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RESEARCH ARTICLE

Effect of pre-harvest fruit covers and calcium fertilization on pineapple thermotolerance and flesh translucency

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ABSTRACT

This study evaluated the effect of pre-harvest fruit covers and calcium fertilization on pineapple thermotolerance and flesh translucency. The treatments were, A (Control: yellow cover), B (white cover + change to yellow in four weeks before harvest), C (black cover + change to yellow in four weeks before harvest), D (Raynox until harvest), E (white cover + change to yellow in four weeks before harvest + Ca from ten weeks until harvest), F (black cover + change to yellow in four weeks before harvest + Ca from ten weeks until harvest), and G (Raynox until harvest + Ca from ten weeks until harvest). MD2 pineapple was employed in this experiment. Translucency incidence, severity and flesh lightness were determined, while calcium content, electrolyte leakage, and fruit temperature were examined to characterized pineapple thermotolerance. The translucency and thermotolerance were positively affected by the flesh temperature (average of 38 °C at 12:00 PM), whereas the thermotolerance was also influenced by the calcium (Ca^{2+}) assimilation in the cell wall. Treatments C and D reduced the thermotolerance (55-65 weight % of Ca^{2+} assimilated), and obtained a higher translucency incidence (> 10 %), while treatment E provided the best performance because decreased the translucency incidence (< 5 %), and increased the thermotolerance (73 weight % of Ca^{2+} assimilated). The calcium content and electrolyte leakage were between the ideal quality values. The dry season hastened the fruit ripening causing a reduced translucency. Further studies should be performed on thermotolerance effects on translucency and the relation to magnesium status.

Keywords: Cell wall; enzyme; incidence; MD2; temperature

INTRODUCTION

Pineapple (*Ananas comosus* L. Merr.) is an economically important crop in many tropical and subtropical areas, cultivated around 82 countries (Montero-Calderón et al., 2008; Chen et al., 2009; Hossain, 2016). For fresh fruit, low acid hybrids are often the most exported and required by the industry. These hybrids deliver continuous harvest and postharvest challenges to pineapple farmers (Zuraida et al., 2011; Žemlička et al., 2013; Cano-reinoso et al., 2021).

The critical problem of these hybrids is the high susceptibility to natural flowering, abrasion injury, and excessively low acidity; as well as a tendency for undesirable increase in total soluble solids, generating physiological disorders like translucency (Zuraida et al., 2011; Žemlička et al., 2013; Cano-reinoso et al., 2021). Translucency is a disorder of the pineapple fruit flesh, with an unknown cause. This is characterized by water soaking and low porosity in the flesh, with a possibility of approximate overall losses of 10 % for fresh fruit, exceeding up to 30 % in the wet season (Paull and Chen, 2015; Chen and Paull, 2017; Paull and Chen, 2018). This physiological disorder has been investigated and described on pineapple since time ago, focused on causes and possible alternative treatments (Paull and Chen, 2015; Paull and Chen, 2018).Chen et al. (2009) and Chen and Paull (2017) suggested temperature, sugar accumulation, and calcium level as essential factors related to translucency. The high temperature in the environment close to the fruit harvest possible favour translucency because of its effect on membrane permeability. In addition, elevated temperature produces a

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solute leakage in the fruit, generating a loss of membrane integrity (Paull and Chen, 2018; Murai et al., 2021). Chen (1999) and Chen and Paull (2001) demonstrated that by using covers to shade the fruit three to one month before harvest, an enhancement in the thermotolerance of the cell of pineapple fruit is provoked. Because of the high temperature and irradiance levels, a reduction of the translucency occurrence is created.

The use of pre-harvest fruit covers has become a normal practice worldwide (Sharma et al., 2014; Ali et al., 2021). Covers can offer physical protection to improve fruit visual quality by promoting peel coloration, like in apple fruit (Sharma et al., 2014; Wang et al., 2021); and reduction of cracking and russeting generated by the microenvironment created, like in Guava (Sharma et al., 2014; Ali et al., 2021) and pomegranate (Sharma et al., 2014; Hamedi Sarkomi et al., 2019).

On the other hand, thermotolerance is a rise in plant cells when grown for an extended period at elevated temperature, or when there is transient heat stress subjected them (Saidi et al., 2011; Song et al., 2012). This circumstance causes a hyperthermal response that synthesizes heat shock proteins (HPSs) (Saidi et al., 2011; Song et al., 2012), and triggers Ca^{2+} influx generating plant tissues with more saturated lipids and rigid membranes (Saidi et al., 2010; Wu and Jinn, 2010; Saidi et al., 2011). Recently, the assumption of elevated temperature as a cause of translucency, close to fruit ripening, has been reinforced by Chen and Paull (2017) and Paull and Chen (2018).

On top of that, calcium is a mineral important in pineapple during the initial stage of crop development due to its role concerning the cell division and differentiation. After flower induction, this mineral gives cellular stability, especially in sink organs (Paull and Chen, 2018; Vásquez-Jiménez and Bartholomew, 2018). Adequate calcium levels in the fruit, primordially close to harvest, can improve cell structures and preventing translucency (Paull and Chen, 2017; Chen and Paull, 2018). Since few papers have been reported on pineapple covers and their influences on constitution and physiological disorders, without a deeply observation on thermotolerance acquisitions, quality variables that may characterized this phenomenon, and calcium interactions; therefore, this study aims to evaluate the effect of pre-harvest fruit covers and calcium fertilization on pineapple thermotolerance and flesh translucency, focused on their impact in a low acid hybrid.

MATERIAL AND METHODS

Fruit, materials, and experimental design

The study was conducted in pineapple fields of Lampung, Sumatra Island of Indonesia, between 2019 and 2020. Pineapple fruit cultivar MD2 was used. Currently this low acid hybrid is one of the most marketable pineapple clones in the industry, known as a "golden ripe". MD2 is characterized by its bright-gold color, sweeter taste, elevated ascorbic acid content, reduced fiber and acidity, with an uniformed size (Bin Thalip et al., 2015).

