

Control of Post-harvest Anthracnose Infection in Guava (*Psidium guajava*) Fruits with Phosphites, Calcium Chloride, Acetyl Salicylic Acid, Hot Water, and 1-MCP

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Abstract. The control of anthracnose (*Colletotrichum simmondsii*) during the post-harvest stage in guava fruits (*Psidium guajava* L.) was performed by the application of phosphites [phosphite-K (40% P₂O₅ and 30% K₂O) and phosphite-Ca (10.7% P₂O₅, 3.89% Ca, and 0.5% B)] including the Carbendazim as reference, calcium chloride (CaCl₂), acetyl salicylic acid (ASA), hot water (HW), and 1-methylcyclopropene (1-MCP). These treatments were applied individually or in combination each other with two or three compounds. The evaluated parameters were diameter of anthracnose lesion (DL), number of lesions (NL), and fruit quality (fresh weight loss, pH, total soluble solids, and titrable acidity). The fruits were disinfested, inoculated, and maintained in an incubator containing fluorescent lights at 75 μmol·m⁻²·s⁻¹ (25°C, 12h photoperiod) for 5 days and were then analyzed. The results showed that the DL and the NL were reduced following treatments, and that the HW (47°C for 20 min) was the strongest and the 1-MCP treatment was the least effective. The physico-chemical characteristics of fruits were affected by some treatments without compromising fruit quality. The combination of treatments was also able to alleviate the anthracnose effect on fruits compared to individual treatments and the control without affect the fruit quality. The combinations which included the HW treatment showed the best performance to control this disease, particularly when combined with the 1-MCP and phosphite.

Additional key words: *Colletotrichum simmondsii*, fruit lesion, fruit quality, titrable acidity

Introduction

There are several bacterial, viral, and fungal diseases associated with guava (*Psidium guajava*) cultivation, whose unicellular or multicellular pathogens degrade complex substances to obtain simple food substrates (Nascimento, 2011). Although fungal pathogens represent the minority in terms of species, they have the most significant effect on the economy of fruit growers and the anthracnose (*Colletotrichum* spp.) is a representative disease in guava at postharvest stage. The primary method of anthracnose control is the eradication of contaminated fruits, and soaking them in

fungicides and compounds that delay ethylene synthesis following harvesting (Agrolink, 2010). These treatments include hot water (HW), K and Ca phosphite, CaCl₂, acetyl salicylic acid (ASA) and 1-methylcyclopropene (1-MCP).

During the 1970s, phosphites were used as systemic fungicides to control oomycete fungi (Bonetti and Ozawa, 1999). This compound also functions as a plant mineral nutrient, by having the merit of fast phosphorus absorption by plants compared with phosphates (Moreira and May-de Mio, 2009) and are easily assimilated by leaves and root cells, having high mobility in plants. In addition to the low cost, they can prolong the post-harvest storage period, with low toxicity,

and can synergistically act as a fertilizer and fungicide (Brackmann et al., 2008).

The CaCl_2 has been demonstrated to be an efficient compound to preserve and maintain the firmness agent in fruit and vegetable due to its capacity to increase fruit firmness and reduce decay in apples after harvesting (Chardonnet et al., 2003). This chemical compound can reduce pathogen spore germination, sporulation, and growth. Physiologically the calcium forms complexes with the cell wall of plants including the middle lamella polygalacturonic acid residues, thus improving structural integrity. The higher calcium concentration in fruits delays ripening and senescence through reduced respiration, ethylene evolution, and loss of fresh mass, thereby prolonging postharvest storage period (Silva et al., 2012).

The ASA is a phenolic compound involved in the germination, synthesis and absorption of ions, and ethylene action in plants (Yin et al., 2013). It is in the ethylene synthesis by inhibiting the 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity, which is responsible for the conversion of ACC to ethylene. The mechanism of ASA action includes binding and inhibiting catalase (Horotan and Oancea, 2014). The inhibition of catalase increases the concentration of hydrogen peroxide (H_2O_2) or reactive oxygen species that are produced from this reaction during respiration, photosynthesis, or during the hypersensitive response to pathogens.

