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Full Length Research Paper

Disease occurrence and fruit quality of pre-harvest calcium treated red flesh dragon fruit (*Hylocereus polyrhizus*)

Muhd Azlan Abd Ghani, Yahya Awang* and Kamaruzaman Sijam

Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

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A study aiming at increasing Ca content in red flesh dragon fruit (*Hylocereus polyrhizus*) via pre-harvest fruit $CaCl_2$ spray in relations to postharvest disease occurrence and fruit quality parameters was conducted. From day 7 after anthesis, red flesh dragon fruits (*H. polyrhizus*) were sprayed at weekly interval for four times with different concentration of $CaCl_2$ (0, 1, 2, 3, and 4 gl^{-1}). Fruit Ca content in fruit peel (DW basis) were markedly increased with the increasing Ca concentration in the applied solution but the Ca application did not affect Ca content in flesh. The compositions of N, P, K and Mg were not affected by the treatment. The severity of anthracnose and brown rot, caused by *Colletotrichum gloeosporioides* and *Monilinia fructicola,* respectively, of artificially wounded fruits was reduced in pre-harvest $CaCl_2$-treated fruits. The concentrations of soluble solids and titratable acidity in fruit were not affected by the treatment. The firmness of fresh cut fruit was markedly enhanced at higher concentration of $CaCl_2$. Beneficial effects of increasing Ca in fruit can be seen in the increase of fruit firmness although this did not contribute in enhancement of fruit quality-related parameters. Increased Ca content in treated fruits, together with no effects of treatment on other mineral nutrients, increased the ratio of Ca to other elements and this may contribute directly to the reduction of anthracnose and brown rot severity in $CaCl_2$-treated fruits.

Key words: Pitaya, anthracnose, brown rot, calcium chloride, fruit quality.

INTRODUCTION

Calcium is a vital macronutrient in plant cycle including fruit development and securing of good fruit quality. Lack of Ca might cause an abnormal growth in fruit and its low mobility into fruit make Ca concentration in fruit de-creasing as the fruit grows (Saure, 2005). Fruits with low Ca are generally poor in its quality (Serrano et al., 2002) and become more sensitive to physiological disorders and disease infection (Fallahi et al., 1997; Biggs et al., 1997; Biggs, 1999; Chardonet et al., 1999; Elmer et al., 2007). Many studies showed a positive relationship between Ca and fruit shelf life and quality retention (Luna-Guzman and Barret, 2000; Alcaraz et al., 2003).

The ability to retain quality or at least to slowdown the quality degradation process probably become the ulti-mate goal for any professional that is involve in the postharvest operations and handling of fresh fruits. Quality of fruit may associate with many attributes but in the present study we are only concern on a few aspects of them, that is, disease infection and selected physico-chemical attributes in relations to pre-harvest fruit Ca treatment. Our earlier reports clearly indicated that, dragon fruit is highly susceptible to many diseases (Masyahit et al., 2009) and such diseases have caused significant losses to the growers. Besides diseases,

*Corresponding author. E-mail: yahya_awg@putra.upm.edu.my. Tel: 603-89466917. Fax: 603-89435973.

Abbreviations: SSC, Soluble solids contents; **TA,** titratable acidity.

degradation of quality is also link to many other quality parameters as seen in the reduction of tissue firmness and moisture content, changes in color and in some other nutritional values.

Fruit tissue softening is associated with many factors, one of them is calcium content of the fruits (Luna-Guzman and Barret, 2000; Aguayo et al., 2007). The role of Ca in developing the resistant of fruit tissue to softening is attributed to the stabilization of membrane systems and the formation of Ca-pectates, which increases rigidity of the middle lamella. Ca also makes the tissue become more resistant to cell wall degradation enzymes such as polygalacturonase (PG) and pectine-methylesterase (PME) (Siti Hajar et al., 2010; Manganaris et al., 2005). Positive relationships between fruit Ca and firm-ness retention were observed in the existence of many type of fruits (Saftner et al., 2003; Omaima and Karima, 2007).

Loss of calcium from cell wall and middle lamella of fruit occurs during the maturing process (Cutting et al., 1992) and causes fruit softening (Stow, 1993), while increasing fruit Ca content through Ca application which reduces Ca loss. Thus, it increases the fruit firmness (Gerasopoulos et al., 1996; Singh et al., 2007), possibly by several mechanisms including an increase in Ca-pectin bond in middle lamella (Grant et al., 1973), a reducing fruit respiration (Eaks, 1985) and maintenance of cell turgor potentials (Mignani et al., 1995).

