absolute and relative growth rates. The post-harvest and quality characteristics of the fruits were also evaluated, as well as the harvest index. The application of CPA significantly reduces the length and weight of the crown of the fruits, however, the exogenous application of auxin provided an increase in the weight of the fruit. Regarding the post-harvest analysis, the application of CPA reduced the content of soluble solids in the fruit pulp.

Keywords: *Ananas comosus var. comosus*, auxin, growth regulator, production

Introduction

Pineapple (*Ananas comosus* var. *comosus*) is a tropical fruit worldwide appreciated due to its characteristic aroma and flavor, which can be consumed fresh or processed. The global production of this fruit reached 2.3 million tons in 2021, with Costa Rica being the main producer, followed by Indonesia, the Philippines, and Brazil (Faostat 2021). The average worldwide pineapple production reached 25.04 tons per hectare in 2019, with the highest yields ranging from 128.32 to 68.67 tons per hectare, obtained by Indonesia, Costa Rica, and Ghana. In Brazil, the average yield was 36.12 tons per hectare (Faostat 2021).

To achieve high yields, it is necessary to use technologies that enhance fruit yield and quality, particularly in terms of size, weight, flavor, and visual appearance, leading to improved economic profitability and sustainability of pineapple crops. Among the various

practices that can be applied, the use of natural or synthetic growth regulators applied directly to the fruits stands out as a simple, safe, and easy-to-use technology. The purpose of these growth regulators is to standardize production and improve fruit quality due to their activities in plant physiology and metabolism, acting on growth and development processes (Suman et al. 2017).

Among these regulators, synthetic auxin applied directly to the flower has the effect of developing parthenocarpic fruits, as it replaces the supply of endogenous auxin in the ovary for fruit growth and development (Godoy and Cardoso 2004), increasing their final size. 2-(3-chlorophenoxy) propionic acid (CPA) is a synthetic auxin, and its application in pineapple cultivation is done directly on the flowers, at the end of the dehiscent phase of flowering (Fahl and Franco 1981, Rebolledo-Martínez et al. 2002). However, it is necessary to adjust the dosage to obtain higher-quality

fruits in terms of visual, physical, and chemical aspects. Furthermore, although there have been studies on the doses, there is still no information on the growth pattern of fruits in response to CPA application. In this sense, the objective of this study was to evaluate the effects of the application of 2-(3-chlorophenoxy) propionic acid on the growth and quality of 'Pérola' pineapple fruits.

Material and Methods

The experiment was conducted at an experimental area located in the municipality of Janaúba, MG (15° 43' 47.4" S and 43° 19' 22.1" W, at an altitude of 533 meters), in a Typic Hapludox (Latossolo Vermelho; Santos et al. 2018). The climate of the region, according to Köppen classification, is of type Aw (hot tropical with a dry and cold winter). The average data for monthly rainfall, maximum and minimum temperature during the study period are shown in **Figure 1**.

Figure 1. Monthly rainfall, maximum, and minimum temperatures during the experimental period.

The experiment was set up in a randomized block design with five treatments and four replications. The experimental units were arranged in 8 double rows using "Pérola" cultivar slips, which were 30 cm in length and had a fresh weight of 78 g. Only slips curing was performed before planting. The treatments tested were as follows: T1) Control (water); T2) 40 mg L-1 CPA; T3) 80 mg L-1 CPA; T4) 120 mg L⁻¹ CPA; T5) 160 mg L⁻¹ of CPA. Each plot consisted of two double rows, totaling 40 plants. Border plants were excluded from the evaluations, and assessments were made on the fruits of the 8 central plants.