The harvesting of the fruit was carried out between 144 to 147 days after flower induction when it is considered MD2 pineapple exhibit the appropriate physical and chemical characteristics to be consumed (Bin Thalip et al., 2015; Ding and Syazwani, 2016). In this study the experiment was first conducted between August and October of 2019 and replicated from November to January of 2020. The first trial corresponds to observations through the wet season, while trial two occurred during the dry season. Table 1 shows the soil physical and chemical characteristics.

Before starting the experiment, the soil was fertilized with 200 kg ha^{-1} Di-ammonium Phosphate, 1000 kg ha^{-1} K_2SO_4, and 200 kg ha^{-1} Kieserite crystal during row preparations; after that, foliar applications of 700 kg ha^{-1} Urea, 700 kg ha^{-1} $(NH_4)_2SO_4$, 1000 kg ha^{-1} K_2SO_4, 170 kg ha^{-1} $MgSO_4$, 60 kg ha^{-1} $FeSO_4$, and 60 kg ha^{-1} $ZnSO_4$, were carried out three months after plating, at intervals of 30 d. Finally, after the flower induction, liquid Ethepon and Borax were sprayed in doses of 2.5 L ha^{-1} and 30 kg ha^{-1}, respectively. A weather station (LSI Lastem, equipped with a CR6 datalogger of Cambell scientific, Italy) calculated an average of 55.78 % of relative humidity (RH), 32.99 °C of ambient temperature, 269.75 w m^{-2} of solar radiation, and a rainfall of 486 mm, for the period of the first trial; while for the trial two these variables were in average 30.26 % RH, 41.14 °C, 86.48 w m^{-2}, and 40 mm, respectively.

A randomized complete block design was set in the field. For the first trial, seven treatments, including six replications, were arranged with 30 fruits per replication.

Table 1: Physical and chemical properties of the soil in the experiment

Texture	1st Trial	2nd Trial
Clay (%)	29.14	21.60
Loam (%)	6.00	9.40
Sand (%)	64.36	69.00
Chemical properties	**1st Trial**	**2nd Trial**
pH (H_2O)	4.18	4.46
C (%)	1.24	1.23
N (mg kg^{-1})	900.00	800.00
P (mg kg^{-1})	3.22	14.10
K (mg kg^{-1})	110.97	125.00
Ca (mg kg^{-1})	310.00	315.00
Mg (mg kg^{-1})	8.25	56.40
Na (mg kg^{-1})	3.22	4.14

*The N, P, K, Ca, Mg and Na represent the available mineral content in the soil

In the case of the second trial, the number of replications organized was four. There were seven rows in each block with a width and length of 0.4 and 3.75 m, respectively. Pineapple plants were organized in two lines of 15 plants inside the row with a separation of 0.25 m. Observations were conducted once every two weeks, from ten weeks before harvest. Table 2 shows the arrangements of the cover treatments.

The yellow cover was implemented as a control cause is a typical material for sunburn protection in Lampung fields. Raynox was implemented as one of the treatments to compare the effect of a sunburn protectant product with the experiments' covers and know the incidence of thermotolerance and translucency occurrence. The material of fabrication for the yellow cover typically implemented in Lampung fields is machine glazed paper (MG), while for the white and black covers were employed non-woven polypropylene bags materials. All the covers had a dimension of 30 x 40 cm^2, 0.1 mm of thickness, with no photosensitive properties. Yellow cover had a grammage of 55 g m^{-2}, meanwhile black and white covers 80 g m^{-2}. The covers were set on the pineapple fruit shell without covering the crown.

Calcium applications were made in ten, eight, six, four, and two weeks before harvest. Simultaneously, Raynox was administrated in ten, eight, five, and two weeks before harvest. Calcium product employed was Calcibor (12.9 % w/v CaO and 2.6 % w/v B) in doses of 4 L ha^{-1} (v/v = 4 L/2000 L); in the same way, Ryanox doses sprayed were 25 L ha^{-1} (v/v = 25 L/1000 L). Calcium and Raynox were sprayed onto the leaf, fruit shell, and crown during night time, employing the recommendations and descriptions on calcium mineral uptake and mobility after forcing for pineapple fruit described by (Chen, 1999; Chen and Paull, 2000).

Determination of fruit temperature

The fruits flesh temperature was calculated similarly to the method of (Chen and Paull, (2001). A thermocouple thermometer was employed for this procedure (Taylor thermocouple thermometer with a k-type probe, 9821, USA). The thermocouple was introduced 1 cm under the pineapple shell to measure the temperature at every observation moment. Fruit shell temperature was taken utilizing an infrared thermometer (BENETECH, GM320 infrared thermometer, USA) that pointed the laser directly to the shell. Both flesh and shell temperature were obtained in the most exposed side of the fruit to the sunlight, three times a day (9:00 AM, 12:00 PM, and 3:00 PM). Three fruits per replication in every treatment were used. The observations were carried out four weeks before harvest to change all the covers to yellow colour. This time was selected as the ideal to calculate this variable because elevated temperatures one month before harvest have been crucial to increasing translucency occurrence in the fruit (Paull and Chen, 2015; Paull and Chen, 2018).

Table 2: Organization of the treatments used in the study

Treatment	Characteristic
A	Control: Yellow cover
B	white cover + change to yellow in four weeks before harvest
C	black cover + change to yellow in four weeks before harvest
D	Raynox until harvest
E	white cover + change to yellow in four weeks before harvest + Ca from ten weeks until harvest
F	black cover + change to yellow in four weeks before harvest + Ca from ten weeks until harvest
G	Raynox until harvest + Ca from ten weeks until harvest

Determination of the electrolyte leakage (EL)

The EL was obtained in each treatment replication as a composition of three fruits during every observation, as described by (Chen and Paull, (2001). Flesh plugs arranged longitudinally were taken using a cork borer (10 mm diameter) and sliced into 2 mm thick disks. After that, around 6 g of disks were washed three times, employing deionized water to remove lysed cell material. The disks were shaken and incubated in 60 mL of 0.3 M mannitol solution for two hours. A radiometer was used to determine the conductivity of this solution. Consequently, the sample was boiled for two hours, looking to release all electrolytes, and finally, total conductivity was measured. The EL is represented as the percentage of the total conductivity.