Since the mobility of calcium in plants is low, Ca root uptake from soil-applied fertilizer is less effective in increasing Ca content in fruit. Direct application of liquid source of Ca on leaf and fruit may offer an alternative solution. In this study, we were aiming to increase Ca content in red flesh dragon fruit (*H. polyrhizus*) *via* pre-harvest fruit $CaCl_2$ spray and to examine its effects on postharvest disease occurrence and fruit quality parameters. As the absorption of Ca into fruit may interact with the uptake and translocation of other nutritional elements, thus affecting their balance which could have impacts on fruit quality, the fruit contents of N, P, K and Mg were also considered in the study.

MATERIALS AND METHODS

Pre-harvest calcium application

The study was conducted at a two-year old commercial dragon fruit farm at Pajam in Negeri Sembilan, Malaysia. Prior to pre-harvest Ca treatment, four well developed flowers on four different plants were tagged at one day after anthesis and their development were closely monitored. The pre-harvest fruit $CaCl_2$ treatment began at 7 DAA. The fruits were sprayed till drip (approximately 20 s) at 0900 to 1000 h with the respective treatment of five different concentrations of $CaCl_2$: 0-distilled water, 1, 2, 3 and 4 gl^{-1} Ca (4 fruits/plot). 5% (v/v) of a non-ionic wetting agent was added into the $CaCl_2$ solution to increase Ca retention on the fruit skin. During the course of experiment, the fruits were sprayed four times, at day 7, 14, 21 and 28 after anthesis. The fruits were wrapped in clear perforated plastic bags after every spray and re-opened again before each $CaCl_2$ application. The fruits were harvested at fully ripened stage (33 ± 2 DAA) and stored overnight at 13°C before being used for the study.

Disease occurrence

Sample fruits (4 fruits/plot x five treatments) were artificially wounded with a cork borer (0.5 cm diameter) and inoculated with 1 x 10^6 spores' ml^{-1} of *C. gloeosporioides* and *M. fructicola* (two fruits for each fungus/plot). The controlled fruits were 'inoculated' with distilled water and placed in moisturized plastic trays, covered with cling-films and incubated for three days at 22°C after which the disease incidence (% of fruits infected) and severity of the infections (size of lesions of the infected fruits) were evaluated.

Fruit N, P, K, Ca and Mg contents

The inoculated fruits were divided into flesh and peel portions, cut into small pieces and dried at 60°C in an air-circulating oven and finely ground once dried. 0.25 g of the fruit peel and flesh were digested in 5 ml H_2SO_4 on hot plate at 450°C in a fume chamber for 7 min. 10 ml H_2O_2 was added into the mixtures and the heating was continued for another 4 min. The solution mixtures were made-up to 100 ml with distilled water. N and P contents in the samples were determined using an auto-analyzer (LACHART Instruments, Model Quikchem IC + FIA 8000 Series, Milwaukee, USA) while K, Ca and Mg were measured using an atomic absorption spectrophotometer (Perkin Elmer, Model AAS 3110, Palo Alto, California, USA).

Fruit quality

To examine the impact of $CaCl_2$ treatment and disease infection on fruit quality, fruit firmness, soluble solids contents (SSC) and titratable acidity (TA) of the inoculated fruits were measured. The firmness of the whole fruit was determined using a texture analyzer (Instron Universal Testing machine, Model 5543, Instron Corporation, USA) by measuring the maximum penetration force required during peel tissue breakage, using a 5 mm diameter flat probe. The measurement of firmness was done at two locations for each fruit at 2.0 cm away from the point of fungus inoculation. SSC of the inoculated fruits was determined using a digital refractometer meter (Model N-α, Atago, Japan) by squeezing the fruit flesh onto the meter's prism. TA was measured using diluted fruit juice (1 juice to 4 distilled water) prepared using the same fruits as for the SSC measurement. 10 ml of the juice for each treatment (with three determinations of each) were titrated with 0.1N NaOH to pH 8.1. The TA was calculated and expressed as percentage of citric acid. The pH of the same fruit juice was also measured using a glass electrode pH meter.

The experiment was conducted in a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and comparison of means was performed using Tukey HSD with SPSS (Version 13).