The HW has often been used to control pests and diseases in fruits, in particular to eliminate the eggs and larvae of fruit flies and control pathogens in fruits for exportation (Armstrong and Follett, 2007). The advantage of this treatment is that it does not leave waste or chemical residues, and the pathogens can be eliminated without damaging the host plant. The HW can reduce the viability of pathogen propagules and physiological disorders during storage, and maintain fruit quality, which improves the resistance to disease (Sponholz et al., 2004).

However, in addition to damaging cell plasma membranes and altering fruit flavor, this treatment may also affect the ripening, ethylene production, respiration, firmness, and color of fruit (Nolasco et al., 2008). In the HW treatment, the temperature and timing of exposure depend on the type, maturity, and size of fruit, and also on the environmental conditions during the growing season (Soares Pessoa et al., 2007).

The efficiency of 1-MCP as an antagonist of ethylene function in many plant products has been well demonstrated. This molecule competes with ethylene at its receptor site, preventing ethylene from binding and activating its signaling pathways in plant cells (Villarreal et al., 2010). Due to its efficiency and irreversible binding to the ethylene receptor site, this compound can protect fruit from endogenous and exogenous ethylene. However, the normal ripening process in fruit is resumed due to the formation of new receptor sites (Jacomino et al., 2003). Several compounds, for example, 2,

5-norbornadiene and diazocyclopentadiene, are capable of blocking the binding of ethylene to its receptor on the cell surface, thereby inhibiting the effects of ethylene. Therefore, the hypothesis that 1-MCP can indirectly control anthracnose in guava fruits, through a delay in ripening should be evaluated.

The objective of this study was to evaluate the effect of several treatments-phosphites, CaCl_2 , ASA, HW, and 1-MCP-individually and in various combinations, on the control of anthracnose in guava, and the physico-chemical characteristics of fruit at the post-harvest stage.

Materials and Methods

Inoculum Preparation and Inoculation

The inoculum was isolated from injuries in guava fruits affected by anthracnose, whose structures of were characterized by a orange mycelium containing conidia in abundance. The fungus was identified by molecular analysis as *Colletotrichum simmondsii*, here represented by the code UNBBCGU01, according to the Genbank (Accession number - JQ247595.1; Similarity 99%). The mycelia were placed on PDA plates, which were maintained in an incubator containing fluorescent lights at $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. These plates were exposed under 12 h photoperiod at 25°C . The cultured fungi were inoculated in guava fruits cv. 'Pedro Sato', obtained from CEASA-DF, Brazil. The fruits, at maturity stage between 1 and 2, between dark and light green (Ferraz, 2010), were sterilized by immersing in 10% alcohol for 1 min, followed by 1% sodium hypochlorite for 1 min, and then washed in sterilized water for 1 min.

Sterilized toothpicks were inserted in PDA containing the mycelia and were then inserted lightly into fruit epidermis and stored in an incubator. After 24 h, the toothpicks were removed and the treatments were applied. The fruits remained in the incubator for a period of 7 to 10 days until measurement of diameter of anthracnose lesion (DL) and physico-chemical characteristics. Another assay was conducted with non-inoculated fruits following the same procedures, where the disease severity was evaluated by the number of lesions (NL).

Application of Phosphite, CaCl_2 , and ASA

Five fruits inoculated and non-inoculated were immersed in a phosphite and carbendazim solution for 20 min as previously described (Blum et al., 2004), with a few modifications. The phosphites used were phosphite-K (40% P_2O_5 and 30% K_2O) and phosphite-Ca (10.7% P_2O_5 , 3.89% Ca, and 0.5% B). The concentration of phosphite-K applied was 0.5, 1.0, or $1.5 \text{ mL}\cdot\text{L}^{-1}$, phosphite-Ca 0.75, 1.5, or $3.0 \text{ mL}\cdot\text{L}^{-1}$, and carbendazim $150 \text{ mg}\cdot\text{L}^{-1}$.