Calcium mineral analysis

This analysis was performed in every observation, using inductively coupled plasma optical emission spectrometry (ICP-OES) (5100 ICO-OES, Agilent Technologies, USA). A composition of three fruit per replication was analyzed in every treatment, based on the method described by (Benton-Jones, 2001). For the analysis, 5 mL of juice from the fruit flesh adjacent to the core was taken, filtrated with a paper to prevent any contaminant or residuals in the liquid sample, and put in a digestion tube. After that, 5 mL of 65 % Nitric Acid through the digestion tube walls was added and left overnight. Later, the sample was heated with a block digester at 125 °C for one hour, and subsequently lifted and cooled. Thereafter, 3 mL of 30 % Hydrogen Peroxide (H_2O_2) through the digestion tube walls were added, reheat for 1 h, removed, and cooled. This step was repeated three to five times until a clear filtrate was obtained. Afterward, HNO_3 was used to prevent the filtrate from drying out (1 mL residue), warming and cooling the sample again. 5 mL of Nitric Acid with distillate water (1:10) was added and shake using a Vortex Shaker.

Finally, the sample was transferred to a flask 25/50 mL quantitatively and pitch with distillate water creating an extract ready for mineral analysis. Results are expressed in dry basis content.

Flesh translucency determination

Flesh translucency was determined by employing six fruits per replication in each treatment at harvest time. A longitudinally cut was make in the fruit, separating them into two parts. A subjective method was used for the severity assessments. Examinations of visible ovaries of the fruit flesh adjacent to the core were necessary to elaborate on this method, following the previous observations of (D'Eeckenbrugge and Leal, 2003; Montero-Calderón et al., 2010)

An ovary number ranging between 10 to 18 in a half of the longitudinal cut of the fruit flesh was obtained in previous trials; usually 10 ovaries for small fruits and 18 for the biggest ones. Later, after observing that naturally, an ovary affected by translucency turned into brown-dark colour (Fig. 1), the determination of those was the last step to follow. The severity in percentage was established in both longitudinal flesh cuts by accounting for the number of brown-dark ovaries, divided by the number of visible ovaries. Finally, the incidence was calculated by measuring the percentage of fruits affected in the observations.

Fig 1. Illustration of the ovary identification method in the flesh. A) Ordinary recognition of the ovary in one side of the flesh, adjacent to the core (green ring). B) Identification of the high translucent ovary, generally with a dark colour in the flesh (red ring).

Additionally, measurements of the flesh lightness (L*) and b* chroma value using a colourimeter (3nh, NH310 - High-quality portable colourimeter, China) were carried out on the flesh of the longitudinal cuts every sample. The purpose was to establish a correlation between L*, b* and translucency severity to identify a more accurate and objective severity determination method for future studies. Every longitudinal cut was divided into four areas (two at the superior part of the flesh and two at the base); after the measurements were taken, the total L* value of the flesh was calculated by computing the result of the four areas in every side of the longitudinal cuts.

Evaluation by scanning electron microscope - energy-dispersive X-ray (SEM-EDX)

SEM analysis was conducted on second trial samples, similar to the method applied by Hu et al. (2012). A fine flesh tissue adjacent to the core (5×5×2 mm^3) was separated from the middle of the flesh with a tweezer. In a desiccator dried at a critical point of liquid CO_2 the slices were dehydrated in a compendium of ethanol solutions before scanning. The samples were grouped onto tubs of an aluminium specimen using conductive silver glue and sputter-coated with gold. SEM was processed with a scanning electron microscope (ZEISS/EVO MA 10, German) equipped with an Energy Dispersive Spectrometer (EDS), which permits a SmartEDX analysis at 15 Kv. EDX analysis was carried out to reveal the calcium and magnesium weight percentage concentration in the cell wall.

Statistical analysis

Statistical analyses were performed using SPSS Version 22.0 software (SPSS Inc., Chicago, IL, USA). All data were analyzed by ANOVA of one-way. Mean significant differences at $P < 0.05$ were determined by Duncan's multiple range tests and Kruskal-Wallis test (specifically for the translucency data).

RESULTS

Flesh translucency assessments

The translucency of the pineapple flesh was recorded in terms of incidence and severity. In the first and second trial, the severity and incidence percentages did not show statistically significant differences however, examinations of the mean values help understand the research outcomes. According to the results, treatment C (17.78 %) produced the highest incidence, followed by D (11.11 %), especially in the first trial, where the percentage was considerably higher (Table 3). In case of the severity, the first trial also had treatment C (1.77 %) and D (2.46 %) with the highest percentage; meanwhile, in trial two, severity had lower values compared to trial one (Table 3). In trial one, mostly

Table 3: The effects of the treatments used on the flesh calcium content, electrolyte leakage (EL), EL/Ca ratio, lightness (L*), chroma b*, and translucency incidence and severity, at harvest time of the fruits