RESULTS

Effects on disease occurrence

Regardless of $CaCl_2$ concentration in the applied solution, fruit sprays did not reduce the incidence percentage of disease infection. After three days of incubation period, all fruits were inoculated with 10^6 spores·ml^{-1} of both fungi exhibited 100% infections. Increasing $CaCl_2$ concentra-

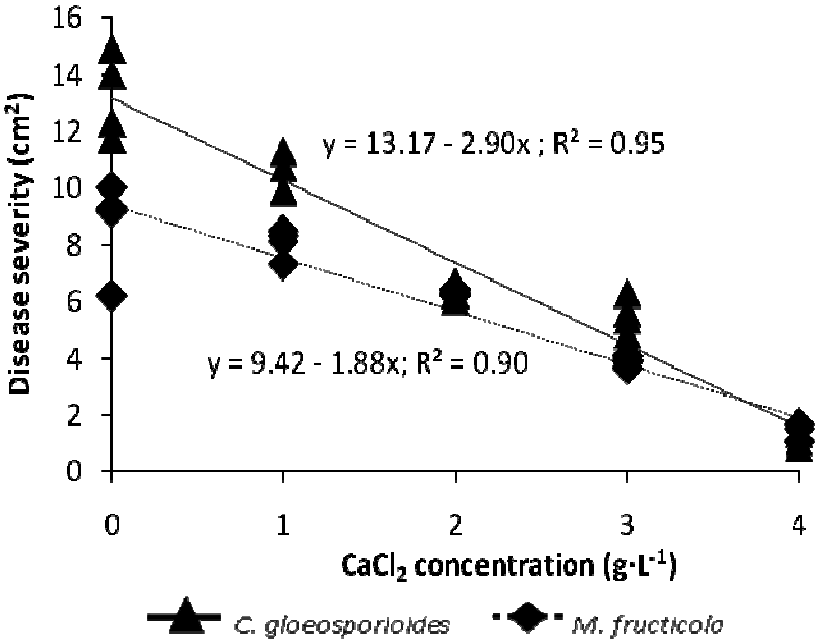

Figure 1. Relationship between $CaCl_2$ concentration and disease severity of pre-harvest $CaCl_2$ treated fruit at three days after inoculated with *C. gloeosporioides* and *M. fructicola* spores (10^6 spores per ml^{-1}).

tion in the spray solution linearly reduced the severity of disease symptom, as seen in the reduction of lesion size at high $CaCl_2$ concentration (Figure 1). The diameter of lesion was reduced from 8.3 to 2.0 cm^2 in fruits inoculated with *C. gloesporioides* as the $CaCl_2$ concentration increased from 0 to 4 gl^{-1}. The corresponding values for *M. fructicola* were 13.4 and 1.8 cm^2. Disease severity caused by *C. gloeosporioides* and *M. fructicola* was negatively correlated with Ca in peel ($r = 0.91$, $p \leq 0.01$) but it has no correlation with Ca concentration ($r = 0.23$) in flesh (Figure 2).

Effects on fruit mineral contents

Fruit Ca content increased markedly with the increasing concentration of $CaCl_2$ applied (Figure 3). However, the effects of treatment on Ca content in fruit tissues were more apparent in peel compared to the flesh. The peel of the fruits treated with Ca concentration at 4 gl^{-1} had the highest concentration of Ca (83.56 mg per 100 g^{-1}), followed by 3 gl^{-1} (66.79 mg per 100 g^{-1}), 2 gl^{-1} (28.62 mg·100 g^{-1}), 1 gl^{-1} (20.62 mg per 100 g^{-1}) and 0 gl^{-1} (22.77 mg per 100 g^{-1}). Although not significant, Ca content in the flesh was also enhanced at higher $CaCl_2$ concentration which is ranged from 6.68 to 10.53 mg per 100 g^{-1}. There was no significant correlation existence between Ca in fruit flesh and in fruit peel (Figure 4). Results in Table 1 show that, the contents of N, P, K and Mg in peel and flesh were not significantly affected with $CaCl_2$ concentration.

Effects on fruit quality

Fruit firmness was progressively increased at higher $CaCl_2$ regardless of fungal treatments (Figure 5). The firmness level of the non-treated fruit ranged from 22.12 to 24.81 N but the firmness of the fruits inoculated with *C. gloeosporioides* and *M. fructicola* ranged from 14.95 to 23.88 N and 14.95 to 23.81 N, respectively. The firmness of the non-inoculated fruit increased as the $CaCl_2$ concentration increased from 0 to 4 gl^{-1}, but the effects were not significantly differed among them. Overall, fruit firmness was positively correlated to Ca concentration in peel, regardless of inoculation types ($r = 0.93$). There was no correlation existed between firmness and Ca concentration in flesh (data not shown).