The CaCl_2 was applied at doses of 0, 1.0, 1.5, 2.0, and 2.5% and ASA was used at concentrations of 0, 0.05, 0.1,

0.2, 0.3, 0.4, and 0.5%. Prior to application, ASA was dissolved in 100 mL ethanol and was then diluted in water to achieve the required concentration. The control treatments followed the same procedures with the same amount of water applied only. These substances were dissolved in a 6 L plastic box and the fruits were then immersed in the solution at room temperature for 20 min. After treatments were applied, the fruits were dried and stored in an incubator in similar light conditions of the PDA plates at 25°C for 5 days, after which analyses were performed.

Application of Hydrothermal Treatment

In this experiment the digital thermostatic water baths (Adamo, mod. 50/9, Adamo Co., Piracicaba, SP, Brazil) consisting of a steel enclosure with a tank capacity of 9 L and a drip tray cover made of the same material were used. The hydrothermal experiment consisted of two assays. In the first one, fruits were immersed in water set at a fixed temperature (47°C) for 0, 5, 10, 20, and 30 min, and in the second assay the immersion in water bath was fixed to 20 min and the temperatures changed (43, 45, 47, 49, 51, and 53°C). The immersion for 20 min in distilled water was used as control treatment for both experiments.

Application of 1-MCP

The 1-MCP solution was prepared in glass jars using the commercial product Ethylbloc® (Dow AgroSciences/Agrofresh, Inc., Springhouse, PA, USA) containing 0.14% of the active ingredient at room temperature. The product was dissolved in hot water at 50°C and the fruits were placed in airtight containers at different doses of the 1-MCP gas (0, 4, 10, and 16 mg·L⁻¹) for 12 h at 25°C. The control treatment was performed in the same container but without MCP treatment.

Application of Combined Treatments

In this experiment, the phosphite-K (1.0 mL·L⁻¹ for 20 min), hydrothermal (47°C for 20 min), CaCl₂ (2.0% for 20 min), ASA (0.3% for 20 min), and 1-MCP (10 mg·L⁻¹ for 12 h) treatments were applied, taking into consideration other results (Ferraz, 2010; Soares et al., 2008) and the intermediate between the minimum and the maximum level for each treatment.

These treatments were combined in two and three, where this last one obeyed the following choice: 1, HW-MCP-phosphite; 2, HW-MCP-CaCl₂; 3, HW-MCP-ASA; 4, MCP-phosphite-CaCl₂; 5, MCP-CaCl₂-ASA; and 6, phosphite-CaCl₂-ASA. In all of these combinations, the fruits were submitted to the subsequent treatment immediately, without any drying or rinsing process. Also, the individual ones and a control (no treatment) were prepared for comparison.

