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Effect of Pre-Harvest Foliar Calcium and Silicon Fertilization on Pineapple Quality and Fruit Collapse Incidence

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ABSTRACT

Pineapple can be affected by fruit collapse, a disease caused by the bacterium *Dickeya zeae*. However, adequate fertilization can increase the fruit's resistance to this illness. Therefore, the impact of pre-harvest foliar calcium and silicon fertilization on pineapple quality and fruit collapse incidence was assessed in this study. The experiment implemented a split-plot design with two factors. The first factor has two terms of inoculation (flower induction and before harvest). The second factor uses a control with three foliar fertilization treatments, A (control: no foliar fertilizers applied), B (Ca from 13 to 11 weeks before harvest/from 6 weeks to harvest), C (Si from 13 to 11 weeks before harvest/from 6 weeks to harvest), and D (Ca + Si from 13 to 11 weeks before harvest/from 6 weeks to harvest). Treatment D gave the best response. It had the lowest fruit collapse incidence (21.70%), highest ascorbic acid (71.64 mg/kg), elevated β-carotene (4.87 mg/kg) and mineral content (Ca: 1851.10 mg/kg, Si: 1164.87 mg/kg), essentially under the before harvest term of inoculation, which was more harmful for the fruit. In conclusion, mixed foliar calcium and silicon fertilization manage to improve the tolerance to fruit collapse incidence, impacting the pineapple quality positively.

INTRODUCTION

Post-harvest diseases in pineapple are frequent problems impacting quality, with infections arising from the field, usually three weeks before harvest (Sipes & de Matos, 2018). Fruit collapse is a disease caused by the bacteria *Dickeya zeae* (formerly named *Erwinia chrysanthemi*) (de Matos, 2019; Sueno, Marrero, de Silva, Sether, & Alvarez, 2014). It is characterized by an exudation of juice and gas bubbles, olive-green shell colour and cavities within the skeletal fibres of the flesh (Aeny, Suharjo, Ginting, Hapsoro, & Niswati, 2020; Sueno, Marrero, de Silva, Sether, & Alvarez, 2014). Previous studies have reported two typical terms for inoculating the pathogen causing this disease. One is during flowering, influenced by two vectors. One vector is linked with latent bacteria entering the plant during this flower induction, brought from previously infected plants and transported by the wind. A second vector is associated with ants, beetles and flies that penetrate the plant attracted by its nectar, bringing the *D. zeae* bacteria. The other term is when three weeks before harvest, an incidence of high temperature on the fruit cause an increase in its transpiration, permitting the bacteria to enter into the fruit by the shell stomata (Sipes & de Matos, 2018; Wang, Fu, Liu, & Hong, 2011). Therefore, the described symptoms tend to appear through fruit development or post-harvest handling, as the bacterium can stay inactive for long periods, even after flowering (Cano-Reinoso, Soesanto, Kharisun, & Wibowo, 2021a; de Matos, 2019).

Nowadays, due to their consumer appeal, low acid pineapple hybrids are currently the most exported by the industry as fresh fruit (Kleemann, 2016; Murai, Chen, & Paull, 2021; Žemlička, Fodran,

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Kolek, & Prónayová, 2013); however, these hybrids have exposed a greater susceptible to fruit collapse (Soteriou, Kyriacou, Siomos, & Gerasopoulos, 2014; Žemlička, Fodran, Kolek, & Prónayová, 2013). Therefore, a solution must be found to confront this problem, looking to maintain the fruit quality and minimaxing cost of production, typically requested by the pineapple industry. In this context, the use of fertilizers commonly employed to improve the quality of the fruits, but that can enhance their tolerance and resistance to this disease appears as an attractive and more economical option for the current farmers.

Calcium has been shown to improve fruit tolerance and disease resistance (de Freitas & de Cássia Mirela Resende Nassur, 2018; Kim, Jacob, & Dangl, 2022; Madani, Mirshekari, Sofo, & Tengku Muda Mohamed, 2016). This characteristic has been associated with the inhibition of ripening and senescence-related processes, causing the cellular responses to reductions in biotic signals and cell wall breakdown (de Freitas & de Cássia Mirela Resende Nassur, 2018; Hocking, Tyerman, Burton, & Gilliham, 2016). Besides, it has been determined that an adequate host defence involves specific cytosolic Ca^{2+} oscillation, known as the Ca^{2+} signal, triggering protein networks generating specific host defence mechanisms against pathogens (de Freitas & de Cássia Mirela Resende Nassur, 2018; Gao, Cox Jr, & He, 2014; Kim, Jacob, & Dangl, 2022). Continuous fertilization, for example, boosted calcium levels in apricot and peach fruit tissues, reducing the incidence and increasing resistance to bacterial canker caused by *Pseudomonas syringae* pv. Syringae (Cao, Duncan, Kirkpatrick, Shackel, & DeJong, 2013); also, in tubers like potato, the severity and incidence of the soft rot caused by *Erwinia carotovora* was reduced by pre-harvest calcium fertilization (Ngadze, 2015). Furthermore, foliar fertilization with this mineral has become necessary in pineapple plantations as complementary treatment. Calcium content in pineapple fruit usually decrease in concomitance with its ripening, as the plant does not accumulate significant quantities after flowering (Bartholomew & Sanewski, 2018; de Freitas & de Cássia Mirela Resende Nassur, 2018).