Soil preparation for pineapple planting involved tilling and furrowing the area. For the physical and chemical characterization of the soil and recommendations for soil amendments and fertilizers, 20 individual samples were collected to obtain a sample from the 0-20 cm layer. The results were as follows: 50 g/kg of sand (sandy textural class); 24 g/kg of silt; 26 g/kg of clay; pH in water 5.4; 25.7 mg/dm³ of P (Mehlich⁻¹); 131 mg/dm³ of K⁺; 0.1 mg/dm³ of Na⁺; 2.0 cmolc/dm³ of Ca²⁺; 0.8 cmolc/dm³ of Mg²⁺;

3.5 cmolc/dm³ of H+Al; 0.6 mg/dm³ of B; 1.1 mg/dm³ of Cu; 18.4 mg/dm³ of Fe; 16.1 mg/dm³ of Mn; 1.5 mg/ dm³ of Zn; and 0.3 dS/m of electrical conductivity. Slips were planted in furrows using a double-row system with a spacing of 1.00 x 0.40 x 0.30 m, resulting in a density of 47,619 plants per hectare. Irrigation was carried out using a conventional sprinkler system. For planting fertilization, 8g of monoammonium phosphate (MAP) and FTE BR12 (9% Zn, 1.8% B, 0.8% Cu, 2.1% Mn, and 0.1% Mo), as a source of micronutrientes, (Cunha and Franco, 2017) were applied per plant. Total topdressing doses of 15 g N (urea) and 15 g $K₂O$ (potassium chloride) per plant were applied until forcing date.

Throughout the experiment, the recommended cultural and phytosanitary practices for pineapple (Sanewski et al. 2018) were carried out. Floral induction (Forcing) was conducted on 645th day after planting (DAP), using 50 mL of Ethrel® (p.a 240 solution) at 1%, with the addition of calcium hydroxide (lime) at a dosage of 0.35 g L⁻¹ of water. The application of 2-(3-chlorophenoxy) propionic acid (CPA) was done when the apical flowers were in the final stage of dehiscence. Only one application of the product in liquid form, with a volume of 25 mL per plant, was made. All treatments were applied with addition of surfactant (except for the control) to facilitate the adherence of the product to the fruit, using a 10-liter backpack sprayer. After the CPA application, the fruits were protected with newspaper to reduce sunburn, and they were harvested when they reached 50% yellow peel (Reinhardt et al. 2000).

To assess fruit growth, the diameter (mm) was measured using a digital caliper, and the length (cm) of the fruit with and without the crown was measured using a tape measure. Weekly evaluations were conducted for 84 days, corresponding to the period between the product application and the final fruit maturation stage.

After harvesting, the following characteristics were also evaluated: pH, titratable acidity, soluble solids, pulp firmness, as well as the weight of fruits with and without the crown and the crown-to-fruit ratio.

Following that, the entire plants were harvested, and the following characteristics were determined: plant height, leaf length, stem diameter, number of propagules (suckers and slips) , fresh weight of leaves, stem, root, and propagules, length and fresh weight of "D" leaf. Using the data on the fresh weight (weight) of the entire plant and the fruit weight, the harvest index (HI) was calculated (fruit weight/total plant weight).

To determine the pulp pH, 10 g of ground and homogenized pulp were used, which were mixed with 90 mL of distilled water. The pH was measured using a digital pH meter (GEHAKA PG 1800). Titratable acidity was determined by titrating the solution obtained from 10 grams of ground and homogenized pulp with 90 mL of distilled water. A 0.2 mol L-1 NaOH solution was used as the titrant, and three drops of 1% phenolphthalein were added as an indicator. The results were expressed in mg of citric acid per 100 g of pulp. Pulp soluble solids (°Brix) were obtained by refractometry using a digital refractometer. Pulp firmness (N) was determined using a benchtop penetrometer (Fruit Pressure Test FT 327). The weights of fruits with and without the crown (g) were measured using a precision digital scale.

For fruit growth data, sigmoid models were fitted $(\hat{Y} = \alpha/1 + \exp(-(x-x0)/b))$, and graphs of the variables over time were plotted. Absolute growth rates (AGR) and relative growth rates (RGR) in centimeters were estimated from the fitted models according to Hunt (1945).

The data were subjected to analysis of variance using the F-test, and when significant, the variables were studied using regression analysis with the assistance of the statistical software R studio with the easyanova package. Sigma Plot 12.5 Demo was used for regression. The models were adjusted based on the adjusted determination coefficient and their ability to explain the biological phenomenon.