1st Trial						Translucency (%)	
Treatment	Ca (mg kg^{-1})	EL (%)	EL/Ca	Lightness (L*)	b*	Incidence	Severity
A	1014.92 ± 26.61 ab	47.08 ± 1.10 a	465.87 ± 18.59 a	75.88 ± 0.37 ab	32.83 ± 0.94 cd	4.44 a	1.54 a
B	857.31 ± 59.81 c	47.28 ± 1.99 a	565.92 ± 49.80 b	76.07 ± 0.38 ab	37.29 ± 0.87 abc	4.44 a	0.56 a
C	1044.86 ± 47.17 ab	40.04 ± 1.70 a	390.34 ± 33.88 a	74.99 ± 0.43 b	33.55 ± 0.93 bcd	17.78 a	1.77 a
D	1060.69 ± 41.65 ab	42.06 ± 1.55 ab	399.80 ± 22.58 a	76.32 ± 0.36 a	36.59 ± 0.85 a	11.11 a	2.46 a
E	1080.02 ± 41.56 a	42.07 ± 1.88 ab	394.67 ± 30.36 a	76.1 ± 0.40 ab	35.73 ± 0.96 ab	4.44 a	0.31a
F	930.50 ± 26.66 bc	39.49 ± 2.30 b	424.68 ± 22.73 a	76.64 ± 0.39 a	31.73 ± 0.96 d	5.56 a	0.34 a
G	816.57 ± 33.32 c	37.26 ± 1.90 b	462.57 ± 36.68 a	75.83 ± 0.41 ab	35.52 ± 1.02 abc	3.33 a	0.25 a
2nd Trial						Translucency (%)	
Treatment	Ca (mg kg^{-1})	EL (%)	EL/Ca	Lightness (L*)	b*	Incidence	Severity
A	1312.83 ± 109.72 a	32.48 ± 0.69 ab	253.21 ± 24.07 a	78.95 ± 0.29 abc	22.75 ± 0.26 d	0.00 a	0.00 a
B	1454.53 ± 73.29 a	36.19 ± 3.09 ab	250.47 ± 22.63 a	78.30 ± 0.31 c	23.53 ± 0.31 abc	2.00 a	0.15 a
C	1520.64 ± 46.79 a	39.19 ± 5.33 ab	256.19 ± 31.32 a	78.44 ± 0.33 bc	24.30 ± 0.44 ab	6.00 a	0.46 a
D	1515.66 ± 12.09 a	40.38 ± 5.49 a	267.24 ± 38.21 a	78.80 ± 0.32 bc	25.14 ± 0.60 a	2.00 a	0.29 a
E	1334.37 ± 88.60 a	35.05 ± 3.31 ab	270.07 ± 43.55 a	79.33 ± 0.30 ab	23.01 ± 0.40 cd	0.00 a	0.00 a
F	1389.98 ± 58.71 a	37.61 ± 0.53 ab	271.62 ± 8.49 a	78.91 ± 0.28 abc	23.61 ± 0.36 abc	0.00 a	0.00 a
G	1484.20 ± 33.45 a	28.67 ± 1.53 b	193.82 ± 13.07 a	79.78 ± 0.34 a	23.20 ± 0.32 bcd	0.00 a	0.00 a

**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 and Kruskal-Wallis test (for the translucency incidence and severity data) (P<0.05).
** A (Control: Yellow cover), B (white cover+change to yellow in four weeks before harvest), C (black cover+change to yellow in four weeks before harvest), D (Raynox until harvest), E (white cover+change to yellow in four weeks before harvest+Ca for ten weeks until harvest), F (black cover+change to yellow in four weeks before harvest+Ca for ten weeks until harvest), and G (Raynox until harvest+Ca for ten weeks until harvest)

those treatments with low incidence values had a lower severity, which impacted more when there was a calcium application; Also, in trial two between more reduced the incidence, lower was the severity; although almost all the severity percentages were zero.

Measurements of L* by the colourimeter provided significant differences in both trials. The first trial exposed representative variances between the treatment with the most reduced value C (74.99) and the one with the most significant F (76.64), while the second trial displayed the most notable significant variances between treatment B and G (78.3 and 79.78, respectively) (Table 3). In the case of b*, this variable also displayed significant differences in both trials. In the trial one, treatment D had the most superior outcome (36.59), while treatment F exposed the lowest one (31.73); besides, for the second trial, treatment D (25.14) provided again the highest value with A having the most reduce outcome (22.75) (Table 3). A negative interaction was noticed between the L* and b* in both trials. As higher was the L* value in each of the treatments, lower was the value of b* displayed.

Calcium determinations

Calcium results displayed significant differences for trial one but no for trial two. In the trial one, treatment A, C, D, and E had a calcium content much higher compared to B, F, and G. The Treatment G (816.57 mg kg^{-1}) and E (1080.02 mg kg^{-1}) had the most reduced and highest values in this trial, respectively (Table 3). Fig. 2 displayed the performance of E and G through the experiment weeks

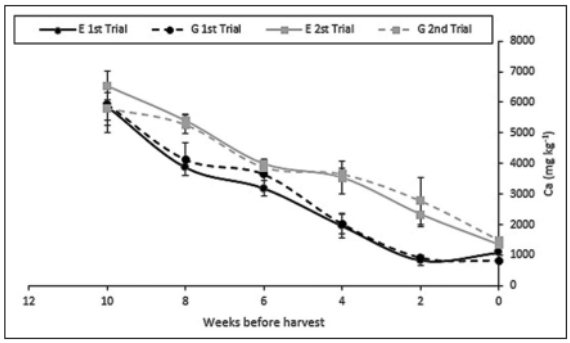

Fig 2. The effect of the treatment implemented on the pineapple flesh's calcium content in treatment E and G for both trials during the experiment weeks. Treatment's composition: E (white cover + change to yellow in four weeks before harvest + Ca from ten weeks until harvest), and G (Raynox until harvest + Ca from ten weeks until harvest). Values are the mean of 6 and 4 replicates for trials one and two, respectively, and error bars represent the standard error.

in both trials, showing that both treatments have a similar trend. Nevertheless, in the first trial starting in four weeks, this trend changes and increases, exposing a remarkable difference until the harvest time.

The decrease of the incidence and severity percentage in the trial one associated with the treatments with calcium applications is not reflected in the fruit's calcium levels (Table 3). Also, not all the treatments with high calcium content delivered a reduced percentage of incidence and severity. There may be a secondary factor that decreases the incidence and severity triggered by calcium applications.

EL determinations

The EL of the fruit flesh revealed significant differences in both trials. For trial one, treatments A and B obtained a percentage higher than C, F, and G; besides, D and E did not have significant variances in their results. Significant differences were determined for G (37.26 %) with the lowest value and B (47.28 %) with the highest one, providing the most notable contrast between the data analyzed. In the same way, for the replication of the experiment, that contrast occurred between treatment D (40.38 %) and G (28.67 %) (Table 3). Fig. 3 shows the trend of the EL for trial one and two, among treatment B and G, and D and G, respectively. There was a significant change in the trend of the percentage in four weeks before harvest, increasing and exposing a gap between treatments for trial one. For trial two, that situation happened two weeks before harvest, when there was a fundamental change in the EL trend.