On the other hand, the favourable effects of silicon on plant disease prevention have been known for many decades, and the number of plant-pathogen interactions influenced by silicon continues to grow (Artyszak, 2018; Laane, 2018). Recent data indicates that silicon interferes with pathogen effector proteins, allowing the plant to establish more effective defense responses (Liang, Nikolic, Bélanger, Gong, & Song, 2015). On top of that, studies on silicon have exposed its influences on cell and membrane properties (Liang, Nikolic, Bélanger, Gong, & Song, 2015). For instance, silicon fertilization improved the quality, reduced the incidence of bacterial fruit blotch caused by *Acidovorax citrulli* (Ferreira et al., 2015), and reduced the internal severity of bacterial spots caused by *Xanthomonas axonopodis* pv. Passiflorae on yellow passion fruit (Brancaglione, Sampaio, Fischer, de Almeida, & de Fatima Fumis, 2009). Additionally, experiments have proved that foliar fertilization with this mineral has an equal or more positive impact on plant physiology and disease tolerance than soil silicate applications, essentially using silicic acid (Artyszak, 2018; Liang, Nikolic, Bélanger, Gong, & Song, 2015).

Currently there is no available data concerning the effect of pre-harvest calcium and silicon foliar fertilization on *D. zeae* and fruit collapse. However, the previous information demonstrating the beneficial effect of these minerals on managing plant diseases suggests that using proper fertilization with them can be a feasible solution to deal with fruit collapse. On the other hand, as no good experiments have been reported about how the typical terms of inoculation of *D. zeae* in pineapple plant affect the fruit, an emphasis should be done on this matter. Therefore, it is necessary to establish which term can cause more disease incidence, their impact on fruit development and quality, and possible interactions with any fertilization implemented. As a result, this study aims to assess the influence of calcium and silicon foliar fertilization on pineapple quality and fruit collapse incidence, focusing on two terms of inoculation.

MATERIALS AND METHODS

Fruits and Field Conditions

The experiment was arranged in the pineapple fields of Nusantara Tropical Farm (5.0669° S, 105.6925° E, elevation: 20 m above sea level), located in Lampung, Sumatra island of Indonesia, between February and June of 2020. MD2 low acid cultivar was employed in this experiment. Pineapple was harvested 148 days after flower induction when MD2 is considered to achieve its

optimal physicochemical characteristics for future consumption (bin Thalip, Tong, & Casey, 2015; Ding & Syazwani, 2016). The characteristics of the soil where the experiment was set are presented in Table 1.

Table 1. Physical and chemical properties of the experiment's soil

Soil Properties	Value
Texture	
Clay (%)	18.56
Loam (%)	13.01
Sand (%)	68.43
Chemical composition	
pH (H_2O)	6.8
C (%)	0.7
N (mg/kg)	800
P (mg/kg)	43.75
K (mg/kg)	319.8
Ca (mg/kg)	638
Mg (mg/kg)	235.2
Na (mg/kg)	4.6

Remarks: The minerals N, P, K, Ca, Mg and Na exposed in this table represent the available content in the soil.

During the row preparations, the soil was fertilized with 200 kg/ha Di-ammonium Phosphate, 1000 kg/ha K_2SO_4, and 200 kg/ha Kieserite crystal; then, three months after plating, 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 used in intervals of 30 days. Finally, following flower induction, 2.5 l/ha of liquid ethepon and 30 kg/ha of Borax were sprayed. A weather station (LSI Lastem, equipped with a CR6 datalogger from Cambell Scientific, Italy) determined an average relative humidity (RH) of 89.34%, an ambient temperature of 26.8°C, solar radiation of 16.83 w/m, and a monthly average rainfall of 133.77 mm during the experiment.

Sampling Tissue for Analysis

For the subsequent analysis carried out in the central laboratory for Great Giant Pineapple Company quality control, tissue and juice samples were extracted from the flesh of the fruit. The composition of four fruits per replication in every one of the treatments implemented was used. Juice samples of 5 ml were employed for the ascorbic acid (AsA), β-carotene, and calcium determinations; meanwhile, 100 mg of flesh tissue were necessary for silicon detection. Besides, for variables like the total soluble solids (TSS), and total titratable acidity (TA), less than 1 ml of juice was used.

On the other hand, employing the method described in Siti Roha, Zainal, Noriham, & Nadzirah (2013), the water content of the samples used for the calcium and silicon determinations was previously determined; because of that, the results of these variables are given in dry bases content using the respective humidity equivalence relations.