Physico-Chemical Analysis of Fruits

These analysis including fresh weight loss (FWL, %), firmness, pH, total soluble solids (TSS, °Brix), and titrable acidity (TA) was conducted after the evaluation of DL and NL, when the fruits were in a stage of maturity at around 4 and 5, i.e. between light green and yellow (Ferraz, 2010). Fresh weight loss (% FWL) was measured by weight lost by fruits between the inoculation and the end of the experiment was monitored using a precision semi-analytical balance (Marte, AS2000C, Marte Balanças e Aparelhos de Precisão Co., - Marte Balances and Precision Equipments Co., São Paulo, SP, Brazil.). The FWL was calculated by this formula % FWL = [(initial weight - final weight)/initial weight] × 100. Firmness was determined using a hand penetrometer (Fruit Pressure Tester, FT 327, 8 mm tip). The firmness was calculated by this formula: $P = F/A$. Where P = firmness (kg·cm⁻²), F = penetration force (kg), and A = area of tip (mm²). Fruit pH was analyzed with 10 g of mashed pulp per 50 mL water using a digital pH meter (PH TEK, PHS-3B, Nanjing T-Bota Sciotech Instruments & Equipment Co., Ltd Beijing, China). The TSS (°Brix) was determined using a portable refractometer (Hand-Held refractometer, Master-PM, Atago Co., Tokyo, Japan), by placing a small amount of fresh pulp on the prism, which then marks the level of light refraction on a metric ruler that represents the TSS. This parameter was adjusted to °Brix by a correction table (IAL, 2008). The determination of the TA was conducted by taking 10 mL of the pulp samples, to which was added two drops of phenolphthalein (1%), and which was then titrated with 0.1 N NaOH (standard solution). The estimation of TA was done with the following formula: % citric acid = $[Vg \times \text{normality of NaOH (0.1N)} \times 0.0064/10] \times 100$, where: Vg = volume of spent NaOH (mL), 0.0064 is a constant for the presence of anhydrous citric acid in the spent 1 mL solution of 0.1N NaOH, 10 = sample fresh weight (g).

The experimental set-up was a completely randomized design with five replicates, i.e. five fruits, per treatment where each compound concentration was totally applied in these fruits with five lesions each one. The statistical analysis were proceeded by subject the data to analysis of variance and comparison of means in each treatment using the Scott-Knott test with statistical software (Assistat Vers. 7.7 beta, UFCG, Brazil).

Results and Discussion

The results revealed that higher concentrations of phosphite can suppress fungal proliferation, as observed by the reduction in the DL and NL as the phosphite concentration increased. However, the significant difference was only detected between the control and other treatments for the DL. The phosphite-Ca

Table 1. Effects of Ca-phosphite (Ca-Ph), K-phosphite (K-Ph), and carbendazim (Car) application in fruits inoculated with *Colletotrichum simmondsii* (UNBBCGU01) and in non-inoculated ones on anthracnose severity and fruit quality.

Treatment (mL·L ⁻¹)	FWL (%) ^z	DL (mm) or NL ^y	Firmness (kg·cm ⁻²)	pH	TSS (°Brix) ^x	TA (%) ^w
Inoculation						
0 (control)	34.5 b ^u	18.4 a	0.9 a	3.6 b	7.9 b	0.9 c
Ca-Ph 0.75	41.8 a	6.9 b	0.8 a	4.4 a	8.2 a	1.2 b
Ca-Ph 1.5	40.6 a	8.1 b	1.0 a	4.0 a	9.1 a	0.9 c
Ca-Ph 3.0	35.1 b	4.8 c	0.8 a	4.2 a	8.1 a	1.3 a
Car 150	34.1 b	8.1 b	0.9 a	4.4 a	7.8 a	1.0 c
K-Ph 0.5	29.6 c	6.2 b	0.7 a	4.4 a	7.9 a	1.6 a
K-Ph 1.0	33.4 b	7.5 b	0.9 a	4.0 a	6.5 c	1.2 b
K-Ph 1.5	41.4 a	6.8 b	0.8 a	4.3 a	8.2 a	1.2 b
Non-inoculation						
0 (control)	5.5 e	59.5 b	1.1 a	4.0 a	10.8 a	1.2 b
Ca-Ph 1.5	13.9 a	18.8 e	1.1 a	3.9 a	10.8 a	1.1 b
Ca-Ph 3.0	11.2 b	13.8 e	1.0 a	3.9 a	11.3 a	1.1 b
Car 150	13.3 a	25.0 d	1.1 a	4.0 a	10.9 a	1.2 b
K-Ph 0.5	9.4 c	16.0 e	1.2 a	4.1 a	9.4 a	1.2 b
K-Ph 1.0	6.3 d	44.0 c	0.9 a	4.0 a	11.7 a	1.5 a
K-Ph 1.5	9.2 c	10.8 f	1.1 a	4.0 a	9.8 a	1.3 b