Results and Discussion

The doses of 2-(3-chlorophenoxy) propionic acid (CPA) did not show a significant effect ($p > 0.05$) on the following variables: plant height $(\hat{Y}=123.56$ cm), stem diameter (\hat{Y} = 50.51 mm), number of propagules (\hat{Y} = 0.67), and fresh weight of leaves (\hat{Y} = 1012.15 g), stem (\hat{Y} = 326.03 g), root $(\hat{Y} = 14.92$ g), and propagules $(\hat{Y} = 75.02$ g). The vegetative growth of the pineapple plant stops after floral induction (Marques et al. 2011, Pegoraro et al. 2014). Since the application of CPA was performed directly on the inflorescence and in the late flowering stage, these characteristics were not significantly influenced by the auxin.

The "D" leaf length $(\hat{Y} = 95.38$ cm) was not significantly affected by the treatments ($p > 0.05$). However, the doses of CPA applied to 'Pérola' pineapple fruits affected the "D" leaf fresh weight. There was an increase of 0.0289 g for each mg $L⁻¹$ of applied CPA, reaching a value of 77.22 g at the highest dose (160 mg L-1) (**Figure 2**).

Although there is a reduction in the growth of pineapple plants with the onset of the reproductive phase (Marques et al. 2011, Pegoraro et al. 2014), the increase in "D" leaf fresh weight may have occurred due

Figure 2 – "D" leaf fresh weight of 'Perola' pineapple plants as a function of the doses of 2-(3-chlorophenoxy) propionic acid. Significance: ***(0.001), *(0.05)

to a modification in its metabolism in response to the auxin application. This is because, among the leaves in the pineapple plant, "D" leaf, which is the most metabolically active (Reinhardt et al. 2000), might have metabolized auxin even after floral induction, resulting in greater fresh weight with an increase in product concentration. Another possibility is that stronger sinks stimulate higher production of photoassimilates by source organs due to increased photosynthetic rates (Smith et al. 2018). Given that the pineapple fruit is the main sink (Lima et al. 2002), the application of auxin can make the fruit a stronger sink, and source organs, such as "D" leaf, may be stimulated to increase photosynthetic rates, thereby fixing a greater amount of carbon into dry matter, and consequently increasing the leaf fresh weight.

The nonlinear sigmoidal model was fitted for the variables fruit length with and without crown, crown length, and fruit diameter of 'Perola' pineapple as a function of days of evaluation after application of different CPA doses (**Figure 3**).

In the case of the variable "growth of fruit without crown," it's evident that there is a more pronounced effect, especially in the later days of evaluation, for the treatment with a dose of 40 mg L⁻¹ of 2-(3-chlorophenoxy) propionic acid (CPA) (Figure 3A). This is further confirmed by the higher values of Absolute Growth Rate (AGR) and Relative Growth Rate (RGR) for most of the evaluation period for the fruits that received this treatment compared to the others (Figure 3B and 3C). Additionally, it's notable that the inflection point (x0) in this treatment occurs later, indicating a longer delay in the decline of AGR (**Table 1**).

All tested CPA doses reduced the length of fruits with crowns (Figure 3D, Table 1). This can be observed by the lowest values of the 'a' parameter in the regression model fitted to estimate fruit growth over time (Table 1). In the model adjusted to estimate the growth of fruits that received the control treatment, the highest inflection

Figure 3 - Growth of 'Perola' pineapple fruits over time and in response to different doses of 2-(3-chlorophenoxy) propionic acid. A - Length of fruits without crown; B - Absolute growth rate of fruits without crown; C - Relative growth rate of fruits without crown; D - Length of fruits with crown; E - Absolute growth rate of fruits with crown; F - Relative growth rate of fruits with crown; G - Length of the crown; H - Absolute growth rate of the crown; I - Relative growth rate of the crown; J - Diameter of the fruits; K - Absolute growth rate of the diameter of the fruit; L - Relative growth rate of the diameter of the fruits

point (14.8995) associated with the highest 'a' value resulted in fruits with larger crowns compared to the other treatments starting on the 18th day of evaluation. This is confirmed by the highest values of AGR (maximum value of 0.403517 cm day-1 at 15 days of evaluation) and RGR of fruits in the control treatment throughout the evaluation period (Figure 3E and F). Therefore, there is a reduction in the length of fruits with crowns, as well as their respective **Table 1-** Parameters estimated from the nonlinear sigmoidal model for Fruit Length with Crown, Fruit Length without Crown, Crown Length, and Fruit Diameter of 'Perola' Pineapple in response to days after the application of 2-(3-chlorophenoxy) propionic acid (3-CPA).