Total Soluble Solids (TSS), Total Acidity (TA), and Fruit Collapse Incidence Determination

The TSS and TA were calculated following the methods described by Shamsudin, Zulkifli, & Kamarul Zaman (2020). First, TSS was calculated using a handheld refractometer (MASTER-53; Atago, Japan). On the other hand, titration to pH 8.1 with 0.1 M NaOH using phenolphthalein as an indicator was implemented to determine TA as a percentage of citric acid. Meanwhile, the fruit collapse incidence was calculated by detecting the number of fruits affected by this disease in each observation and converted to a respective percentage.

Ascorbic Acid (AsA) Content Determination

Pineapple AsA was determined using the method described by Siti Roha, Zainal, Noriham, & Nadzirah (2013), which involved the use of the High-Performance liquid chromatography (HPLC) - (Hitachi, USA) model L-2000 apparatus with a Refractive Index detector model L-2490. A standard solution of AsA was dissolved in distilled water and filtered through a 0.45 µm membrane filter from a Millipore. A chromatographic technique was used to calculate the AsA content by comparing the peak area. The chromatographic conditions for the procedure as follows:

Column: Purospher® STAR NH2 (250 x 4 (mm), 5 µm). Guard column: LiChocart® 4-4 / LiChrospher® 100 NH2, 5 µm. Column temperature: Room temperature (22°C). Mobile phase: Pipette of 0.14 ml H_2SO_4 (0.0025 M) concentrated at 97% is introduced in a volumetric flask, then add 1000 ml of distilled water. Injection volume: 20 µl. Duration of analysis: 15 minutes.

β-carotene Detection

The method described by Owolade et al. (2017) was used to determine the β-carotene content in pineapple fruits. First, 25 mg of β-carotene were weighed, dissolved in 2.5 ml of chloroform, and then diluted with petroleum ether to make 250 ml for this procedure. Following that, various concentrations of this solution were utilized for the subsequent absorbance test using a spectrometer (Spectroquant® Pharo 300, Thomas Scientific, USA) at 452 nm and 3% of acetone in petroleum ether as a blank. Finally, the corresponding standard curve was used to calculate the β-carotene content.

Calcium Content Determination

Based on the Benton-Jones (2001) method, the calcium content of the samples was determined using atomic absorption spectrometry (AAS) - (932 Plus, GBC scientific equipment, USA). First, a digestion tube containing a selection of juice was arranged. Five ml of 65% nitric acid was then added and put overnight. After that, the sample was cooked in a block digester for an hour at 125°C. Next, 3 ml of 30% hydrogen peroxide (H_2O_2) was added and heated for one hour before being utilized with HNO_3 (1 ml residue). The next step was adding and shaking 5 ml of nitric acid with distillate water (1:10). Finally, the sample was quantitatively transferred to a 25/50 ml flask and pitched with distillate water to create an extract suitable for mineral analysis. In a dry base content, the results are presented.

Silicon Content Determination

A spectroscopic approach proposed by Liang, Nikolic, Bélanger, Gong, & Song, 2015) was used to calculate the silicon concentration as silica (SiO_2). The process consisted of two steps: sample preparation using the autoclave-induced digestion method (AID) and silica determination using the molybdenum blue method. The first step regarding the sample preparation was grinding, pressing, and drying the samples of flesh tissue before putting them in a polyethylene tube. Two ml of 50 % H_2O_2 and 4.5 ml of 50 % (w/w) NaOH were then added. After digesting the resultant suspension for 1 hour at 138 kPa in an autoclave, the samples were diluted with distilled water to measure the silica concentration colorimetrically.

The second step began after placing a 1 ml of sample solution into a volumetric flask. Then, 10 ml of ammonium molybdate and 30 ml of 20% acetic acid were added and left for 5 minutes. Next, a reducing solution of 1 ml and 5 ml of 20% tartaric acid were added immediately. After that, the solution was adjusted to 50 ml with the addition of 20% acetic acid, giving 30 minutes for this procedure. Consequently, a computerized spectrometer calculated silica's absorbance at 650 nm.

Experimental Design

The research was elaborated using a split-plot design with two factors. The first factor concerns the inoculation of the bacteria causing fruit collapse, and the second factor involves the foliar fertilization treatments implemented with the control. There were three replications of each treatment containing 44 fruits. From six weeks before harvest, observations were made once every two weeks. A juice from previously contaminated fruits collected from their flesh (having the *Dickeya zeae* bacterium) was used to infect all of the samples of every treatment, including the control. Before the inoculation, a bacteriological test was done to the juice to corroborate the existence of the bacterium. Table 2 summarises the experiment design used with the two factors implemented.