Parallel to that, EL/Ca ratio did not show any significant variances in the second trial results; meanwhile, for trial one, treatment B and C received the most representative and lowest ratios (565.92 and 390.34, respectively) (Table 3). This was performed to compare the EL/Ca ratio results with the translucency incidence and severity. There is a possible linkage between the cell wall membrane permeability (EL), calcium status in fruit, and translucency that wanted to be examined.

Estimation of the fruit temperature

Fruit temperatures assessed at 3:00 PM did not indicate any significant differences between values obtained. On the contrary, at 9:00 AM, treatments evidenced representative variance in the results. For 9:00 AM in the first trial, the most reduced temperature of 25.53 °C and highest of 27.27 °C was measured for treatment C and D, respectively. In the second trial, a value of 35.85 °C in B and 35.60 °C in F represented the most elevated and lowest results (Table 4). In the same way, at 12:00 PM in the trial one, the fruit flesh exposed significant differences with the lowest and highest temperature in treatment B (38.78 °C) and D (40.93 °C). Besides, in the same hour, trial two had that contrast result between treatment A and C (37.97 °C and 38.1 °C, respectively) (Table 4). Fruit shell temperate for the second trial did not show representative differences in the results obtained. Conversely, the first trial showed significant differences at 9:00 AM and 12:00 PM. At 9:00 AM, temperatures of 27.36 °C for treatment B and 31.03 °C for D were assessed as the lowest and the highest. Similarly, at 12:00 PM obtained values of 35.96 °C in treatment B and 39.65 °C in G were the most reduced and elevated results, respectively (Table 4). Temperature data at 12:00 PM in flesh and shell of the pineapple fruit were always higher than the data at 9:00 AM and 3:00 PM. Moreover, differences in temperature between shell and flesh ranged around 2 to 3 °C in each treatment for both trials (Table 4). The results suggested that for the most critical condition (12:00 PM), treatments B and E are more efficient to control and set the flesh and shell temperature in the lowest values, meanwhile treatments C, D, and G facilitate the increase of this in the flesh and shell, being less effective in that aspect.

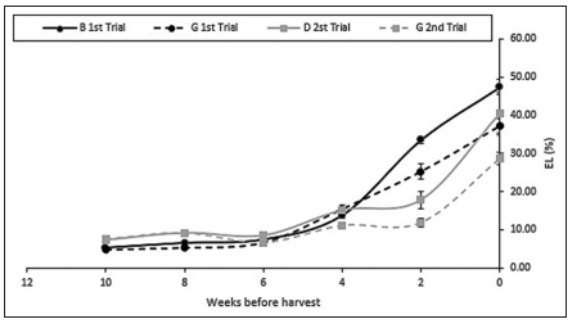

Fig 3. Effect of the treatments employed on the pineapple flesh's electrolyte leakage (EL) in treatment B and G (trial one) and treatment D and G (trial two) during experiment weeks. Treatment's composition: B (white cover + change to yellow in four weeks before harvest), D (Raynox until harvest), and G (Raynox until harvest + Ca from ten weeks until harvest). Values are the mean of 6 and 4 replicates for trials one and two, respectively, and error bars represent the standard error.

Effect of the covers on the cell wall structure of pineapple fruit flesh

SEM analysis of the fruit flesh was carried out at harvest in all treatments for the second trial. Fig. 4 shows that treatments A, B, D, E, and G maintained the cell wall integrity through the experiment time. On the contrary, C and F exposed some symptoms of integrity losses in their cell wall structure. The symptoms were exhibited by clear broken holes across the area of examination. This result suggested that C and F employing black covers would be more susceptible to translucency than A, B, D, and E.

Table 5 displays the EDX results of the flesh tissue examined for trial two. The results were calculated in terms of calcium and magnesium weight percentage. Calcium percentages show that treatments C, F, and G, are the most susceptible to have integrity losses in the cell wall, especially in F. Moreover, in these treatments, magnesium's result tends to be higher than A, B, D, and E, suggesting an antagonism interaction between calcium and magnesium in the cell wall structure. Treatments B and E, both using white cover initially, exposed the highest calcium and the lowest magnesium percentages in the samples. This statement infers that white cover could help maintain the cell wall structure and reaffirm calcium and magnesium interaction's antagonism.

Table 4: The effects of the treatments employed on the pineapple flesh and shell temperature four weeks before harvest, at 9:00 AM, 12:00 PM, and 3:00 PM