^zPercentage of fresh weight loss.^yDiameter of anthracnose lesion (DL) was measured for inoculated fruits and number of lesions (NL) for non-inoculated ones.^xTotal soluble solids.^wTitrate acidity.^uMeans followed by the same letters in columns are not significantly different according to Scott-Knott test ($p \leq 0.05$).

treatment led to a significant increasing in FWL without altering the fruit firmness, whereas the results obtained with the phosphite-K treatment showed that only the 0.5 (mL·L⁻¹) concentration decreased the FWL (Table 1). For the same parameter similar trends were observed in non-inoculated fruits with larger difference between the treatments results. The pH, TA, and TSS had a small increasing by the treatments in inoculated fruits (Table 1). The dose of 1.5% CaCl₂ showed the best control of anthracnose as demonstrated by DL and NL, with no significant change in higher treatments (Table 2). Concentrations of 0.5 and 2.5% showed the highest FWL with little effect on firmness. The pH was not altered by these treatments, but the TSS and TA had the higher values at 0.5% and control, respectively (Table 2). The data analysis of ASA treatments showed a reduction of DL by all of treatments, where the 0.4 and 0.5% reached the lowest value. The firmness was unaltered, until 0.2% ASA, which revealed the highest level of firmness, similarly the pH was not affected by the treatments, TSS and TA reached the highest value at 0.4% in inoculated fruits. In non-inoculated fruits treated with 0.1% ASA or higher, the FWL and NL decreased, but the firmness increased after 0.2% ASA. For the pH, TSS, and TA similar results of the inoculated fruits occurred, i.e. non alteration of the pH and higher values of TSS and TA at 0.4% (Table 3).

Other publications (Ferraz, 2010), corroborating with our data, showed some consistency in the results and that both treatments were effective in delaying fungal mycelial growth. The fruits responded as expected to the treatments with CaCl₂, carbendazim, potassium phosphite, and calcium phosphite. In another study (Dutra, 2008), all phosphite treatments reduced disease development, suggesting that phosphites had anti-fungal properties. Favorable results were obtained with dragon fruits (*Hylocereus polyrhizus*) (Awang et al., 2013) and 'Pedro Sato' guava (Linhares et al., 2007) following treatment with CaCl₂, with a reported reduction in FWL, an increase of TSS, and maintenance of TA by application of this compound. However, it should be emphasized that application of 2.0% CaCl₂ promoted calcium incorporation into tissues, and consequently extended the postharvest storage period. However, different results were obtained in grapes treated with this compound, where the chemical characteristics were improved in those treated with 2.0% CaCl₂ (Carvalho et al., 2008). Pomegranates treated with ASA after the harvesting and stored under chilling temperature of 2°C following by 4 days at 20°C could had the exhibit chilling injury symptoms alleviated during storage. In these fruits, the ASA treatments were able to maintain the high contents of sugars, organic acids, total phenolic content, and anthocyanins. This indicated that ASA treatment could potentially increase the antioxidant

Table 2. Effects of calcium chloride (CaCl₂) application in fruits inoculated with *Colletotrichum simmondsii* (UNBBCGU01) and in non-inoculated ones on anthracnose severity and fruit quality.