Table 2. The organization of the experiment design employed in the research

Factor	Description
Factor 1. (Term of inoculation)	1. Flower induction (FI)
	2. Before harvest (BH)
Factor 2. (Control/Foliar fertilization)	A. Control (No foliar fertilizers applied)
	B. Ca from 13 to 11 weeks before harvest/ from 6 weeks to harvest
	C. Si from 13 to 11 weeks before harvest/ from 6 weeks to harvest
	D. Ca + Si from 13 to 11 weeks before harvest/ from 6 weeks to harvest

For the inoculation during the FI, doses of 20 ml juice/plant fruit were employed using a hand sprayer. These doses were selected after previous field trials demonstrated that with their applications, some fruits were exposed to symptoms of fruit collapse just after the beginning of their development. Therefore, the plants were sprayed two and one week before the FI and one week after the FI. Those were executed at night. The moment for spraying the bacteria represents the normal vectors of infections in the field. A latent bacteria in the plant went through the nectarthodes before and after the FI, or insects brought the inoculum after that (Sipes & de Matos, 2018; Wang, Fu, Liu, & Hong, 2011).

In the case of the inoculation term BH, doses of 0.2 ml juice/fruit were implemented using a syringe. These doses were employed after previous studies described in Barral, Chillet, Minier, Léchaudel, & Schorr-Galindo (2017) demonstrated that injections with these doses in pineapple are sufficient to inoculate a disease before harvest. The fruits were injected six, four, and two weeks before harvest. For this method, four eyes of the pineapple shell were inoculated by pushing the syringe through the fruit shell. Two eyes were inoculated on the upper part, and two on the lower part of the fruit, similar to the method reported (Barral, Chillet, Minier, Léchaudel, & Schorr-Galindo, 2017). The time chosen for this term of inoculation tried to emulate another critical moment of infection. It was through the shell stomata because of the high fruit transpiration weeks before harvest, as described in Sipes & de Matos (2018).

The foliar fertilizer's implementation was done weekly from 13 to 11 weeks before harvest. After that, starting at six weeks, the fertilization was executed every two weeks until harvest. The silicon product used was NewSil (0.8% w/v Silicic acid-Si $(OH)_4$, 0.18% w/v H_3BO_3, 49% w/v Polyethylene glycol) in dosages of 1.5 l/ha (v/v = 2 ml/l), whereas the calcium product employed was Calcibor (12.9% w/v CaO and 2.6% w/v B) in doses of 4 l/ha (v/v = 4 l/2000). Moreover, following the findings regarding uptake and mobility of minerals within the pineapple fruit reported by Vásquez-Jiménez & Bartholomew (2018), calcium and silicon were sprayed on the fruit shell and crown during the night. Finally, four rows were equivalent to the treatments and control in each split-plot of the second factor with 0.4 m width and 3.75 m length. Pineapple plants were set in two lines of twenty-two plants per row with a separation of 0.25 m.

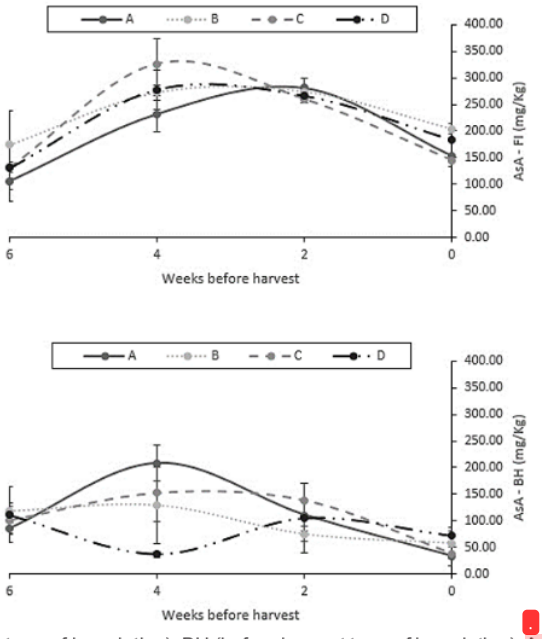
Statistical Analysis

For statistical analysis, SPSS Version 22.0 software was used. All of the variables in this experiment were analyzed using two-way ANOVA. Mean significant differences at $p < 0.05$ were determined by Duncan's multiple range test (DMRT) (concerning the TSS, TA, AsA, β-carotene, calcium and silicon content) and Kruskal-Wallis test (in case of the fruit collapse incidence).

RESULTS AND DISCUSSION

TSS and TA Content

The TSS demonstrated significant differences in the interaction outcomes. Treatment D using the FI had the highest value (15.33%); in contrast, treatment B in the BH had the lowest TSS value (12.13%). More premium content of TSS in the fruits was linked to a lower incidence of fruit collapse (Table 3). In common low acid hybrids like MD2, a minimal TSS of 12% has been established as the optimal (Bartholomew & Sanewski, 2018; Cano-Reinoso, Soesanto, Kharisun, & Wibowo, 2021b; Lu, Sun, Wu, Liu, & Sun, 2014). The results guaranteed the assessment of this TSS parameter. A better disease tolerance has been documented in fruits with high TSS content (Morkunas & Ratajczak, 2014; Naseem, Kunz, & Dandekar, 2017). Usually, pathogens interfere with the metabolism of their hots, causing an increment of sugar uptake to their benefit and subsequently decreasing the final content of TSS in sink organs of the plant (Morkunas & Ratajczak, 2014; Naseem, Kunz, & Dandekar, 2017). This previous finding explains why TSS results in the BH were lower than in the FI. The BH generated a higher incidence of fruit collapse; therefore, the host infection and pathogen interaction in this term of inoculation were more intense. Studies on pre-harvest calcium applications did not report the influences of this mineral on the TSS content (Uthairatanakij, Aiamla-or, & Jitareerat, 2015). Although, experiments on silicon pre-harvest effects on fruit TSS content revealed an increment of this variable at harvest and a steady concentration during post-harvest handling (Weerahewa & Wicramasekara, 2020); also, in fruits like bananas (Hanumanthaiah et al., 2015) and tomato cherry (Islam, Mele, Choi, & Kang, 2018). This fact can explain why in both terms of inoculation, the treatments employing silicon applications (like treatments C and D) obtained a superior TSS content.