1st Trial						
Treatment	Flesh temperature (°C)			Shell temperature (°C)		
	9:00 AM	12:00 PM	3:00 PM	9:00 AM	12:00 PM	3:00 PM
A	26.5 ± 0.52 ab	39.62 ± 0.39 abc	36.19 ± 0.24 a	30.2 ± 0.96 ab	36.85 ± 1.10 ab	34.48 ± 0.38 a
B	26.22 ± 0.60 ab	38.78 ± 0.47 c	35.67 ± 0.28 a	27.36 ± 0.80 b	35.96 ± 1.06 b	35.82 ± 0.83 a
C	25.53 ± 0.50 b	39.28 ± 0.58 bc	35.99 ± 0.31 a	28.32 ± 1.11 ab	36.75 ± 1.12 ab	35.49 ± 0.54 a
D	27.27 ± 0.62 a	40.93 ± 0.41 a	35.75 ± 0.23 a	31.03 ± 0.91 a	38.46 ± 0.97 ab	35.41 ± 0.71 a
E	25.88 ± 0.36 ab	40.35 ± 0.43 ab	36.21 ± 0.28 a	28.75 ± 0.81 ab	35.99 ± 0.54 b	36.05 ± 0.60 a
F	26.10 ± 0.45 ab	39.07 ± 0.60 bc	36.16 ± 0.42 a	29.36 ± 1.02 ab	37.24 ± 1.11 ab	35.03 ± 0.56 a
G	26.56 ± 0.61 ab	40.74 ± 0.18 a	36.09 ± 0.26 a	31.03 ± 1.14 a	39.65 ± 0.92 a	35.09 ± 0.58 a
2nd Trial						
Treatment	Flesh temperature (°C)			Shell temperature (°C)		
	9:00 AM	12:00 PM	3:00 PM	9:00 AM	12:00 PM	3:00 PM
A	35.73 ± 0.6 abc	37.97 ± 0.08 b	34.40 ± 0.51 a	32.08 ± 0.58 a	39.85 ± 0.22 a	37.18 ± 1.25 a
B	35.85 ± 0.07 a	38.03 ± 0.01 ab	34.41 ± 0.49 a	31.32 ± 0.48 a	39.00 ± 0.37 a	37.33 ± 1.36 a
C	35.83 ± 0.07 ab	38.1 ± 0.02 a	34.41 ± 0.50 a	31.48 ± 0.55 a	39.34 ± 0.30 a	37.13 ± 1.74 a
D	35.64 ± 0.09 bc	38.07 ± 0.03 ab	34.53 ± 0.46 a	32.44 ± 0.33 a	39.41 ± 0.36 a	37.38 ± 1.26 a
E	35.63 ± 0.08 bc	38.04 ± 0.03 ab	34.40 ± 0.51 a	31.19 ± 0.45 a	39.18 ± 0.29 a	37.57 ± 1.35 a
F	35.60 ± 0.02 c	38.08 ± 0.03 ab	34.43 ± 0.50 a	32.48 ± 0.34 a	39.40 ± 0.21 a	37.23 ± 1.49 a
G	35.63 ± 0.05 bc	38.08 ± 0.03 ab	34.49 ± 0.49 a	31.38 ± 0.30 a	39.24 ± 0.39 a	37.39 ± 1.40 a

** 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: Yellow cover), B (white cover+change to yellow in four weeks before harvest), C (black cover+change to yellow in four weeks before harvest), D (Raynox until harvest), E (white cover+change to yellow in four weeks before harvest+Ca for ten weeks until harvest), F (black cover+change to yellow in four weeks before harvest+Ca for ten weeks until harvest), and G (Raynox until harvest+Ca for ten weeks until harvest)

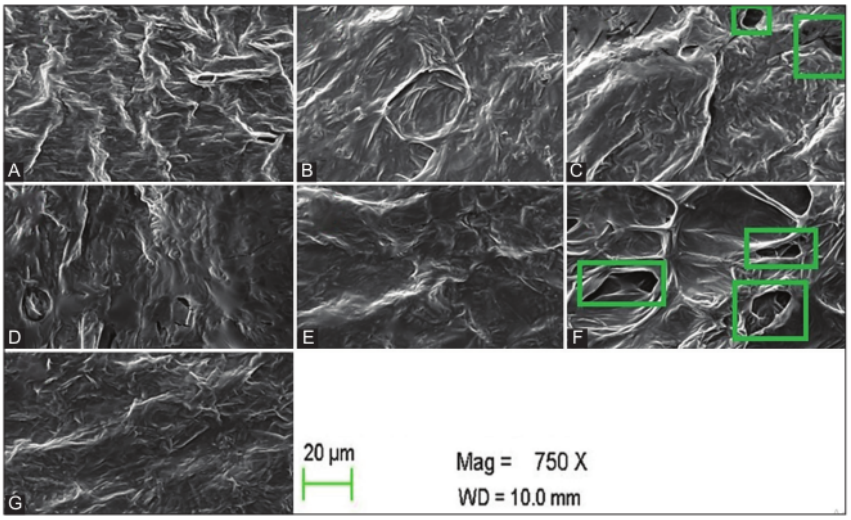

Fig 4. Effects of the treatments implemented on the cell walls of pineapple flesh samples detected by SEM (20 µm size, 750 x of magnification). Integrity losses and discontinuities of the cell wall (green square) were observed. Treatment's composition: A (Control: yellow cover), B (white cover + change to yellow in four weeks before harvest), C (black cover + change to yellow in four weeks before harvest), D (Raynox until harvest), E (white cover + change to yellow in four weeks before harvest + Ca from ten weeks until harvest), F (black cover + change to yellow in four weeks before harvest + Ca from ten weeks until harvest), and G (Raynox until harvest + Ca from ten weeks until harvest).

DISCUSSION

The purpose of the treatments combinations arrangements was to increase the thermotolerance during the fruit's initial development and to maintain the temperature at the lowest level possible among the experiment's last weeks. The use of the yellow cover in each treatment was essential for that.

Table 5: The results of the cell wall EDX analysis, for each of treatment

	Weight (%)		
Treatment	Ca	Mg	Total
A	69.11 ± 0.10	30.89 ± 0.06	100
B	72.92 ± 0.05	27.08 ± 0.04	100
C	57.38 ± 0.04	42.62 ± 0.04	100
D	64.86 ± 0.07	35.14 ± 0.05	100
E	73.31 ± 0.05	26.69 ± 0.04	100
F	23.70 ± 0.03	76.30 ± 0.04	100
G	56.44 ± 0.05	44.00 ± 0.04	100

Flesh translucency

Chen and Paull (2001) reported one of the few studies on the influences of covers on translucency occurrence in pineapple; they demonstrated that shading the fruit with white-opaque paper highly increases the translucency incidence in case that the treatment is applied up to five weeks before harvest. Furthermore, pineapple cultivars are susceptible to sunburn because of the elevated irradiance when the fruit is close to harvest (Hepton, 2003; Lu et al., 2011; Prabha et al., 2018). Therefore, to look for an alternative cover that protects the shell against sunburn and subsequently decrease the fruit temperature, yellow cover was selected as the optimal option to be employed in combination with some of the treatments starting four weeks before harvest.