CaCl ₂ (%)	FWL (%) ^z	DL (mm) or NL ^y	Firmness (kg·cm ⁻²)	pH	TSS (°Brix) ^x	TA (%) ^w
Inoculation						
0 (control)	8.8 b ^v	19.5 a	0.6 b	4.1 a	9.4 a	1.3 b
0.5	10.6 a	16.4 b	0.8 b	4.0 a	9.9 a	1.7 a
1.0	6.2 c	13.4 c	1.1 a	4.0 a	10.2 a	1.3 b
1.5	8.3 b	5.9 d	1.0 a	4.1 a	9.8 a	1.3 b
2.0	8.5 b	6.3 d	1.0 a	4.1 a	9.3 a	1.1 c
2.5	9.5 a	4.1 d	0.6 b	4.1 a	9.1 a	3.1 b
Non-inoculation						
0	16.5 c	47.0 a	1.4 a	4.2 a	6.8 c	1.4 a
0.5	15.4 c	34.4 b	1.8 a	4.0 a	9.2 a	0.7 b
1.0	18.0 b	26.5 c	1.2 a	4.3 a	8.2 b	0.7 b
1.5	23.9 a	15.7 d	1.5 a	4.0 a	7.7 b	0.8 b
2.0	20.2 a	15.9 d	1.4 a	3.9 a	7.9 b	1.1 a
2.5	18.9 b	12.7 d	1.6 a	3.9 a	6.5 c	1.1 a

^zPercentage of fresh weight loss.

^yDiameter of anthracnose lesion (DL) was measured for inoculated fruits and number of lesions (NL) for non-inoculated ones.

^xTotal soluble solids.

^wTitrate acidity.

^vMeans followed by the same letters in columns are not significantly different according to Scott-Knott test ($p \leq 0.05$).

Table 3. Effect of acetyl salicylic acid (ASA) application in fruits inoculated with *Colletotrichum simmondsii* (UNBBCGU01) and in non-inoculated ones on anthracnose severity and fruit quality.

ASA (%)	FWL (%) ^z	DL (mm) or NL ^y	Firmness (kg·cm ⁻²)	pH	TSS (°Brix) ^x	TA (%) ^w
Inoculation						
0 (control)	15.7 b ^v	15.2 a	1.8 c	4.6 a	8.5 b	0.8 c
0.05	19.2 a	10.0 c	2.3 c	4.3 a	8.7 b	0.9 b
0.1	13.6 b	11.8 c	2.7 c	4.3 a	9.2 b	0.9 b
0.2	18.9 a	13.5 b	2.3 c	4.4 a	9.7 a	0.8 c
0.3	14.5 b	7.3 d	3.2 b	4.2 a	8.2 b	1.1 b
0.4	19.1 a	5.0 e	3.8 a	4.4 a	10.5 a	1.6 a
0.5	15.3 b	4.8 e	4.5 a	4.2 a	8.7 b	1.3 a
Non- inoculation						
0	10.3 a	47.0 a	0.9 c	4.2 a	8.9 b	1.7 a
0.05	9.3 b	42.5 a	0.7 c	4.4 a	7.9 b	1.5 a
0.1	7.7 c	17.0 c	1.1 b	4.1 a	8.4 b	1.7 a
0.2	9.0 b	20.0 c	1.2 a	4.0 a	9.4 b	1.6 a
0.3	9.6 b	29.5 b	1.4 a	4.1 a	10.4 a	1.3 a
0.4	9.1 b	22.0 c	1.3 a	4.0 a	10.6 a	1.5 a
0.5	9.3 b	21.0 c	1.4 a	4.2 a	9.7 a	1.4 a

^zPercentage of fresh weight loss.

^yDiameter of anthracnose lesion (DL) was measured for inoculated fruits and number of lesions (NL) for non-inoculated ones.

^xTotal soluble solids.

^wTitrate acidity.

^vMeans followed by the same letters in columns are not significantly different according to Scott-Knott test ($p \leq 0.05$).

Table 4. Effects of immersion time in hot water at 47°C in fruits inoculated with *Colletotrichum simmondsii* (UNBBCGU01) and in non-inoculated ones on anthracnose severity and fruit quality.