Remarks: FI (flower induction term of inoculation), BH (before harvest term of inoculation). A (Control: No foliar fertilizer applied), B (Ca from 13 to 11 weeks before harvest/ from 6 weeks to harvest), C (Silicon from 13 to 11 weeks before harvest/ from 6 weeks to harvest), D (Ca + Si from 13 to 11 weeks before harvest/ from 6 weeks to harvest). Vertical bars show the ± standard error, and values are the mean of three replicates.

Fig. 1. Effects of the treatments and control employed using both terms of inoculation on the ascorbic acid (AsA) content during the experiment.

Table 3. Effect of the interaction between the control, treatments and terms of inoculation implemented on the fruit quality at harvest

Treatments*Term of inoculation	TSS (%)	TA (%)	AsA (mg/kg)	β-carotene (mg/kg)
A*FI	13.73 ± 0.64 abc	0.60 ± 0.06 a	152.77 ± 4.88 ab	3.58 ± 0.26 a
B*FI	14.53 ± 0.29 bc	0.57 ± 0.02 a	204.19 ± 10.56 a	2.21 ± 0.19 cd
C*FI	14.93 ± 0.24 bc	0.52 ± 0.01 a	144.58 ± 10.41 ab	2.45 ± 0.22 bc
D*FI	15.33 ± 0.18 a	0.53 ± 0.00 a	183.22 ± 1.75 ab	1.86 ± 0.13 d
A*BH	12.93 ± 0.47 ab	0.54 ± 0.04 a	33.38 ± 16.83 c	2.95 ± 0.08 ab
B*BH	12.13 ± 0.71 c	0.60 ± 0.09 a	57.96 ± 29.65 c	2.29 ± 0.28 cd
C*BH	13.00 ± 0.90 ab	0.61 ± 0.09 a	37.98 ± 21.90 c	5.12 ± 0.12 a
D*BH	13.47 ± 0.85 abc	0.60 ± 0.02 a	71.64 ± 9.03 bc	4.87 ± 0.06 a

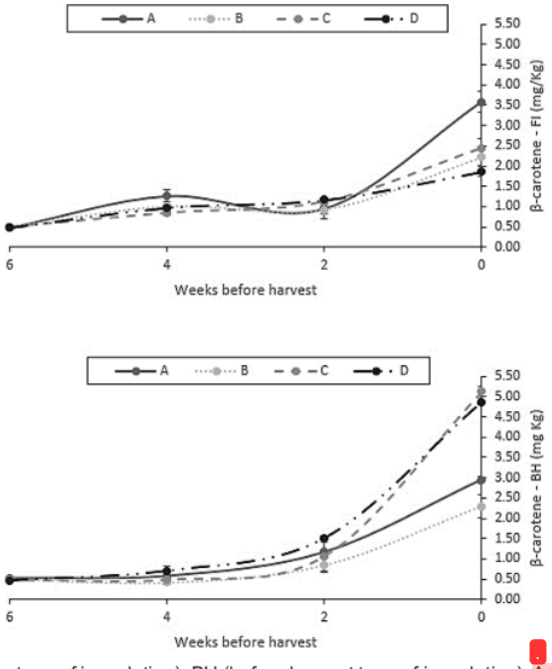
Remarks: **Each value of the table represents a mean ± standard error. Mean values shown in each column followed by the same lower-case letters are considered not statistically different by Duncan's multiple range test ($p < 0.05$). Total soluble solids (TSS), total acidity (TA), and ascorbic acid (AsA); ***A (Control: No foliar fertilizers applied), B (Ca from 13 to 11 weeks before harvest/ from 6 weeks to harvest), C (Silicon from 13 to 11 weeks before harvest/ from 6 weeks to harvest), D (Ca + Si from 13 to 11 weeks before harvest/ from 6 weeks to harvest). FI (flower induction term of inoculation), BH (before harvest term of inoculation).

In the case of TA, there were no significant differences in the interaction results (Table 3). In MD2, it has been determined that an ideal value for TA should be between 0.4 and 0.7% (Lu, Sun, Wu, Liu, & Sun, 2014; Murai, Chen, & Paull, 2021; Paull & Chen, 2018). Values between this range were obtained in all the outcomes observed for both inoculation terms. Besides, previous studies have determined no significant impact of any pre-harvest foliar calcium or silicon fertilization On TA, more than a slightly increase among the ideal range reported (Weerahewa & Wicramasekara, 2020).