Thermotolerance is the ability of an organism to deal with extremely high temperatures (Song et al., 2012). This ability is possibly provoked by the exposition to short but sublethal high temperatures, together with a temperature progressing towards deadly high levels, habitually under natural conditions (Larkindale and Vierling, 2008; Pumisutapon et al., 2012; Song et al., 2012). During these heat periods, several heat shock proteins (HSPs) are synthesized, and those help to provide thermoprotection to plants to subsequent heat stress (Song et al., 2012). This is why raising the environmental temperature weeks before harvest increases the translucency incidence, in case an adequate thermotolerance in the fruit has been undeveloped. In trial one, the thermotolerance could have been more remarkable in the treatments with calcium spraying because of the lower incidence and severity results, while treatment C and D, without the calcium application, would make the fruit more susceptible to translucency.

Second trial results on translucency incidence and severity were affected by the severe dry season and elevated temperature. Elevated temperatures are able to retard and hasten fruit development by decreasing metabolic activities (Bartholomew et al., 2003; Bartholomew and Sanewski, 2018). The optimal harvest time for the second trial was possibly delayed because of this condition. Therefore, when the fruit was harvested, the high sugar accumulation and reduced calcium content, usually triggering and evidenced in translucency, were not acquired (Paull and Chen, 2015; Chen and Paull, 2017). In this trial, treatment C and D had the highest incidence, reaffirming the low thermotolerance hypothesis obtained when these treatments were used. The severity outcomes of the experiment suggest that the treatments had a greater impact on the incidence. In trial one, not all the severity results were correlated with the incidence, as the treatment with the most elevated incidence (in case of C), did not obtain the highest severity; moreover, treatment having a lower incidence (like A and D), had a superior severity value. Therefore, the severity is possibly more dependent on the individual calcium status, sugar content, and temperate influence of the analyzed sample (Chen et al., 2009; Chen and Paull, 2017; Murai et al., 2021); although this cannot be concluded, as the severity percentage of trial two were mainly zero, opposite to the first trial, indicating an influence of the high temperature on the severity as well. Therefore, further studies between these variables are suggested. Furthermore, due to this situation, some of the discussions of this research's outcomes are focused more on the impact of the variables studied in the translucency incidence, while in some cases, like the second trial outcomes, this variable was considered more appropriate to be taken into account for interpretation of results.

In both trials, L* and b* provided similar results as observed in previous research ($L^* \geq 70$ and $b^* \geq 20$) (Nadzirah et al., 2013; Ding and Syazwani, 2016). This condition is attributed to flesh in an optimal ripened stage, but is not enough to detect flesh translucency (Nadzirah et al., 2013; Ding and Syazwani, 2016). Translucency exposes opaque flesh symptoms associated with more reduced L* and more elevated b* value (Ding and Syazwani, 2016; Paull and Chen, 2018). Furthermore, unfortunately few experiments have been reported on b* values and their association with flesh optimal conditions. Mostly those experiments have been focused on shell colour determinations. Therefore, still work need to be done to stablish a relation between b* and flesh translucency symptoms.

The flesh changes from white to bright yellow close to harvest. Carotenoids are responsible for that yellow colour change, linked to a normal reduction of L*and elevation of b* values (Syazwani et al., 2013). Typically carotenoids increase during the final ten days before the fruit's fully ripe stage (Paull and Chen, 2003; Paull and Chen, 2018). Those are constituted by β-carotene, zeaxanthin, and β-apoδ'-carrotenal (Steingass et al., 2020). β-carotene is the most predominant in the flesh (Sun, 2011; Vásquez-Jiménez and Bartholomew, 2018; Steingass et al., 2020). In addition, a relation between potassium and carotene content in the

fruit has been previously proposed (Saradhuldhat and Paull, 2007). Although, this previous approach should be further investigated in the future.

The treatments could have induced a delay or decrease in the accumulation of β-carotene and potassium, especially those employing covers, with more impact in trial two, where the L* results were higher and b* were lower (Table 3). This affirmation can explain the difficulties in establishing a relation between L*, b*, and translucency severity, regardless of the significant differences exposed in this experiment's outcomes.

Calcium content

De Freitas and Nassur (2017) reported that as a signaling molecule, calcium (Ca^{2+}) could delay the ripening and senescence-related processes of the fruit, possibly regulating signaling responses inhibiting ethylene biosynthesis and respiration. This infers that the assimilation of the Ca^{2+} molecule could help regulate the sugar accumulation and hydrolyzing of the cell wall in the fruit, which ends in the more reduced content of solutes in the pineapple flesh and, as a consequence, the decrease of the translucency severity and incidence.

Flesh translucency in pineapple has been associated with increased cell wall hydrolysis and membrane permeability (Paull and Chen, 2015; Chen and Paull, 2017; Paull and Chen, 2018). Meanwhile, application of calcium during the fruit's development led to an increase in the concentration of this mineral in the flesh and, consequently, a decrease in the translucency occurrence. Furthermore, in the absence of sufficient calcium, cell membranes tend to lose integrity, producing leakage, and as a result, translucency appears (Chen and Paull, 2017; Dayondon and Valleser, 2018; Paull and Chen, 2018).

In trial one, treatment E could permit to absorb and assimilate more calcium ions (Ca^{2+}) and maintain the cell wall membrane's integrity. This treatment may have regulated the membrane permeability through fruit development, an opposite process happening in treatment B and G. Moreover, treatment E in the first trial was able to provided more thermotolerance to the flesh tissue than the others, perhaps because of that calcium assimilation. After a few minutes of temperature increase, a preserved transient calcium influx has been detected in several plants (Saidi et al., 2010; Wu and Jinn, 2010; Saidi et al., 2011). Furthermore, dry season and elevated temperatures also affect the calcium content in the fruit of trial two. Although the concentration of calcium in the flesh of pineapple fruit declines with the fruit development (Fig. 2) (Chen and Paull, 2017; Dayondon and Valleser, 2018; Paull and Chen, 2018), the higher calcium content in the outcomes of the trial two is more related to the delaying of the fruit ripening caused by the elevated temperatures.