Immersion time in hot water (min)	FWL (%) ^z	DL (mm) or NL ^y	Firmness (kg·cm ⁻²)	pH	TSS (°Brix) ^x	TA (%) ^w
Inoculation						
0	45.3 a ^v	16.0 a	1.0 a	4.1 a	9.8 a	1.1 b
5	38.5 c	6.2 b	0.93 a	3.9 a	8.7 b	1.4 a
10	41.7 b	6.8 b	0.77 b	4.3 a	9.2 a	1.2 a
20	40.8 b	4.9 c	0.83 b	4.0 a	9.1 a	1.0 b
30	40.9 b	6.8 b	0.66 c	3.8 a	10.1 a	1.0 b
Non-inoculation						
0	9.9 a	32.2 a	1.0 a	4.0 a	10.6 a	0.9 c
5	9.8 a	22.0 b	0.8 b	4.2 a	9.9 a	1.2 b
10	6.9 b	9.2 d	0.9 a	4.1 a	9.4 a	1.2 b
20	6.8 b	13.2 c	1.1 a	4.1 a	9.9 a	1.5 a
30	7.1 b	13.2 c	0.9 a	4.0 a	11.6 a	1.3 a

^zPercentage of fresh weight loss.^yDiameter of anthracnose lesion (DL) was measured for inoculated fruits only. Number of lesions (NL) for non-inoculated ones only.^xTotal soluble solids.^wTitrate acidity.^vMeans followed by the same letters in columns are not significantly different according to Scott-Knott test ($p \leq 0.05$).

capacity of fruits during the post-harvest stage (Sayyari et al., 2011). Treatment of strawberry fruit (*Fragaria ananassa* Duch Camarosa) with ASA delayed ripening, leading to higher TA, less weight loss, and a higher vitamin C content, which affected the quality of strawberry fruit and increases their storekeeping (Lolaei et al., 2012).

All fruits experienced reduction of FWL by increasing the exposure time in HW (Table 4) and the temperature (Table 5). There was no variation in the pH of inoculated fruits compared to the control. At 47°C, the effects of hot water on DL were evident from 5 min compared to the control, however exposing at longer later (10, 20, and 30 min) did not significantly augmented. In inoculated fruits the immersion in 47°C for 30 min lead the lowest firmness, whereas the other treatments showed no change compared with the control. The pH was not changed by the treatments. Analyzing the TA the values at 5 min showed some difference to the control, i.e., the fruit did not ripen as much as in the other treatments and the control (Table 4). At 5 min, there was some variation in TSS when compared with other treatments (Table 4).

In non-inoculated fruits, the NL suffered varied with the duration of treatment, with the strongest suppression occurring at 10 min. The firmness was not affected, and all of the treatments resulted in a higher TA compared with the control. The fruits immersed for 20 min showed the best value of TA. Furthermore there was no change in TSS in the control fruits at 5, 10, and 20 min, but at 30 min the best TSS was

observed. This was an advantage because it meant that the treatments did not induce ripening above control levels (Table 4).

With temperature variation the DL of fruits with inoculation reduced starting from 43°C from the control; especially at 47°C which showed a smaller DL compared to the other treatments and the control (Table 5). The firmness was affected by the treatments, particularly in those fruits immersed in water at 51°C, which were firmer than those immersed at lower temperatures. The TSS reached was the highest value at 45°C, and the TA at 49°C (Table 5). In fruits without inoculation, the NL reduced after 43°C. Furthermore, there was a significant difference in FWL after 45°C. No variation was detected in the pH, and TA. In TSS and firmness fruits treated at 51°C had the highest values (Table 5).

During heat treatment, the physiological processes in fruits were inhibited or inactivated. The delay of ripening, senescence, and the degradation of antifungal compounds might occur during the application of HW; moreover, it could control the activity of pectinmethylesterase and polygalacturonase (Chávez-Sánchez et al., 2013). Treatment of this type associated with *Rhodotorula glutinis* was shown to reduce blue mold (*Penicillium expansum*) in pears, where fruits treated for 15 min for 46°C showed a lower incidence of this disease (Zhang et al., 2008). