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Studying the impact of foliar fertilization with calcium and silicon close to harvest on pineapple physico-chemical characteristics

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Abstract

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Calcium is an essential mineral for pineapple development and quality. On the other hand, silicon is another mineral that lately has been investigated due to its positive effects on fruit quality. Nonetheless, no sufficient information has been document in pineapple, primordially with applications close to harvest, when pineapple plant tends to exposed mineral deficiencies. Therefore, this study aimed to evaluate the effect of foliar fertilization with calcium and silicon close to harvest on pineapple physico-chemical characteristics. The treatments arranged were, A (control: Without fertilization), B (Ca from ten weeks before harvest until harvest), C (Ca from six weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest), E (Si from six weeks before harvest until harvest), F (Ca + Si from ten weeks before harvest until harvest), and G (Ca + Si from six weeks before harvest until harvest). MD2 pineapple hybrid was used in this experiment. Fruit total soluble solids, total acidity, sugar, acid, water content, β-carotene, fruit and crown weight, and flesh firmness were determined in two experimental trials. Treatment D delivered the best performance by obtaining an ideal level of total soluble solids, water, sugar and acid content, fruit and crown weight, and flesh firmness. Besides, this treatment provided the highest citric acid ($\geq 0.6\%$) and β-carotene content (≥ 3.5 mg/kg), representative antioxidants in pineapple. In conclusion, the employment of silicon close to harvest, from ten weeks before harvest until harvest can be used as an ideal treatment to provide an optimal pineapple quality.

Keywords: Antioxidant; MD2; rainfall; stress; waterlogging

Introduction

Pineapple (*Ananas comosus* L. Merr.) is a valuable crop in many tropical areas, where low acid hybrids have become the priority to be cultivated and exported; however, these hybrids have risen new challenges to growers and shippers (Sipes & Pires de Matos, 2018; Cano-Reinoso et al., 2021a). For example, susceptibility to natural flowering, shell burning, and excessively low acidity are typical problems these hybrids exhibit, causing physical and physiological disorders (Cano-Reinoso et al., 2021a, 2022a). Due to these inconveniences, there is a need to control the adequate mineral levels in pineapple plant tissues, especially those essentials to obtain fruits with optimal physico-chemical characteristics.

Calcium is a vital mineral in pineapple plant during its initial crop development because of its influences in cell division and differentiation, delivering cellular stability (Pires de Matos, 2019; Sipes & Pires de Matos, 2018). Calcium helps to maintain an adequate cell wall structure in the fruit, preventing physiological disorders like water soaking, flesh translucency and internal browning (Cano-Reinoso et al., 2021b, 2022a). Furthermore, recently several studies have remarked the importance of this mineral in fruit sugar and acid accumulation, affecting its quality positively (Žemlička et al., 2013; Kleemann, 2016). For example, pre-harvest calcium applications have been associated with an increase in the acid content and reduction of internal browning in pineapple, extending its shelflife significantly (Uthairatanakij & Jitareerat, 2015).

Calcium (Ca^{2+}) is a mineral mobile in the xylem but not in the phloem (Žemlička et al., 2013; Soteriou et al., 2014). Therefore, because the fruit is considered the main sink for the plant, the fruit's calcium content typically decreases with pineapple ripening (Madani et al., 2016; De Freitas & Resender Nassur, 2017). On top of that, the pineapple plant after flowering usually does not assimilate significant quantities of minerals by the root absorption system (De Freitas & Resender Nassur, 2017; Cano-Reinoso et al., 2022b). For that reason, foliar applications of elements like calcium are becoming a necessary solution to supply the fruit under mineral deficiencies, essentially during the period close to harvest (De Freitas & Resender Nassur, 2017; Cano-Reinoso et al., 2022b).

For example, pineapple low acid hybrids from planting to flower induction can employ between 14 and 18 months, especially in tropical areas. Thereafter, from flowering to harvest, this period can take between 20 and 22 weeks (Bartholomew & Sanewski, 2018). After flower induction the plant stop the formation of new leaves, directing almost all its photo-assimilates into the fruit sink, like sugars, acids and vitamins. As a consequence, due to this shift in the plant metabolism, a deficient of essential minerals can be exhibited during the last stage of fruit development, primordially close to harvest (Cano-Reinoso et al., 2022b; Vásquez-Jiménez & Bartholomew, 2018).

On the other hand, another mineral that has become important for plant physiology studies is silicon due to its outstanding signalling regulator characteristics, usually influencing the cell and membrane properties (Liang et al., 2015; Artyszak, 2018; Laane, 2018). Although silicon's metabolism and its association with the cell constitution are still under study, evidence suggests its relevant impact when used as a foliar fertilizer (Liang et al., 2015). Furthermore, experiments have proved that crop quality improvements are caused by silicon foliar applications (Liang et al., 2015). Typically, in fertilization with stabilized silicic acid, the root, plant growth, yield, and quality have been enhanced, especially in monocots (Liang et al., 2015). For instance, an increase in the sugar and acid content, together with the improvement of shelflife was detected in mandarin and mango using pre-harvest spraying of silicon (Laane, 2018). Nevertheless, not many papers have been reported having clear information about silicon's impact on pineapple quality; and some of them were focused more on its positive influences on the fruit mineral status (Barral et al., 2017; Cano-Reinoso et al., 2022b). As a result, more studies are needed to establish the effect of this mineral on pineapple physico-chemical properties.

Because of the documented beneficial effects on plant and fruit physiology, the employment of silicon and calcium, primordially during the last stages of the fruit development, arises as a possible solution to produce pineapples with optimal quality. For this reason, this study aims to evaluate the effect of foliar fertilization with calcium and silicon close to harvest on pineapple physico-chemical characteristics, focused on its effect in a low acid hybrid.