AsA and β-carotene Content in the Fruits

The AsA exposed significant differences in the interaction outcomes. Treatment A in the BH has the lowest AsA content (33.38 mg/kg), while B in the FI delivers the most elevated level (204.19 mg/kg) (Table 3). Fig. 1 exposes the AsA content in the fruits throughout the experiment until harvest. This graph shows a noticeable shift in the AsA trend in both inoculation terms. Essentially, the BH shows a more usual pattern four weeks before harvest. In both periods, AsA content peaks and decreases at that point, with a slight increase after two weeks, for treatments like B and D in the FI and C and D in the BH.

The interaction results for the β-carotene also showed significant differences. Treatment C in the BH had the maximum level (5.12 mg/kg), while treatment D in the FI had the lowest concentration (1.86 mg/kg) (Table 3). Fig. 2 shows the fruit's β-carotene content from the start of the experiment until harvest. This figure shows that two weeks before harvest there is a notable shift in the trend of β-carotene in both terms of inoculation, particularly regarding the final highest and lowest values.



Remarks: FI (flower induction term of inoculation), BH (before harvest term of inoculation). A (Control: No foliar fertilizer applied), B (Ca from 13 to 11 weeks before harvest/ from 6 weeks to harvest), C (Silicon from 13 to 11 weeks before harvest/ from 6 weeks to harvest), D (Ca + Si from 13 to 11 weeks before harvest/ from 6 weeks to harvest). Vertical bars show the ± standard error, and values are the mean of three replicates.

Fig. 2. Effects of the treatments and control employed using both terms of inoculation on the β-carotene content during the experiment.

Hybrids like MD2 are known for their higher AsA level at harvest, between 300 to 600 mg/kg (Cunha et al., 2021; Lu, Sun, Wu, Liu, & Sun, 2014; Paull & Chen, 2018). Previous research on pineapple demonstrated a strong correlation between the antioxidant activity in fruits and the AsA level; besides, it has been established that AsA tends to increase with higher irradiation and temperature, affecting the fruits (Cunha et al., 2021; Ferreira et al., 2015; Paull & Chen, 2018). However, the AsA values observed in this experiment at harvest were lower than the range formerly determined. Data in Fig. 1 manage to identify that starting at four weeks before harvest when the organic acids accumulations usually begin to happen, the AsA content for mostly all the treatments in both inoculation terms never achieve values close to or higher than 300 mg/kg. The irradiation, rainfall and temperature of the experiment area could have affected the quality of the fruits concerning this variable.

AsA is a soluble vitamin that serves as a scavenger agent to protect cells from oxidative stress. In addition, AsA has been found to stimulate the activity of several enzymes, including catalase (CAT), peroxidase (POD), and ascorbic peroxidase (APX) (Akram, Shafiq, & Ashraf, 2017; Lu, Sun, Wu, Liu, & Sun, 2014; Noichinda, Bodhipadma, & Wongs-Aree, 2018). These enzymes are known for enhancing the fruit scavenging process through its senescent or pathogen infections causing diseases. Pathogens can produce an increase in the reactive oxygen species (ROS). To deal with this ROS production, fruits increase the creation of more AsA through their metabolism (Lu, Sun, Wu, Liu, & Sun, 2014; Noichinda, Bodhipadma, & Wongs-Aree, 2018). Although there is not enough information in pineapple concerning pre-harvest foliar calcium applications and increase or decrease in AsA content, the present data suggest a correlation between the APX enzyme and the assimilation of calcium in the fruits. APX has involved in AsA metabolism in plant cells' apoplast and their oxidative stress perception and amplification (Akram, Shafiq, & Ashraf, 2017). Several studies reported an enhancement of Ca^{2+} molecule in plant tissues under AsA increase, characterized by a superior activity of APX (Farouk, 2011). In the case of silicon, despite the lack of information concerning its effects on AsA content in pineapple, pre-harvest foliar applications of silicic acid were associated with increased AsA in tomatoes (Stamatakis, Papadantonakis, Savvas, Lydakis-Simantiris, & Kefalas, 2003) and grapes (Laane, 2018). On top of that, the enhancing activity of the APX has been reported in silicon supplies experiments (Tale Ahmad & Haddad, 2011; Zargar, Mahajan, Bhat, Nazir, & Deshmukh, 2019).

Based on this information, the peak of the AsA at four weeks before harvest in treatments B, C and D in the FI could be associated with enhanced APX activity, especially in D (Fig. 1). In the case of the BH, a more elevated APX activity, picking four weeks before harvest but decreasing after that remarkably, could has been exhibited in treatment A. On the contrary, in the other treatments, the APX activity increased progressively, causing a peak after two weeks and a higher AsA content at harvest, primordially in treatment D (Fig. 1). Nevertheless, more studies should be performed on this matter with precise APX activity detection to corroborate the previous information described.