Results in Table 2 show that the pH of fruit was not significantly affected by $CaCl_2$ treatment. In contrast, pH of fruit inoculated with *C. gloeosporioides* and *M. fructicola* decreased significantly compared to the controlled fruit. There was no interaction effect between $CaCl_2$ concentration and inoculums type on pH. The pH of fruit was correlated positively with severity of infection ($r = 0.80$). The negative correlation was also observed between pH and Ca concentration in peel ($r = - 0.87$).

Soluble solids contents of fruit inoculated remains constant after experiencing $CaCl_2$ treatment and SSC was significantly lower ($p \leq 0.05$) in fruit inoculated with

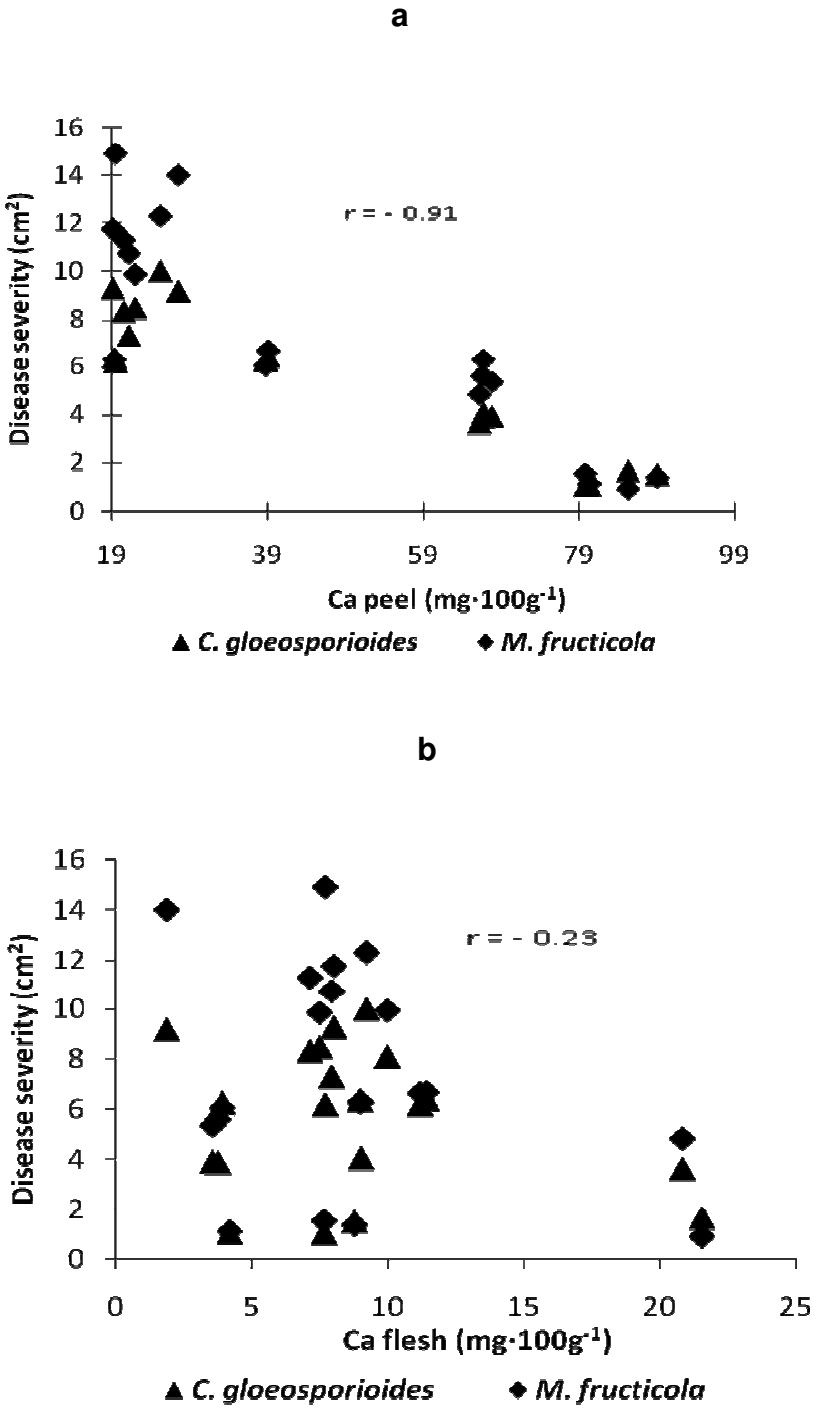

Figure 2. Correlation between disease severity and Ca concentration in peel (a) and disease severity and Ca concentration in flesh (b) of pre-harvest $CaCl_2$ treated fruit at three days after inoculated with *C. gloeosporioides* and *M. fructicola* spores (10^6 spores·ml^{-}).

either *C. gloeosporioides* or *M. fructicola* compared to the non-inoculated fruit (Table 2). Results of correlation analysis show that SSC was negatively correlated to severity of infection (r = - 0.84) and positively correlated with Ca concentration in peel (r = 0.88), TA of the inoculated fruit was not influenced by the treatment. In fruit inoculated with *C. gloeosporioides, M. fructicola* and TA increased significantly with $CaCl_2$ concentration