Material and Methods

Experiment design and treatments

This research was conducted in Lampung, Indonesia, located in the south of Sumatra island, in a pineapple plantation during 2020. MD2 pineapple was employed for this experiment. MD2 is a low acid hybrid attractive to consumers due to its yellow shell colour characteristics, higher sugar content, and uniformity; moreover, nowadays it is the hybrid more exported as fresh fruit worldwide (Bin Thalip et al., 2015; Paull & Chen, 2018). The harvesting was done between 144 and 147 days after flower induction when it is considered MD2 can exhibit its best physico-chemical characteristics (Bin Thalip et al., 2015; Ding & Syazwani, 2016). This experiment was set in the field, employing two trials. Trial one from February to April and the second trial from May to July of 2020. The research was elaborated when the rainy season was influencing the pineapple cultivation area. The data in Table 1 show the physical and chemical characteristics of the soil in the plantation, similar to the conditions reported in the previous experiment of Cano-Reinoso et al. (2022b).

In the same way described by Cano-Reinoso et al. (2021b) and (2022b), the soil was fertilized with 200 kg/ha diammonium phosphate, 1000 kg/ha K_2SO_4, and 200 kg/ha Kieserite crystal during row preparation; thereafter, foliar applications of 700 kg/ha Urea, 700 kg/ha $(NH_4)_2SO_4$, 1000 kg/ha K_2SO_4, 170 kg/ha $MgSO_4$, 60 kg/ha $FeSO_4$, and 60 kg/ha $ZnSO_4$, were done from three months after plating in intervals of 30 days. Finally, during and after the flower induction, liquid Ethepon and Borax were sprayed in dos-

Table 1. Physical and chemical characterization of the soil in the research

Texture	1st Trial	2nd Trial
Clay, %	8.00	21.92
Loam, %	39.56	10.72
Sand, %	52.44	67.36
Chemical properties*	1st Trial	2nd Trial
pH (H_2O)	7.69	5.33
C, %	3.64	0.90
N, mg/kg	950.00	830.00
P, mg/kg	1.32	11.50
K, mg/kg	6.68	156.00
Ca, mg/kg	66.30	212.00
Mg, mg/kg	104.00	100.80
Na, mg/kg	10.80	6.90

*The *N, P, K, Ca, Mg and Na represent the available mineral content in the soil. Soil data taken from Cano Reinoso et al. (2022b)

es of 2.5 L/ha and 30 kg/ha, respectively. A weather station (LSI Lastem, equipped with a CR6 datalogger of Cambell scientific, Italy) measured an average of 71.66% of relative humidity (RH), 23.45°C of ambient temperature, and 16.83 w/m^2 of solar radiation, associated with the first trial; while for the trial two, these values were in average 89.34% RH, 26.8°C, and 9.18 w/m^2, respectively. The monthly rainfall data through the experiment time linked to both trials are presented in Table 2, similar to the conditions reported in Cano-Reinoso et al. (2022b).

Table 2. Data of rainfall in every month through the research

Month, mm	1st Trial	Month, mm	2nd Trial
February	457.7	May	106.4
March	282.1	June	199.9
April	262.9	July	95
Cumulative, mm	1002.7	Cumulative, mm	401.3

*Rainfall data taken from Cano-Reinoso et al. (2022b)

Furthermore, in the same manner described by Cano-Reinoso et al. (2022b), the experiment design and treatments administrated were as follows: A randomized complete block design was used. Seven treatments with four replications were implemented with 44 fruits per replication. Seven rows in each block with a width and length of 0.4 and 3.75 m were prepared in the field. Pineapple plants were organized in two lines of 22 plants inside the row with a separation of 0.25 m. Observations were realized once every two weeks, from eight weeks before harvest. The treatments were implemented close to harvest time, during the last stages of the fruit development, based on Bartholomew & Sanewski (2018). The organization was: A (Control: Without fertilization of Ca and Si), B (Ca from ten weeks before harvest until harvest), C (Ca from six weeks before until harvest), D (Si from ten weeks before harvest until harvest), E (Si from six weeks before harvest until harvest), F (Ca + Si from ten weeks before harvest until harvest), and G (Ca + Si from six weeks BH until harvest).

Calcium product used was Calcibor (12.9% w/v CaO and 2.6% w/v B) in doses of 4 L/ha (v/v = 4 L/2000 L); meanwhile, the silicon product employed was NewSil (0.8% w/v Silicic acid – Si $(OH)_4$, 0.18% w/v H_3BO_3, 49% w/v Polyethylene glycol) in doses of 1.5 L/ha (v/v = 2 mL/L). Calcium and silicon were sprayed on the fruit shell and crown at night, following the information about uptake and mobility of minerals after flower induction in pineapple plants and fruit (Cano-Reinoso et al., 2021b, 2022b).

Total soluble solids (TSS) and total acidity (TA) content

The TSS and TA content was determined according to the procedure described in Shamsudin et al. (2020), as a composition of four fruits per replication of each treatment implemented. The TSS was calculated using a hand-held refractometer (MASTER-53 α; Atago, Japan). Meanwhile, TA was measured by titration to pH 8.1 with 0.1 M NaOH using phenolphthalein as an indicator and expressed as a percentage of citric acid.

Fruit water content and β-carotene

The fruit water content was calculated based on the method described in Siti Roha et al. (2013) and Cano-Reinoso et al. (2022c). Twenty-five grams of a composition of four fruits per replication of every treatment were oven-dried at 60°C for 24 h to constant weight for moisture determination. In the case of β-carotene, its content was calculated according to the method reported in Owolade et al. (2017). A juice taken from the adjacent part to the flesh core was employed. Like the water content, the samples were collected as a composition of four fruits per replication from each of the treatments arranged. Consequently, 25 mg of β-carotene were weighed and dissolved in 2.5 mL of chloroform and diluted to 250 mL, using petroleum ether. After that, concentrations of 2, 10, 20, 30, 40 and 50 mg/L were employed for the subsequent absorbance examination using a spectrometer (Spectroquant® Pharo 300, Thomas Scientific, USA) at 452 nm with 3% of acetone in petroleum ether as blank. The β-carotene content was determined using the respective standard curve.