Adjusted parameters for the sigmoid model: a = maximum value of fruit length, crown, and fruit diameter; b = slope of the curve within the range of days of evaluation where the greatest variation in fruit length, crown, and fruit diameter occurs; x0 =
inflection point of the curve where the maximum rate of length and diameter occurs. Significance: ***(0.001), **(0.01), *(0.05), º (0.1) ns (Not Significant).

AGR and RGR, in response to CPA application.

The use of CPA reduced the length of fruits with crown due to the reduction in crown length (Figure 3G). This effect is pronounced in this variable and can be evidenced both by the parameters of the adjusted models, especially 'a' and 'x0' (Table 1), and in AGR and RGR (Figure 3H and I). In Figure 3H, the AGR changes its

behavior, decreasing in value as the CPA doses increase. In the control treatment $(0 \text{ mg } L^{-1})$, the crown's AGR reaches its maximum point (0.2385 cm day-1) between the 25th and 26th day after treatment. As the CPA dose increases, the AGR value decreases, at least for most of the evaluation period. As for the RGR of crown length (Figure 3I), a decline in values is observed in all treatments, as expected. However, the decline becomes more pronounced in response to the CPA doses.

Regarding the growth of fruit diameter, it is evident that the behavior of this variable is quite similar among the studied CPA doses (Figure 3J and Table 1), as well as their respective AGR and RGR (Figures K and L). Therefore, it can be observed that CPA does not have an effect on increasing the fruit diameter.

The results observed in Figure 3 and Table 1 may indicate that fruits treated with CPA are reaching their maximum growth in a shorter time, given the short duration of the highest absolute and relative growth rates of the fruits. This effect can make the fruit an even stronger sink, causing the translocation of assimilates to it and not to the crown, resulting in a reduction in weight and length of this structure. The stage of crop development influences the allocation of assimilates in the plant (Wu et al. 2013), and since the fruit is the main sink and a strong sink, most of the assimilates are being directed to its development.

The final crown length and weight of 'Pérola' pineapple fruits were also affected by the applied CPA doses. With an increase in the dose, there is a linear reduction in crown length of 0.0568 cm for every mg L-1 of CPA, reaching 5.03 cm with the highest dose (160 mg L-1) (**Figure 4**a). Similarly, a decrease in crown weight of the fruits in response to CPA doses can be observed. In this case, for every mg $L⁻¹$ of the product applied, the crown decreases by 0.4569 g, reaching a minimum value of 5.37 g at the dose of 160 mg L-1 (Figure 4B). Results of

weight reduction and crown length are also found in other studies on the application of 3-Chlorophenoxy-2-propionic acid (3-CPA) to pineapple fruits (Fahl and Franco 1981, Rebolledo-Martinez 2002).

The doses applied to 'Pérola' pineapple fruits were not significant for the length of fruits measured without the crown $(\hat{Y} = 25.24)$ (p > 0.05). However, since there was a linear reduction in crown length, the length of fruits with crowns was also affected by the doses of 3-Chlorophenoxy-2-propionic acid (3-CPA). It can be observed that with the increase in the 3-CPA dose, there is a linear reduction of 0.0627g in fruit length, reaching the smallest size of 29.29cm at the highest applied dose (160 mg L-1) (**Figure 5**).

Figure 5 – Length of 'Pérola' pineapple fruits with crowns as a function of the doses of 3-Chlorophenoxy-2-propionic acid (3- CPA). Significance: ***(0.001), **(0.01).