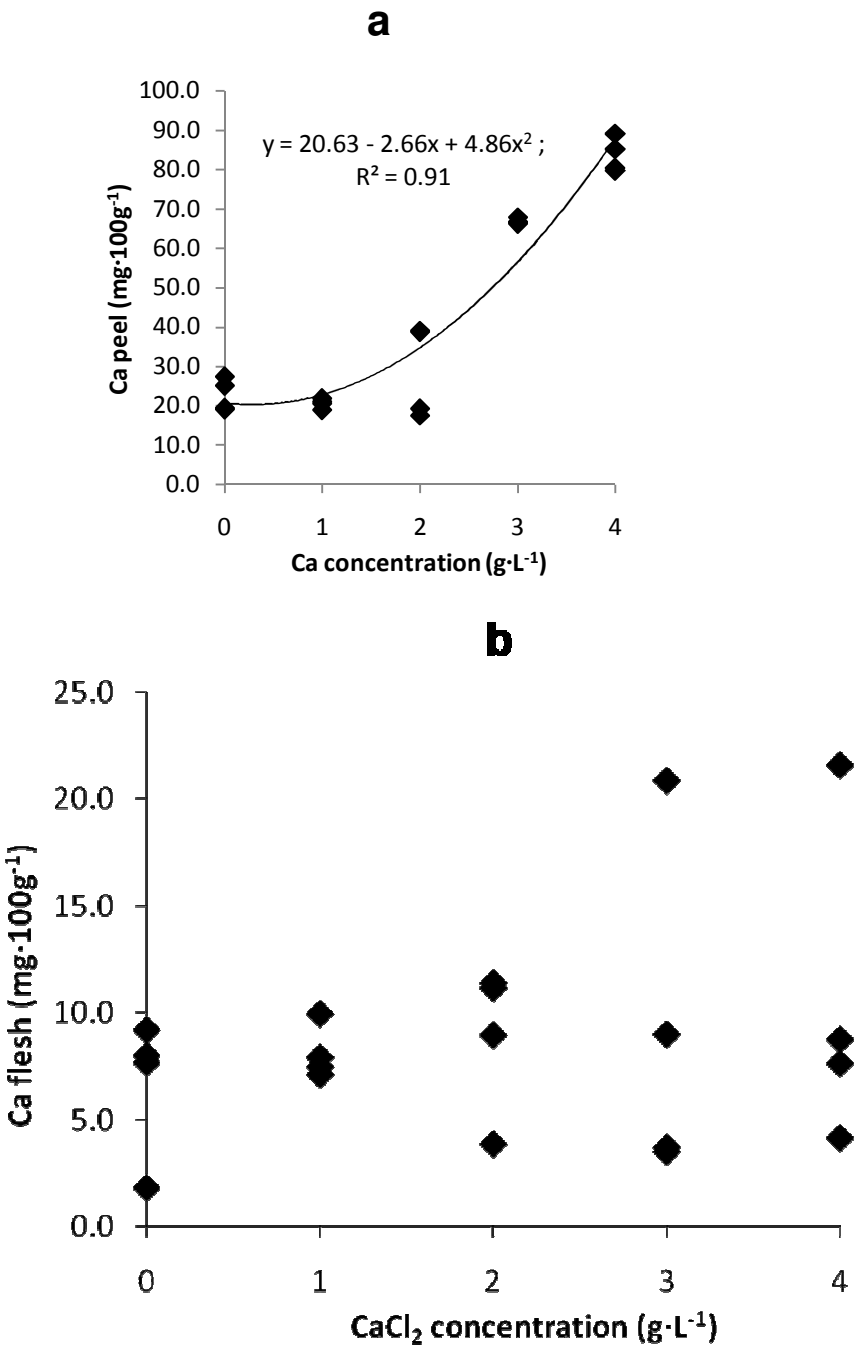

Figure 3. Relationship between $CaCl_2$ concentrations of the applied solution with Ca content of peel (a) and flesh (b) of dragon fruit.

compared to the non-inoculated fruits but TA was negatively correlated with severity of infection (r = - 0.76).

DISCUSSION

Regardless of its concentration in the applied solution, $CaCl_2$ fruit sprays did not reduce the incidence percentage of disease infection. The results recorded here were paralleled with studies involving other fruit species (For example, Kiwi - Gerasopoulos et al., 1996; Strawberry-Hernandez-munoz et al., 2006; Nectarine-Vasilakakis et al., 2006; Peach-Elmer et al., 2007). Increasing $CaCl_2$ concentration in the spray solution however has reduced the severity of symptoms, as seen in the reduction of lesion area. The roles of Ca in reducing disease severity