Fruit sugar and acid content

Pineapple acid and sugar content was calculated using the method described in Siti Roha et al. (2013) and Cano-Reinoso et al. (2022c), employing a High-Performance Liquid Chromatography (HPLC) – (Hitachi, USA) model L-2000 instrument with a Refractive Index detector model L-2490. Briefly, juice extracted from the fruit flesh adjacent to the core was used for this determination. Samples were obtained from a composition of four fruits per replication of each of the treatments implemented. For the sugar content, standard solutions of 500, 1000, 1500, 2000 mg/L of glucose, fructose, and sucrose were prepared with the objective of developing a curve of sugar level; in the case of the acids, a standard solution of 1000 mg/L was used for the citric and malic acid detection, having the same goal in mind. The standard solutions were dissolved in distilled water and filtered through a Millipore 0.45 µm membrane filter. The sugars and acids were quantified, comparing the peak area by a chromatographic procedure.

Firmness and weight of the fruit and crown

The flesh firmness, fruit (including crown) and crown weight were determined based on the procedure documented in Cano-Reinoso et al. (2021b). The firmness was measured in the centre region of each fruit, adjacent to the core, employing four fruits for every replication of each treatment used. A penetrometer (CT3 texture analyzer, BROOKFIELD AMETEK, USA) with a 7 mm diameter flat probe was manipulated, applying the respective test parameters (regular test, trigger: 10 g, deformation: 7 mm, and speed: 1.0 m/s); the results are expressed in N. Moreover, the fruit and crown weight was obtained using weighing scales in four fruits per replication of each treatment.

Statistical analysis

Statistical analyses were performed using SPSS Version 22.0 software (SPSS Inc., Chicago, IL, USA). All data were analyzed by ANOVA. Mean significant differences at $P < 0.05$ were determined by Duncan's multiple range test.

Results and Discussion

TSS and TA content

The TSS exposed significant differences in the outcomes of both trials. In trial one, the highest value was obtained in treatment E (16.27%) and the most reduced in F (14.98%); for trial two, E had the most superior outcome again (14.20%), with the lowest in B (13.10%). Thus, the TSS results of trial one were higher than trial two (15% and 13%, respectively). These outcomes suggested a beneficial influence on TSS from silicon fertilization six weeks before harvest (Table 3).

The TSS for pineapple low acid hybrids should be as minimal as 12% (Lu et al., 2014; Vásquez-Jiménez & Bartholomew, 2018). This minimal requirement was obtained

Table 3. Influences of the treatments applied on the physico-chemical characteristics of the fruit at harvest

Physico-chemical variables 1st trial				
Treatment	TSS, %	TA, %	Water content, %	β-carotene, mg/kg
A	15.69 ± 0.38 ab	0.87 ± 0.04 ab	94.01 ± 0.27 ab	3.04 ± 0.13 ab
B	15.37 ± 0.39 ab	0.78 ± 0.03 ab	93.90 ± 0.14 ab	2.76 ± 0.12 bc
C	14.96 ± 0.16 b	0.73 ± 0.04 c	94.03 ± 0.14 ab	3.07 ± 0.07 ab
D	15.16 ± 0.17 b	0.92 ± 0.02 a	93.85 ± 0.08 ab	3.21 ± 0.15 a
E	16.27 ± 0.56 a	0.81 ± 0.03 bc	94.50 ± 0.38 a	2.61 ± 0.04 c
F	14.98 ± 0.20 b	0.93 ± 0.02 a	94.17 ± 0.42 a	3.02 ± 0.13 ab
G	15.40 ± 0.19 ab	0.92 ± 0.01 a	93.23 ± 0.17 b	2.78 ± 0.02 bc
Physico-chemical variables 2nd trial				
Treatment	TSS, %	TA, %	Water content, %	β-carotene, mg/kg
A	13.83 ± 0.33 ab	0.64 ± 0.03 cd	93.55 ± 0.27 ab	3.45 ± 0.15 cd
B	13.10 ± 0.33 b	0.62 ± 0.03 d	93.19 ± 0.14 ab	2.83 ± 0.12 e
C	13.60 ± 0.14 ab	0.62 ± 0.04 d	93.81 ± 0.14 a	3.26 ± 0.07 d
D	13.55 ± 0.15 ab	0.80 ± 0.01 a	93.40 ± 0.08 ab	4.05 ± 0.19 ab
E	14.20 ± 0.48 a	0.71 ± 0.03 bc	93.85 ± 0.38 a	3.68 ± 0.06 bc
F	13.60 ± 0.18 ab	0.75 ± 0.02 ab	93.93 ± 0.42 a	4.39 ± 0.19 a
G	13.90 ± 0.17 ab	0.75 ± 0.01 ab	92.88 ± 0.17 b	3.53 ± 0.03 cd

*Each value represents a mean ± standard error. Mean values in each column followed by the same lower-case letters are not statistically different by Duncan's multiple range test ($P < 0.05$).

**A (Control: Without fertilization of Ca and Si), B (Ca from ten weeks before harvest until harvest), C (Ca from six weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest), E (Si from six weeks before harvest until harvest), F (Ca + Si from ten weeks before harvest until harvest), and G (Ca + Si from six weeks before harvest until harvest) for all outcomes of each treatment in both trials. Research on pre-harvest silicon fertilization on pineapple demonstrated that this mineral increased the TSS content at harvest and retarded its degrading during postharvest (Weerahewa & Wicramasekara, 2020). Similar results were obtained in tomato cherry (Islam et al., 2018) and banana (Hanumanthaiah et al., 2015). Although treatments D, F and G employed pre-harvest silicon applications, the more superior result delivered by treatment E in trials one and two indicates that those applications are more effective in increasing TSS if done closer to harvest.