Concerning β-carotene, it is a pigment responsible for the flesh's yellow colour in pineapple (Steingass et al., 2020; Vásquez-Jiménez & Bartholomew, 2018). β-carotene belongs to plant carotenoids known for their antioxidant and scavenger properties, eliminating singlets of ROS (Fanciullino, Bidel, & Urban, 2014; Noichinda, Bodhipadma, & Wongs-Aree, 2018). A ROS increase could be more evident in the outcomes of the BH due to the more marked infection by *D.zeae*. Therefore, the fruits could have increased their antioxidant capacity by increasing the β-carotene content to cope with this situation. In the case of treatment A in the FI, as this does not employ any mineral fertilization, the rise in the fruits of the β-carotene level can be caused by systematically acquired resistance (SAR). SAR typically are developed by plants under a pathogen attack to tolerate their infections (Goñi et al., 2018).

Furthermore, the peak of the β-carotene two weeks before harvest in both terms of inoculation (Fig. 2) is connected with a normal physiological response in pineapple fruit. In the fruit, the flesh tends to develop faster than the shell during its repining, changing from white to yellow three to two weeks before harvest (Bartholomew & Sanewski, 2018). Furthermore, another reason behind the more remarked peak in the treatment C and D in

the BH (Fig. 2) is associated with a possible impact of the silicon on the biosynthesis of the β-carotene molecule via the mevalonate pathway (Noichinda, Bodhipadma, & Wongs-Aree, 2018). This pathway is positively influenced by silicon in case a more intense bacterial infection affects the fruits (Fig. 2). However, as there are no sufficient data regarding the employment of calcium and silicon fertilization and their impact on β-carotene molecular biosynthesis, more studies should be proposed to clarify this matter.

Calcium and Silicon Mineral Content

Significant differences were revealed by the calcium content interaction results, particularly between the two inoculation terms. The mean value of BH was highest in treatment B (2064.09 mg/kg), while the mean value of FI was lowest in treatment A (1437.89 mg/kg). Furthermore, the silicon content revealed notable changes between both terms of inoculation, much like the calcium did. The mean value of FI was found to be highest in treatment C (2025.36 mg/kg), whereas the one associated with BH was lowest also for this treatment (982.44 mg/kg) (Table 4).

Regarding calcium, by the already described influence enhancing cellular response to any biotic signal and cell wall breakdown, this mineral has been related to improved resistance and tolerance to pathogen infections in fruits (de Freitas & de Cássia Mirela Resende Nassur, 2018; Loekito et al., 2022; Madani, Mirshekari, Sofo, & Tengku Muda Mohamed, 2016). Cytosolic Ca^{+2} support the activation of calcium-dependent protein kinases (CDPKs), known as protein networks essential for the function of the cell wall well-functioning. CDPKs convert the Ca^{2+} hallmark into specific phosphorylation incidents, causing signalling responses as part of defence mechanisms (de Freitas & de Cássia Mirela Resende Nassur, 2018; Gao, Cox Jr, & He, 2014; Loekito et al., 2022).

Concerning silicon, two mechanisms related to its role in improving plant disease resistance have been put forth. The first one involves creating a mechanical barrier often deposited beneath a plant tissue's surface, such as a leaf cuticle, preventing germs from penetrating it (Kandhol, Singh, Peralta-Videa, Corpas, & Tripathi, 2021; Wang et al., 2017; Zargar, Mahajan, Bhat, Nazir, & Deshmukh, 2019). The second mechanism attends to the induction of natural defence compounds like lignin, phenolic compounds, phytoalexins, and enhancing activities of antioxidant enzymes like described in the production of AsA and β-carotene previously (Kandhol, Singh, Peralta-Videa, Corpas, & Tripathi, 2021; Wang et al., 2017; Zargar, Mahajan, Bhat, Nazir, & Deshmukh, 2019).

Table 4. Effect of the interaction between the control, treatments and terms of inoculation implemented on the mineral content and fruit collapse incidence at harvest.

Treatments*Term of inoculation	Ca (mg/kg)	Si (mg/kg)	Fruit collapse Incidence (%)
A*FI	1437.89 ± 84.33 b	1828.65 ± 104.92 a	3.33 bc
B*FI	1476.85 ± 144.98 b	1867.80 ± 175.10 a	0.00 c
C*FI	1556.02 ± 40.63 b	2025.36 ± 90.60 a	0.00 c
D*FI	1611.45 ± 21.97 b	1858.26 ± 153.30 a	0.00 c
A*BH	1939.51 ± 93.88 a	1068.33 ± 64.97 b	30.00 a
B*BH	2064.09 ± 173.00 a	986.21 ± 99.25 b	33.30 a
C*BH	2036.70 ± 55.33 a	982.44 ± 26.14 b	21.70 ab
D*BH	1851.10 ± 24.79 a	1164.87 ± 33.95 b	21.70 ab

Remarks: **Each value of the table represents a mean ± standard error. Mean values shown in each column followed by the same lower-case letters are considered not statistically different by Duncan's multiple range test and Kuskal-Wallis test (in the case of the incidence data) ($p < 0.05$); ***A (Control: No foliar fertilizers applied), B (Ca from 13 to 11 weeks before harvest/ from 6 weeks to harvest), C (Silicon from 13 to 11 weeks before harvest/ from 6 weeks to harvest), D (Ca + Si from 13 to 11 weeks before harvest/ from 6 weeks to harvest). FI (flower induction term of inoculation), BH (before harvest term of inoculation).