The results of the length of fruits with crowns differ slightly from the findings of Rebolledo-Martinez (2002), where there was a tendency for the fruit length to increase with higher auxin doses. However, in the present study, this did not happen, possibly due to the crown, as it was the part of the plant most affected by the treatments and was measured together with the fruit.

The crown-to-fruit ratio was also affected by the CPA doses used. Similarly to the behavior of the physical assessments of the crown, there is also a reduction in the crown-to-fruit ratio of 0.0022 for each 1 mg L-1 of CPA applied. The highest crown-to-fruit ratio was obtained at the 0 mg $L⁻¹$ dose (0.56), and the lowest ratio was at the 160 mg L-1 dose (0.21) (**Figure 6**).

This result can be explained by the previously described results regarding the crown length, which reduced due to the application of CPA without a significant effect on the length of the fruit without a crown. Consequently, this leads to a reduction in the proportion between the crown and the fruit. Additionally,

Figure 6 – Crown-to-fruit ratio of 'Perola' pineapple fruits as a function of the doses of 2-(3-chlorophenoxy) propionic acid (3- CPA). Significance: **(0.01), º (0.1).

a lower crown-to-fruit ratio is associated with the stronger vigor of the pineapple plant during flowering induction, as it produces larger fruits with a smaller crown weight (Hotegni et al. 2015).

The weight and yield of 'Pérola' pineapple fruits, both with and without crowns, were influenced by the applied doses of CPA. A cubic trend was observed with the increasing concentration of propionic acid (**Figure 7**). Two points of increase in these variables can be observed. The first one between the doses of 0 and 40 mg L⁻¹, and the second between the doses of 120 to 160 mg L-1. In the dose range from 40 to 120 mg L⁻¹, there appears to be a stabilization of fruit weight and yield.

This effect may be related to the compartmentalization of auxin within the plant cell (Casanova-Sáez 2019, Suman et al. 2017, Taiz et al. 2017), preventing the biological response of cells to auxin from continuing at the same intensity, leading to what can be characterized as a stationary phase. This could result in the stability of fruit weight and yield at intermediate doses. However, with the increase in the CPA dose, the plant cell may lose this ability to compartmentalize synthetic auxin, resulting in a new increase in fruit weight and yield as a consequence of the increased CPA dose applied.

One can also observe a more pronounced increase in the weight and yield of fruits without crowns. The initial weight of these fruits was lower when compared to the initial weight of fruits with crowns. However, at a dose of 40 mg L⁻¹, it already reached a similar weight and yield, just like at the highest dose (160 mg L⁻¹) with a fruit weight of 1900 g (Figure 7A) and a yield of 90 t ha-1 (Figure 7B). The exogenous application of auxin plays a stimulating role in fruit development since it is a strong sink, and thus, photoassimilates are translocated for its development, increasing its weight, as found in the studies of Suman et al. (2017). The author also explains that the plant

Figure 7 - (A) - Weight of fruits with and without crowns, and (B) - total and commercial yield of 'Pérola' pineapple in response to doses of 2-(3-chlorophenoxy) propionic acid. Significance: ***(0.001), **(0.01), *(0.05), º (0.1)

balances its hormonal content by mobilizing nutrients to developing organs, allowing for greater weight gain in fruits subjected to auxin doses, consequently resulting in higher production in the harvest.

The lower weight and length of the crown with increased fruit weight may be due to the exogenous application of auxin. The addition of this hormone can influence carbohydrate translocation in the plant, stimulating the mobilization of photosynthates from the leaves and crown towards the fruit (Albacete et al. 2014), resulting in larger and heavier fruits (**Figure 8**) and a shorter crown weight and length (Figure 4ab).

Figure 8- Pineapple fruits (cultivar 'Pérola') treated with different doses of 2-(3-chlorophenoxy) propionic acid (3-CPA), Janaúba, MG.

Furthermore, fruits with smaller crown size have greater acceptance in the foreign market due to a higher proportion of edible parts, which also facilitates storage and transportation (Sampaio et al. 1997). Similar results with reduced crown length and weight were also reported by Fahl and Franco (1981) and Rebolledo-Martinez (2002).