Like TSS, the TA content in the fruit exposed significant differences in the outcomes of both trials. The highest value between 0.92 and 0.93% was obtained in treatments D, F and G for the first trial, while in the second trial, this value was 0.8% but just for the treatment D. In both trials, the most reduced value was obtained in treatment C (0.73% in trial one, 0.62% in trial two). In this variable, the mean value was higher in the first trial than in trial two (0.8% on average for trial one, 0.65% on average for trial two) (Table 3). Moreover, in this case, an increased impact of silicon fertilization could be observed, especially under the application of ten weeks before harvest.

A TA value Between 0.4 and 0.7% is recommended for pineapple low acid hybrids (Paull & Chen, 2018; Cano-Reinoso et al., 2022a). The results obtained in this research were higher than the recommended values in almost all the treatments of trials one and two. The available information concerning pre-harvest foliar silicon and calcium fertilization suggests some increase in this variable (Benítez et al., 2014; Weerahewa & Wicramasekara, 2020; Cano-Reinoso et al., 2021b). In this research, silicon fertilization ten weeks before harvest (treatment D) enhances the TA accumulation in pineapple, over the ideal range; also, calcium fertilization either, ten or six weeks before harvest (treatment B and C), encourages the TA accumulation, although between the suggested optimal range. Therefore, to have an ideal quality of TA, foliar fertilization with calcium close to harvest is sufficient and should not be mixed with silicon because this mineral could increase the TA level over non-desired values.

On top of that, the most elevated TSS and TA content in the first trial results could be attributed to an environmental factor that could cause abiotic stress to the plants. As reported by Cano-Reinoso et al. (2022b), this abiotic stress could be associated with the monthly rainfall during trial one development (Table 2). The monthly rainfall necessary for optimal pineapple plant development should be between 50 and 100 mm (Carr, 2012; Vásquez-Jiménez & Bartholomew, 2018; Cano-Reinoso et al., 2022b). The outcomes of the monthly rainfall for both trials were over that reported range; nevertheless, the values of the first trial were superior, and the cumulative result was higher than trial two (1002.7 mm, 401.3 mm, respectively). In pineapple, a crassulacean acid metabolism (CAM) plant, high rainfall levels can cause waterlogging stress (Carr, 2012; Vásquez-Jiménez & Bartholomew, 2018; Cano-Reinoso et al., 2022b). Typically, plants suffering from waterlogging stress generate a reduction in the stomatal conductance, producing water uptake limitations in roots, more apoplast water accumulation due to the impact on the plant hydraulic conductivity, affecting the photo-assimilates assimilation in the fruit (Irfan et al., 2010; Muhammad Arslan Ashraf, 2012). These circumstances could explain the elevated TSS and TA acidity values in the first trial, despite being in an ideal quality range. The photo-assimilates linked to the TSS and TA level in fruit were increased due to the more apoplast water availability and the outcomes obtained. Besides, as there are not sufficient experiments reported about the effects of calcium and silicon foliar fertilization close to harvest on TSS and TA in pineapple fruit, and because the previous studies employed different doses and application times, future researches are suggested to determine a clear impact on these variables.

Fruit water content and β-carotene

The water content of the fruit delivered a similar mean value in both trials (between 93 and 94%) and provided significant differences. Treatment E and F had the most superior values of water content in both trials (94.50 and 94.17% in trial one, 93.85 and 93.93% in trial two); meanwhile, treatment B provided the most reduced outcomes (93.23 and 92.88%, respectively) (Table 3). These results suggested an influence of silicon fertilization increasing the water content, but with a minor effect when mixed with calcium six weeks before harvest. In pineapple, the moisture content of the fruit can be around 91%, which can increase regarding the maturity stage, in which the fruit is harvested (Ding & Syazwani, 2016; Loh et al., 2020). The water content results of this experiment in both trials are around this value. The results were higher than 91% because of the days after flower induction, in which the fruit was harvested. It is possible to observe that most treatments had a similar water content value in each trial, except for treatment G, especially in trial two. Although all the outcomes obtained in this experiment can be considered as ideal concerning the fruit quality, it can be inferred that the mix of foliar fertilization of calcium and silicon six weeks before harvest does not accumulate the moisture content with the same intensity that the other treatments, especially under waterlogging stress conditions, like the trial two.

In the case of β-carotene, this variable also had similar mean values in both trials (mostly between 2.5 and 3.5 mg/

Table 4. Influences of the treatments applied on the sugar and acid content of the fruit at harvest