Due to the more remarked infection caused by the BH, the fruits as a part of its SAR could have generated the increase in the mineral uptake of calcium; besides, the more production of organic sources into the fruit to give the energy necessary to activate the respective protection mechanisms against the bacteria infection. Although sufficient data on the effects of foliar silicon application on pineapple plant and fruit mineral status have not yet been documented, earlier studies in other fruits have connected silicon fertilization with an increase in mineral uptake and accumulation of N, K, Ca, and Mg in plant tissues (Cano-Reinoso, Kharisun, Soesanto, & Wibowo, 2022; Javaid & Misgar, 2018; Zargar, Mahajan, Bhat, Nazir, & Deshmukh, 2019).

In this experiment, silicon turned out to be opposite to the calcium content in both terms of inoculaion, as the higher was calcium, the lower the silicon level. Nevertheless, the higher the silicon level, the lower the incidence of fruit collapse, especially in the BH, like in treatment D. Silicon arrangements in plant tissues are in phytoliths (typically formed by SiO_2) (Frew, Weston, Reynolds, & Gurr, 2018; Katz, 2015). The plant translocates silicon as mono silicic acid, which polymerizes to form phytoliths deposited within the plant tissues, increasing the plant rigidity and physical toughness (Frew, Weston, Reynolds, & Gurr, 2018; Ma & Yamaji, 2015). Therefore, there may be an effect of the treatments implemented together with the inoculation terms affecting the silicon translocation into the fruits, impacting the phytoliths compositions; this circumstance could have created competition with calcium molecules. Nevertheless, the negative interaction between calcium and silicon should be further investigated in pineapple fruit collapse.

Fruit Collapse Incidence

The interaction outcomes provided significant differences for this variable. The most superior incidences are obtained in treatments A (30%) and B (33.30%), using the BH, while the lowest is observed in B, C, and D, being 0%, in the case of the FI (Table 3). Based on the analysis of the physicochemical variables (primordially the antioxidants) and mineral content, treatment D can be considered the best option in the BH to obtain optimal quality and control fruit collapse incidence. Meanwhile, treatments A (control) and B can be less effective for reducing fruit collapse incidence, providing a lower fruit quality than D.

Because of the inoculation terms employed in this experiment, with a juice extracted from previously infected fruits, the determination of the number of colonies forming units (CFU) was complicated to be established in every juice sample concentrated. However, former trials carried out show that the minimal number of CFU required to inoculate the pathogen causing this disease should be in the range between 10^7 and 10^9 CFU/ml (*Dickeya zeae*), which was in agreement with previous experiments documented (Aeny, Suharjo, Ginting, Hapsoro, & Niswati, 2020; Sueno, Marrero, de Silva, Sether, & Alvarez, 2014).

The previously exposed information can explain why the FI was less effective in causing fruit collapse. Due to the characteristic of the inoculation terms implemented, the number of CFU necessary to generate an infection by *D. zeae* could have been different in each period, more superior in the FI. Furthermore, it has been demonstrated that silicon has more significant effects on plants under biotic or abiotic stress than on plants that are not under particular stress (Frew, Weston, Reynolds, & Gurr, 2018; Zargar, Mahajan, Bhat, Nazir, & Deshmukh, 2019). This knowledge can explain why silicon-based treatments, particularly treatment D, had a more significant impact on the BH variables.

CONCLUSION

The pre-harvest foliar fertilization with calcium and silicon affected the pineapple quality and fruit collapse incidence. The BH caused more incidence and severity of fruit collapse than the FI. Treatment D (Ca + Si from 13 to 11 weeks before harvest/from 6 weeks to harvest) delivered the best response with the most superior resistance to fruit collapse, having the lowest incidence and optimal fruit quality, with the highest AsA, an elevated β-carotene and mineral content, essentially under the BH. Meanwhile, treatments A (control: No foliar fertilizers applied) and B (Ca from 13 to 11 weeks before harvest/from 6 weeks to harvest) are considered the less effective in mitigating the impact of fruit collapse, having a high incidence, despite delivering some ideal fruit quality outcomes.

The number of CFU of the bacterium *Dickeya zeae* could cause the BH to be more harmful and effective in producing a superior incidence of fruit collapse. More studies concerning the impact of pre-harvest foliar calcium and silicon fertilization on pineapple physicochemical characteristics,

especially antioxidants and enzyme-related activities, were suggested for the future. Also, a future examination of silicon's effects on the calcium content and molecule assimilation in the pineapple plant is proposed.

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