The harvest index was affected by the CPA doses, showing a quadratic behavior in response to the

propionic acid doses. An increase in the harvest index is observed, from 0.50 at the 0 mg L-1 dose to 0.55 at the 93.2 mg L⁻¹ dose, which provided the maximum value for this variable. These results suggest that the plant became more efficient in translocating dry matter (carbon) to the fruit. However, starting from the 93.2 mg L⁻¹ dose of CPA, a reduction in the harvest index value is observed, reaching 0.52 at the 160 mg L-1 dose (**Figure 9**).

Figure 9 – Harvest index of 'Pérola' pineapple fruits as a function of 2-(3-chlorophenoxy) propionic acid (3-CPA) doses. Janaúba-MG. Significance: ***(0.001), *(0.05).

 The pineapple plant has the ability to allocate and compartmentalize auxin present in its system. This process is involved in controlling the growth and development of fruits under specific conditions (Su et al. 2017, Zhao et al. 2021). Therefore, the plant's efficiency in translocating carbohydrates to the fruits increases up to the 93 mg L⁻¹ dose. However, as observed in Figure 2, there was an increase in the weight of leaf D, which has a positive correlation with the weight of the plant (Vilela et al. 2015). This indicates that, from the 93 mg L-1 dose, there was a proportionally greater increase in plant weight compared to the increase in fruit weight (Figure 6), contributing to the reduction of the harvest index. Therefore, 3-CPA alters translocation patterns, even varying with the dose used.

The applied doses of 3-CPA did not significantly affect (p>0.05) the pH (\hat{Y} = 3.5), titratable acidity (\hat{Y} = 0.68 mg 100g-1), and pulp firmness of pineapple fruits $(\hat{Y} = 11.68 \text{ N})$. However, the soluble solids content was influenced by the 3-CPA treatments. It was observed that with an increase in 3-CPA dose, there was a reduction of 0.0096 ºBrix in the fruits, decreasing from 12.2 ºBrix at 0 mg L-1 to 10.7 ºBrix at 160 mg L-1 (**Figure 10**).

Figure 10 – Soluble solids content (ºBrix) of 'Perola' pineapple fruit pulp as a function of 3-chlorophenoxy-2-propionic acid doses. Significance: ***(0.001), º (0.1).

It can be observed that in all treatments, the soluble solids values are below those observed for this variable and genotype in semi-arid Brazilian cultivation (Mota et al. 2021, Oliveira et al. 2021) and the minimum required value for pineapple commercialization in Brazil is 12 ºBrix (Ceagesp 2003).

It is possible that the low total soluble solids (TSS) content is related to the environmental conditions under which the treatments were applied, with a minimum temperature of 16.9 and a maximum of 30.5 (Figure 1). In an experiment conducted in Mexico, a reduction in TSS content was observed when treatments (different doses of CPA) were applied under winter environmental conditions, with a minimum temperature of 16.2 and a maximum of 28.9°C (Rebolledo–Martinez et al., 2002). However, when these authors tested the same concentrations under spring conditions (with a minimum temperature of 20.9 and a maximum of 36.6°C), they found that TSS levels increased.

The reduction in TSS content observed in the study may be a consequence of the fruit's growth induced by CPA treatments. One of the biological effects of auxin is cell division and elongation (Taiz et al. 2017). Therefore, the increase in the quantity and size of cells may have led to a dilution effect on sugar concentration, resulting in lower TSS content. An additional possibility is the delay in the fruit's ripening process, which, even with the external appearance of being ripe (skin color), may not have completed the pulp's ripening process entirely. Other

non-climacteric fruits also exhibit similar ripening delay responses to synthetic auxin application (Pattison et al. 2014).

Conclusions

The length and weight of the crown in 'Pérola' pineapple fruits are reduced with the application of 2-(3-chlorophenoxy) propionic acid (3-CPA). Additionally, fruits treated with 3-CPA reach their maximum weight in less time and achieve the highest harvest index at a dose of 93 mg L-1. However, the product reduces the crown-to-fruit ratio and the total soluble solids content of the pineapple fruits. For fresh consumption, the dose of 40 mg L-1 provides fruits that are more marketable and acceptable.

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