Sugar and acid content 1st trial					
Treatment	Fructose, %	Glucose, %	Sucrose, %	Citric acid, %	Malic acid, %
A	1.65 ± 0.04 d	1.02 ±0.03 b	6.32 ± 0.13 d	0.33 ± 0.01 d	0.06 ± 0.11 ab
B	1.90 ± 0.07 c	1.05 ±0.09 b	9.12 ± 0.08 a	0.22 ± 0.01 e	0.09 ± 0.011 a
C	2.11 ± 0.02 bc	1.26 ±0.03 a	6.45 ± 0.15 d	0.16 ± 0.00 f	0.07 ± 0.02 ab
D	2.09 ± 0.03 bc	1.29 ±0.07 a	7.42 ± 0.04 c	0.83 ± 0.01 a	0.08 ± 0.04 ab
E	2.38 ± 0.06 a	1.41 ±0.03 a	6.44 ± 0.17 d	0.47 ± 0.01 c	0.04 ± 0.00 b
F	2.30 ± 0.17 ab	1.35 ±0.11 a	6.60 ± 0.20 d	0.30 ± 0.01 d	0.06 ± 0.00 ab
G	1.99 ± 0.07 c	1.22 ± 0.08 ab	8.28 ± 0.11 b	0.68 ± 0.04 b	0.06 ± 0.01 ab
Sugar and acid content 2nd trial					
Treatment	Fructose, %	Glucose, %	Sucrose, %	Citric acid, %	Malic acid, %
A	1.57 ± 0.04 ab	1.50 ± 0.05 ab	9.76 ± 0.16 ab	0.16 ± 0.01 c	0.05 ± 0.01 ab
B	1.42 ± 0.05 b	1.29 ± 0.11 b	9.54 ± 0.09 ab	0.18 ± 0.01 ab	0.10 ± 0.01 a
C	1.62 ± 0.01 a	1.55 ± 0.04 a	9.76 ± 0.22 ab	0.17 ± 0.01 bc	0.08 ± 0.02 ab
D	1.65 ± 0.02 a	1.47 ± 0.08 ab	9.55 ± 0.05 ab	0.20 ± 0.00 a	0.07 ± 0.03 ab
E	1.65 ± 0.04 a	1.50 ± 0.03 ab	9.91 ± 0.26 a	0.19 ± 0.01 ab	0.04 ± 0.02 b
F	1.71 ± 0.13 a	1.52 ± 0.13 ab	9.18 ± 0.28 b	0.19 ± 0.01 ab	0.06 ± 0.00 ab
G	1.59 ± 0.06 ab	1.51 ± 0.10 ab	9.82 ± 0.14 a	0.19 ± 0.01 a	0.06 ± 0.01 ab

*Each value represents a mean ± standard error. Mean values in each column followed by the same lower-case letters are not statistically different by Duncan's multiple range test ($P < 0.05$).

**A (Control: Without fertilization of Ca and Si), B (Ca from ten weeks before harvest until harvest), C (Ca from six weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest), E (Si from six weeks before harvest until harvest), F (Ca + Si from ten weeks before harvest until harvest), and G (Ca + Si from six weeks before harvest until harvest)

kg), with representative differences exposed. Treatment D gave the most superior value in trial one, while in trial two, it was F (3.21 and 4.39 mg/kg, respectively); although in trial two, D also had a considered high result (4.05 mg/kg). Besides, the lowest values were observed in F for this first trial and in B for trial two (2.61 and 2.83 mg/kg, respectively) (Table 3). Therefore, it is possible to infer a representative silicon fertilization impact, increasing or decreasing the β-carotene in the fruit. Foliar silicon fertilization ten weeks before harvest (treatment D) could be considered the most beneficial regarding this variable.

β-carotene is the pigment responsible for the yellow colour of the flesh in pineapple (Lu et al., 2011; Vásquez-Jiménez & Bartholomew, 2018; Steingass et al., 2020). This pigment belongs to the plant carotenoids, characterised for their scavenger properties, eliminating singlets of oxygen generated by reactive oxygen species (ROS) (Fanciullino et al., 2014; Noichinda et al., 2017; Cano-Reinoso et al., 2022c). Moreover, it has been recognized that silicon cause more beneficial impacts on plants under biotic or abiotic stress than unstressed plants (Frew et al., 2018; Majeed et al., 2019). This information can explain why the highest results of this variable in both trials were obtained in treatments that employed silicon (D and F, respectively), especially in the conditions of the first trial. Waterlogging stress in plants can cause the generation of ROS like superoxide (O^{2-}), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH) due to a reduction of O_2 assimilation in plant tissues, and the already described lower stomatal conductance and hydraulic conductivity (Irfan et al., 2010; Muhammad Arslan Ashraf, 2012). These ROS typically cause the plant cell wall degradation and constitution (De Freitas & Resender Nassur, 2017; Paull & Chen, 2018). Therefore, the plant to cope with the damage produced by these ROS in the fruits of the trial one could have employed more β-carotene as a scavenger agent, especially under some silicon influences, causing more inferior values. This fact needs to be further investigated. Although foliar silicon fertilization has been associated with the increase of antioxidant enzyme activities like catalase (CAT), peroxidase (POD) and ascorbic peroxidase (APX), which usually eliminate the singlets of oxygen produced by ROS (Liang et al., 2015; Noichinda et al., 2017); Future experiments are recommended about the influences of foliar fertilization with calcium and silicon close to harvest on fruit water content and β-carotene in pineapple.

Fruit sugar and acid content

The sugar content of the fruit delivered significant differences. The sucrose results evidenced a higher mean value in the second trial than in the first one (9% on average for trial two, 6.5% on average for trial one). Treatments A and B had the lowest fructose and glucose content in both trials; treatment E had the most superior result in the first trial, with treatment F in trial two, concerning these two sugars. For sucrose, the most reduced value was obtained in treatment A for the first trial (6.32%) and F (9.18%) for trial two, while the highest results were observed in B and E (9.12 and 9.91%, respectively) (Table 4). The second trial outcomes demonstrated that the gap between the mean average values was narrower than trial one, primordially for sucrose.

Furthermore, concerning the fructose and glucose content, fertilization with calcium and silicon can encourage their increase, although there was no evidence of this association for the sucrose content. Figure 1 shows the sugar content trend during the experiment for the treatments with the highest and lowest values in each trial. In this graphic, it can be observed that in four weeks before harvest starts a sugars differentiation, with a remarkable sucrose content increase; also, this graphic corroborated the results previously described, with the fructose and glucose content having similar outcomes in both trials and a major value of sucrose for trial two.