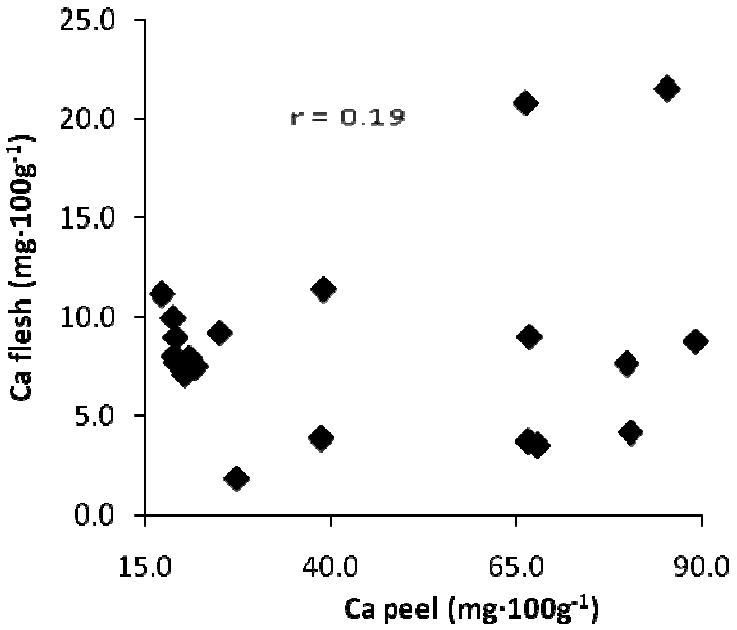

Figure 4. Correlation between Ca peel and Ca flesh of $CaCl_2$ treated fruit.

Table 1. Effects of pre-harvest $CaCl_2$ treatment on mineral concentrations in flesh and peel of *H. polyrhizus*

Part of fruit	$CaCl_2$ treatment (gl^{-1})	Nutrient concentration (mg $100g^{-1}$)			
		N	P	K	Mg
Peel	0 (control)	126.90	10.28	243.30	25.10
	1	100.80	10.81	251.17	25.36
	2	97.10	12.37	250.83	25.36
	3	95.50	10.76	249.97	25.09
	4	100.70	10.99	243.91	26.16
Flesh	0 (control)	208.90	19.53	148.68	20.19
	1	234.40	21.76	144.74	21.19
	2	238.40	21.49	147.47	20.68
	3	173.50	17.38	173.43	18.59
	4	208.90	18.80	157.33	20.57
Tukey $HSD_{0.05}$		55.40	4.50	25.30	1.20

HSD, Honestly significant difference.