EL in the fruit

EL is an image of the response to stress in the entire plant cells. This phenomenon is used to detect any stress-induced injury and measuring plant stress tolerance (Bajji et al., 2002; Demidchik et al., 2014). EL is universal among several species, tissues, and cell types and can be triggered by heat (Liu and Huang, 2000; Demidchik et al., 2014; Luengwilai et al., 2018). After applying any stress factor, this is detected instantaneously and tends to occur from a few minutes to several hours (Demidchik et al., 2014). Results showed that treatment G in both trials reduces the EL, while the other treatments in trials one and two have irregular outcomes. This suggested that the results would be more related to electrolyte losses because of a normal physiologic process opposite to induced temperature stress.

In the Smooth Cayenne pineapple cultivar, the flesh's EL increases rapidly from six weeks before harvest, in concomitance with the sucrose accumulation (Chen and Paull, 2000; Paull and Chen, 2018). Fig. 3 shows identical patterns; however, a rapid rise occurs four weeks before harvest, proving that this sucrose accumulation is possibly delayed for low acid hybrids. This finding confirms that both trials' EL values are more associated with the expected increase of the sugar content in the flesh than the temperature stress factor derived from the treatments and environmental influences.

The EL of flesh with severe translucency symptoms (>70 %) is about 6 % higher than normal fruit flesh (Chen and Paull, 2000; Paull and Chen, 2018). The slight difference between ordinary and high translucent fruit evidence that membrane permeability could be related; however, is not crucial in establishing flesh translucency (Paull and Chen, 2015; Paull and Chen, 2018). This deduction explained why the EL and EL/Ca ratio results in trials one and two are unrelated to any decrease or increase of translucency incidence and severity in this experiment (Table 3).

Fruit temperature

The fruit flesh and shell temperature results in both trials contradict the previous theory. It has been reported that fruit temperature can be elevated because of the exposure to direct sunlight, and the section exposed of the fruit can exceed 50 °C, when there is an ambient temperature close to 30 °C (Bartholomew and Sanewski, 2018; Youryon and Supapvanich, 2021). In this experiment, the flesh and shell did not reach that value, having an ambient temperature higher than previous authors suggested (32.99 and 41.14 °C, trial one and two respectively). This situation shows that there could be any other factor that did not let the

fruit to reach that critical value documented; also, this result proves why treatment E obtained higher thermotolerance, opposite to C and D, when the results of temperature are linked to the translucency incidence (Tables 3 and 4, respectively). This situation could be observed mostly at 12:00 PM in the flesh for both trials. Regarding pineapple translucency, a significant impact of the fruit temperature occurs when the heat-tolerance limit in the flesh has been surpassed (Chen and Paull, 2001; Paull and Chen, 2003; Paull and Chen, 2018). Seemly, treatment C and D could have overcome that limit and generated an increase in the incidence.

SEM-EDX analysis

Analysis of the SEM results in trial two shows that because of the lower thermotolerance achieved by treatment C, a disruption and metabolic modification in the fruit cell wall constitution was produced. This circumstance generated the higher translucency incidence in this treatment; nonetheless, there may be an influencing factor that prevents that from happening in treatment F. Physiological disorders like translucency are related to damage in the cell membrane (Hu et al., 2012; Tucker et al., 2017). This damage initiates several reactions, including the disruption of cellular and subcellular structures (Hu et al., 2012; Tucker et al., 2017); besides, elevated temperature in fruit speeds up metabolic processes and the activities of the cell wall degrading enzymes like Polygalacturonases (PG) (Hocking et al., 2016; De Freitas and Nassur, 2017). PG are enzymes that break down glycosidic links between units of desterified galacturonic acids, creating a cell wall breakdown (Conway, 1988; De Freitas and Nassur, 2017). PG activity in C could have been higher in opposite to the other treatments.

The antagonism interaction exhibited between calcium and magnesium could be related to a translucency incidence. Treatment C had a weight percentage of magnesium higher than the calcium (Table 5). Meanwhile, calcium assimilation in the cell wall causes a positive effect in the flesh firmness, related to a decrease in translucency occurrence (Paull and Chen, 2015; Ding and Syazwani, 2016; Chen and Paull, 2017), magnesium has a negative impact on this variable (Gerendás and Führs, 2013). In addition, competition between the main base cations K, Ca, and Mg has been proven to be a causal factor responsible for lower firmness in fruit, a crucial storage quality parameter (Marcelle, 1995; Gerendás and Führs, 2013). However, several studies have been conducted on magnesium deficiency and excessiveness in pineapple, without providing negative results (Vélez-Ramos and Borges, 1995; Ramos et al., 2010); more experiments are required on this matter. Currently, there is insufficient information to state positive or negative effects on all fruit metabolism. Therefore, further studies ought to be performed concerning the effects of thermotolerance on the magnesium status in the fruit and the relation to translucency occurrence.

CONCLUSION

The treatments implemented influenced the thermotolerance and flesh translucency. The flesh temperature and calcium (Ca^{2+}) assimilation in the cell wall were considered as the variable more influential in the experiment, affecting the thermotolerance acquisition and translucency incidence. Treatment C (black cover + change to yellow in four weeks before harvest) and D (Raynox until harvest) obtained lower thermotolerance and higher translucency incidence in both trials. On the contrary, treatment E (white cover + change to yellow in four weeks before harvest + Ca from ten weeks until harvest) delivered the most efficient performance, decreasing the translucency incidence and gaining more thermotolerance. The dry season and elevated temperature affected the results of the second trial. This condition generated a lower translucency incidence and severity, concerning more to the fruit's ripening delay than a thermotolerance influence. Further studies on the effects of thermotolerance on translucency occurrence as well as the pineapple magnesium relation status, ought to be conducted.

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Author contributions

Diego Mauricio Cano-Reinoso: Data curation, Writing- Original draft preparation, methodology, Software, investigation. Condro Wibowo: Visualization, supervision, methodology, Writing- Reviewing and Editing. Loekas Soesanto: Visualization, supervision, methodology. Kharisun: Visualization, supervision, methodology.

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