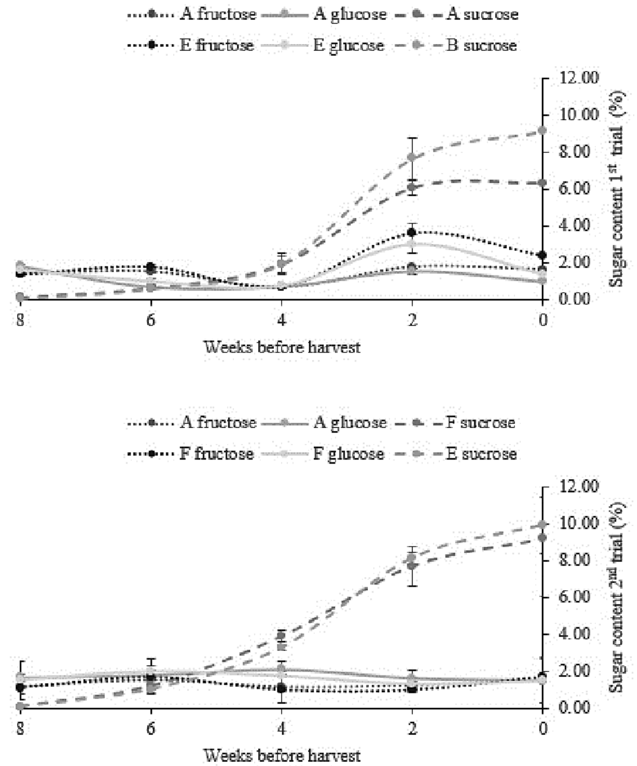

Fig. 1. The sugar content trend during the experiment for the treatments with the highest and lowest values in each trial

Treatments A and E (fructose and glucose), and A and B (sucrose) in the first trial, and treatments A and F (fructose and glucose), and F and E (sucrose) in the second trial. A (Control: Without fertilization of Ca and Si), B (Ca from ten weeks before harvest until harvest, E (Si from six weeks before harvest until harvest), and F (Ca + Si from ten weeks before harvest until harvest). Values are the mean of 4 replicates, and error bars represent the standard error

Sugar content in pineapple is associated with glucose, fructose, and sucrose. Typically, the TSS calculates the sucrose level in the fruit (Paull & Chen, 2015, 2018). The fructose and glucose content has been reported typically between 1 and 2%, while the sucrose content is between 7 and 9% (Nadzirah et al., 2013; Lu et al., 2014). The previously recommended values for fructose and glucose were achieved in this experiment. Concerning the sucrose content, in trial one, not all treatments fulfil this requirement with outcomes lower than 7%. During plant and fruit development, field environmental conditions can retard and affect pineapple sugar and acid accumulation (Bartholomew & Sanewski, 2018; Sipes & Pires de Matos, 2018). This information suggests that not all the fruit harvested had the same maturity stage in trial one. The heavy rainfall linked to waterlogging could have been the reason linked to the exhibition of this phenomenon, causing the sucrose outcomes obtained.

The apoplast sugar content in pineapple increase with fruit maturation, especially close to harvest (Paull & Chen, 2018). This circumstance can be observed in Figure 1, where the speed up on sucrose content in four weeks before harvest could be ascribed to a higher sucrose apoplast level in that stage of the fruit development, as mentioned previously by Paull & Chen (2015) and (2018). Furthermore, in trial one, treatment B had a sucrose result higher than 9%. This situation can be attributed to the influence of calcium fertilization. Calcium (Ca^{2+}) has been linked to the triggering of signalling molecules causing fruit ripening and sugar uptake like abscisic acid (ABA) and ethylene (Hocking et al., 2016; De Freitas & Resender Nassur, 2017). Besides, based on this experiment results, this phenomenon could be more remarkable if the calcium application is executed ten weeks before harvest. Moreover, Paull & Chen (2003, 2018) have reported a high sucrose accumulation in pineapple fruit using pre-harvest foliar calcium fertilization.

The highest result of treatment E in trial two and G in both trials suggested a silicon influence. Although there is no available information on silicon effects on sugar content in pineapple, a beneficial effect in sugar accumulation and uptake has been determined in mandarin (Laane, 2018) and sugar beet (Artyszak, 2018). Nevertheless, it is still unclear how silicon impacts fruit sugar assimilation and the optimal doses and moment of application necessary for it; for example, in this experiment, not all treatments employing silicon under the stress condition of trial one generated a more superior sucrose content.

In the case of the acid content, significant differences were observed in their outcomes. The mean value of the citric acid was higher in the first trial than in trial two, although it delivered a high variability, while for the malic acid, this circumstance was not exhibited. For the citric acid, the treatment D had the most superior value in trials one and two (0.83 and 0.20%, respectively); besides, the most reduced outcomes were observed in treatment B for the first trial and A for trial two (0.22 and 0.16%, respectively). In the malic acid, treatment B had the highest results in both trials (0.09 and 0.10%, respectively), and E had the most reduced outcomes (0.04% in both trials) (Table 4). These results suggested that silicon fertilization ten weeks before harvest can encourage citric acid accumulation in pineapple, while the calcium applications could not cause this situation.

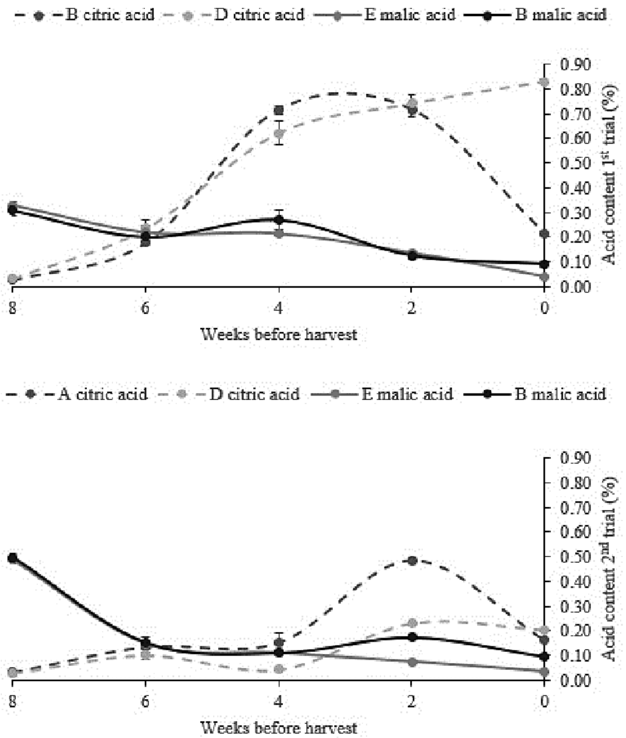

Fig. 2. The acid content trend during the experiment for the treatments with the highest and lowest values in each trial