can be discussed in two ways; by direct effect of Ca to fungal itself or by its indirect effect. The direct effect of Ca in the reduction of disease severity could begin at the spore germination stage. Droby et al. (1997) reported that, calcium reduced the germination of *Penicillium digitatum* spores on Ca treated grapefruit thus reducing the severity of infection. Maintenance of low basal concentrations of internal calcium is essential for normal cell functions of organisms and the inability of cells to regulate calcium may affect the organism's normal growth (Biggs, 1999). Increasing $CaCl_2$ concentration might have increased free Ca in fruit tissues thus elevates the free calcium in the cytosol of fungus and hence inhibit the growth of germ tube and mycelial growth of *C. gloeosporioides* (Biggs et al., 1997; Biggs, 1999) The same mechanism might apply in Ca treated fruit inoculated with *M. fructicola*. Ca also was reported to have the ability to inhibit the activity of pectolytic enzymes secreted by fungus (Droby et al., 1997), thus reduce disease severity in fruit treated with high Ca concentration.

Indirect effect of Ca in reducing disease severity could also relate to the role of Ca on fruit cell wall integrity. Calcium in the cell wall has a role in fruit texture (Quiles

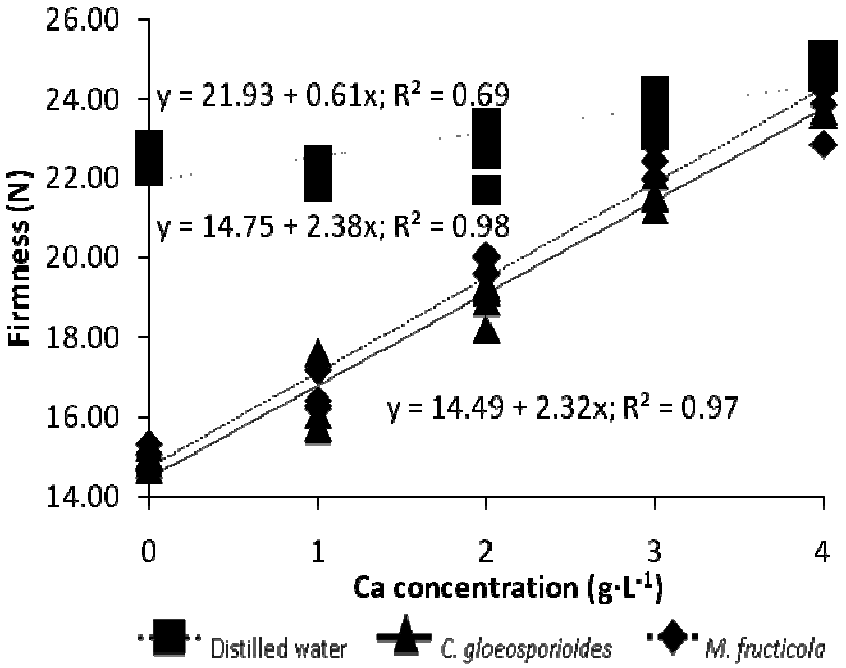

Figure 5. Relationship between $CaCl_2$ treatment and fruit firmness after three days of incubation.

Table 2. Effects of pre-harvest $CaCl_2$ treatment on fruit pH, soluble solid content and titratable acidity of red flesh dragon fruits infected by *C. gloeosporioides* and *M. fructicola* after three days of incubation.

Inoculum (10^6 spores. ml^{-1})	$CaCl_2$ treatment (gl^{-1})	Quality parameter		
		pH	SSC (%)	TA (%)
Sterilized water	0 (control)	5.43	9.26	0.10
	1	5.24	9.80	0.13
	2	5.28	10.12	0.12
	3	5.31	9.88	0.11
	4	5.31	9.77	0.14
C. gloeosporioides	0 (control)	5.76	6.16	0.05
	1	5.68	7.05	0.06
	2	5.60	8.21	0.07
	3	5.47	9.45	0.09
	4	5.36	9.97	0.12
M. fructicola	0 (control)	5.76	6.99	0.06
	1	5.68	7.21	0.06
	2	5.55	8.94	0.09
	3	5.51	9.64	0.09
	4	5.45	9.93	0.12
F-test				
Inoculum		**	**	**
Calcium concentration		ns	ns	ns
Interaction		ns	ns	ns
Tukey $HSD_{pooled\ (0.05)}$		0.26	0.98	0.04

SSC, Soluble solids contents; TA, titratable acidity; ns, * and ** indicate non-significant, significant at $P < 0.05$ and $P < 0.01$, respectively.