Treatments B and D (citric acid), and E and B (malic acid) in the first trial, and treatments A and D (citric acid), and E and B (malic acid) in the second trial. A (Control: Without fertilization of Ca and Si), B (Ca from ten weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest), and E (Si from six weeks before harvest until harvest. Values are the mean of 4 replicates, and error bars represent the standard error

Moreover, calcium employed ten weeks before harvest can increase the malic acid content; meanwhile, the silicon used six weeks before harvest could not be beneficial to accumulate this acid in the fruit. Figure 2 exposed the citric and malic acid trend for the treatments with the highest and lowest values in each trial. In this graphic, citric acid increases as close the fruit is to harvest, contrary to the malic acid content. In six weeks before harvest, a clear differentiation between the accumulations of these acids was evidenced, with an abrupt increase in citric acid; although this acid decreased close to fruit ripening in almost all treatments, more noticeable in trial two.

In low acid hybrids like MD2, the typical percentage value is around 0.1 to 1% for the citric acid and 0.01 to 0.1% for the malic acid (Saradhuldhat & Paull, 2007; Lu et al., 2014). In this research, the values displayed for these two variables were inside the optimal range previously studied. Treatment D had the highest results for the citric acid in both trials, and also, the treatments employing silicon fertilization had more superior results than those using calcium and the control. This outcome suggested that concerning citric acid, pre-harvest silicon applications are beneficial in enhancing its accumulation in the fruit, mainly when those are used ten weeks before harvest. Opposite to citric acid, malic acid exhibited more superior outcomes in those treatments using calcium fertilization and the control, especially when calcium is sprayed ten weeks before harvest. In low acid hybrids it has been discovered that citric acid decreased slightly weeks close to harvest, opposite to the acid hybrids (Saradhuldhat & Paull, 2007; Paull & Chen, 2018). The silicon fertilization in both trials could have inhibited this phenomenon close to harvest and as a consequence the higher citric acid level. In the case of malic acid, the calcium fertilization could have influenced its organic production more than the treatments using silicon or mixed calcium and silicon. Malic acid is highly related and usually inversely to citric acid production in plants (Sun et al., 2019). Typically, in pineapple the malic acid content is higher during the first stage of fruit development, thereafter as close to harvest is the fruit, its level decreases parallel to the increase in citric acid (Saradhuldhat & Paull, 2007; Paull & Chen, 2018).

Furthermore, the more elevated citric acid results observed in the first trial could be associated with the impact of the lower stomatal conductance presented in waterlogging, affecting the plant respiratory metabolism. It has been proved that plants employ their organic sugars to create more malate pool, citric, pyruvic acids, and CO_2 as products of their respiration process (Paull & Chen, 2018; Sun et al., 2019; Yang et al., 2019). Subjected to waterlogging, the plant was private of the regular gas interchange, causing a modification of its respiratory metabolism. This situation could have forced the fruit to use the sugars available in its constitution to maintain its normal respiratory process under these conditions. Besides, the increase of ROS caused by waterlogging encouraged the plant to generate more citric acid as part of its systematic acquired resistance (SAR) under these oxidative circumstances. Studies on citric acid have determined that this organic acid catalyzes the breakdown of ROS molecules (Sadak & Orabi, 2015; Sun et al., 2019; Yang et al., 2019). Therefore, the combination of the previous situations described can explain the most reduced sucrose content linked to a more superior citric acid level in the fruits of trial one. Figure 2 exposed how the citric acid increase in trial two started around four weeks before harvest exhibiting higher values, opposite to trial one, where it happened in six weeks. In trial one, six weeks before harvest could have been when the normal respiration process of the fruit started to be affected, increasing the ROS and, consequently, the acid accumulation started to speed up. For the future, more studies are recommended on the influences of foliar fertilization with calcium and silicon, essentially close to harvest, on sugar and acid content, and a possible enzyme's activities approach.

Fruit and crown weight and firmness

The weight of the fruit and crown provided significant differences, although not in both trials; for the fruit, weight differences were observed in trial one, while for the crown weight, just in trial two. The fruit weight in trial one shows that treatment C had the most inferior value (1625.92 g), while the rest of the treatments had a similar and more superior outcome (1800 g on average) (Table 5). The mean average results of the two were almost similar for this variable. In the case of the crown weight in the second trial, treatment B provided the most superior value and A the lowest one (155.38 and 130.33 g, respectively). Moreover, the second trial mean results were much lower for this variable than trial one (360 g on average of the first trial) (Table 5). These outcomes suggested that treatments employing calcium fertilization impacted the fruit and crown weight more than those using silicon.

For the fruit firmness, the trial one and two exposed that treatment C had the most superior values (5.39 and 5.92 N, respectively); meanwhile, the most inferior results were observed in treatment D (3.87 N) for trial one and G (4.71 N) for the trial two (Table 5). These outcomes infer that calcium applied six weeks before harvest increases the firmness of the flesh. In comparison, applications in ten weeks before harvest or mixed with silicon do not cause a remarkable impact on this variable. Overall, with the minor exception of treatment D in the first trial, the average mean values of the treatments in both trials were similar (Table 5).

In MD2 pineapple, the fruit weight usually ranges from 1300 to 2500 g (Li et al., 2011; Bin Thalip et al., 2015). In

Table 5. Influences of the treatments applied on the fruit and crown weight, and flesh firmness at harvest

Weight and firmness 1st trial			
Treatment	Fruit weight, g	Crown weight, g	Fruit firmness, N
A	1783.42 ± 62.81 a	354.33 ± 20.41 a	4.33 ± 0.28 b
B	1834.88 ± 85.39 a	358.71 ± 16.78 a	4.29 ± 0.19 b
C	1625.92 ± 57.03 b	368.08 ± 15.88 a	5.39 ± 0.21 a
D	1869.92 ± 68.67 a	372.92 ± 9.03 a	3.87 ± 0.19 b
E	1850.71 ± 62.78 a	380.88 ± 15.17 a	4.48 ± 0.27 b
F	1738.17 ± 85.92 a	365.67 ± 19.25 a	4.25 ± 0.20 b
G	1809.63 ± 66.97 a	376.17 ± 14.39 a	4.39 ± 0.25 b
Weight and firmness 2nd trial			
Treatment	Fruit weight, g	Crown weight, g	Fruit firmness, N
A	1675.33 ± 94.95 a	130.33 ± 6.27 b	4.67 ± 0.30 b
B	1774.00 ± 73.62 a	155.38 ± 7.75 a	4.35 ± 0.25 b
C	1590.54 ± 104.31 a	146.21 ± 8.68 ab	5.92 ± 0.28 a
D	1740.33 ± 127.67 a	142.25 ± 8.21 ab	4.44 ± 0.22 b
E	1807.58 ± 73.25 a	144.88 ± 6.48 ab	4.70 ± 0.28 b
F	1767.75 ± 96.32 a	144.54 ± 8.12 ab	4.75 ± 0.22 b
G	1840.25 ± 88.40 a	148.54 ± 7.18 ab	4.31 ± 0.25 b

*Each value represents a mean ± standard error. Mean values in each column followed by the same lower-case letters are not statistically different by Duncan's multiple range test ($P < 0.05$).

**A (Control: Without fertilization of Ca and Si), B (Ca from ten weeks before harvest until harvest), C (Ca from six weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest), E (Si from six weeks before harvest until harvest), F (Ca + Si from ten weeks before harvest until harvest), and G (Ca + Si from six weeks before harvest until harvest) the case of the crown, its weight has not been adequately established due to the minor work done on its physiological constitution (Bartholomew & Sanewski, 2018). However, some studies have reported that its weight depends on the fruit due to competition for photo-assimilates (Chen & Paull, 2017; Bartholomew & Sanewski, 2018). The values obtained in both trials were inside the proposed ideal range regarding the fruit weight, having the lowest results in treatment C.

There is a correlation between a high weight of the plant at flowering and higher fruit weight (Bartholomew & Sanewski, 2018; Paull & Chen, 2018). Moreover, a relation between the cell enhanced area and the weight of the fruit at harvest has been proposed (Li et al., 2011). However, as mentioned in the sugar and acid content results, seemly calcium application six weeks before harvest (like treatment C) does not encourage the triggering of hormones like ethylene. This circumstance can cause a retard of fruit ripening if the environmental conditions does not favour the fruit development, and also can impact the increase of the cell wall area as a part of its metabolism to start photo-assimilates accumulation via apoplast. Therefore, this situation can lead to weight at harvest lower than the genetic potential determined for the fruit.

Regarding the crown, there was no correlation between high fruit weight and a low crown weight. The result of the first trial in each of the treatments was higher than trial two. It has been established that irradiance influences the crown weight at harvest. High irradiance during fruit development can encourage crown photosynthetic activity, causing increased weight and acid accumulation (Londers et al., 2011; Paull & Chen, 2018). Both trials were carried out during the rainy season, and trial two had lower irradiation conditions than trial one (16.83 and 9.18 w/m^2, respectively). This circumstance could have provoked a lower photosynthetic activity in the crown of the fruits of the second trial, and as a consequence, the more inferior weight observed at harvest.

An ideal value of fruit firmness in pineapple should be between 4 and 7 N (Ding & Syazwani, 2016). The degradation of the cell wall is considered the primordial factor for fruit softening (Gao et al., 2014; Tucker et al., 2017). Besides, fruit firmness declines in association with pectin modification in the cell (Gao et al., 2014; Tucker et al., 2017). In this research, the most superior results were obtained in treatment C in both trials. This outcome indicates that calcium fertilization from six weeks before harvest can cause more rigid cell walls. Hocking et al. (2016) and De Freitas & Resender Nassur (2017) reported that calcium (Ca^{2+}) could delay the ripening and senescence-related processes linked to cell wall modification. This mechanism acts by the influences of this mineral in the ethylene production, retarding the triggering of the enzyme associated with the softening; also, by its ability to bind into the cell wall matrix to maintain its integrity during fruit development. Although mostly all the firmness values were inside the ideal range, it was necessary to notice the more superior value evidenced in treatment C. Fruits with severe low firmness (usually lower than 3 N) are prone to suffer mechanical damage like bruises during postharvest handling. Also, these fruits are susceptible to shorter shelflife during storage (Ding & Syazwani, 2016; Paull & Chen, 2018).

Conclusion

Foliar fertilization with calcium and silicon close to harvest affected the pineapple physico-chemical quality. Treatment D (Si from ten weeks before harvest until harvest) provided the most optimal performance in both trials implemented by obtaining an ideal value of TSS, water, sugar and acid content, fruit and crown weight, and flesh firmness. Also, this treatment delivered the highest citric acid and β-carotene levels, antioxidant scavengers associated with optimal fruit quality, longer shelflife, and inferior decay symptoms. The outcomes of the first trial were affected by waterlogging. This circumstance caused the fruits of this trial to have a more superior TSS, TA, water, and acid content as a response to this abiotic stress; besides, those fruits had a lower sugar content and a more elevated fruit and crown weight. Future experiments are recommended concerning the impact of calcium and silicon on MD2 pineapple, to clarify some outcomes obtained in this experiment, including application times and fertilization doses suitable for obtaining an optimal fruit quality.

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