et al., 2007) but Ca content in the fruit could have reduced through the maturing process and resulting in the lack of Ca at the end of fruit maturity period. Deficiency of Ca would increase cell membrane permeability thus permits ions to escape and lead to the breakdown of intercellular compartmentalization, as well as the escapes of enzymes, such as polygalacturonase and pectin methylesterase which accelerates fruit ripening and softening processes (Deytieux-Belleau et al., 2008). This predisposes fruit to fungal attack. Reduction in fungal infection in Ca treated fruits as observed here could also be attributed to the increase of cell wall-bound Ca (Chardonnet et al., 1999) which stabilize cell wall structure and protect it from pectolytic enzymes produced by the fungi (Conway et al., 1994). The roles of Ca treatment in increasing cell wall integrity thus protect the wall from fungal pectolytic enzymes that have been proposed and discussed in many studies (Fallahi et al., 1997; Elmer et al., 2007; Singh et al., 2007). In another perspective, plants are known to produce phytoalexin and phenolics compound as a self-defense mechanism and Ca application increases the synthesis of these compounds thus inhibit the activity of fungal pectolytic enzymes (Kohle et al., 1985).

Increased Ca content in fruit and specifically in fruit peel following pre-harvest $CaCl_2$ application recorded here is in agreement with the results of studies reported by Dris (1998) for apples, Elmer et al. (2007) for peaches and Singh et al. (2007) for strawberry. As fruit is an organ with high metabolic rate and dependent on continuous supply of Ca, it is highly demanded during fruit development and rapid fruit growth causes a dilution of Ca in fruit tissues (Saure, 2005). Fruit Ca spray creates a concentration gradient of calcium between exogenous and endogenous portion of fruit, resulted in passive uptake of Ca into fruit (Alcaraz et al., 2003). In its early development, Ca is evenly distributed but when the fruit is becoming more mature, Ca will be transported to peel of fruit (Dris, 1998), making the concentration of Ca in peel higher than that in the flesh.

The concentration of N, P, K and Mg contents in fruit were not affected by the treatment. Such results would give a good indication on the positive effects of increasing Ca in the fruit tissue as this did not affect the balance of nutrient composition. This leads to higher ratios of Ca to other elements, thus elevating the possible benefit of the $CaCl_2$ treatment. Higher Ca:N ratio in fruit tissue for example could reduce fruit physiological disorders (Ferguson and Boyd, 2002).

Lack of interaction between the content Ca and other mineral nutrients in fruit could be attributed by differences in the regulation of uptake and absorption process for Ca when calcium is directly applied on fruits, whereby calcium enters the fruit tissue mainly via stomata, lenticels and fruit cleavage (Harker and Ferguson, 1988; Eichert and Burkhardt, 2001; Schlegel and Schonherr, 2002; Paul and Srivastava, 2006). In contrast to soil applied calcium, the element is absorbed and moved radially across the roots before being transported upward via the xylem. In the process, Ca^{2+} may have to compete with other ions such as K^+ and NH_4^+ at the site of absorption and with Mn^+ in upward translocation (Mengel et al., 2001). However, such competition does not occur when Ca is directly sprayed on fruit surfaces.

The infected fruits were observed to have high pH. Upon infection, the production and activity of cell wall degrading enzymes secreted by fungus could be influenced by host pH (Prusky et al., 2001). Fungus secretes NH_3 in order to activate the enzymes thus increase the pH of host tissue (Drori et al., 2003). Although not significant, $CaCl_2$ application seems to reduce to fruit pH, possibly related to the reduction of fungal activity in the treated fruits.

Generally, the fruit SSC and TA are not directly influenced by $CaCl_2$ application. TA naturally decreases during ripening (Park et al., 2006) but fungal infection accelerates the decrease. Working with strawberry, Wang and Galletta (2002) reported that anthracnose reduced the fruit SSC and TA, which may link to the utilization of the carbon (C) skeleton in sugars and organic acids by the fungus (Steinberg et al., 1999). For this reason, infected fruit would eventually have lower sugar and acid content, as recorded in this study.

Conclusion

$CaCl_2$ application as liquid spray at pre-harvest stage elevated Ca content in fruit. Beneficial effects of increasing calcium in fruit can be seen in the increase of fruit firmness although this did not contribute in enhancement of fruit quality-related parameters. Except for fruit Ca content, the composition of other mineral nutrients measured were not affected by the treatment, thus increasing the ratio of Ca to other elements. This may contribute directly to the reduction of anthracnose and brown rot severity observed in Ca-treated fruits.

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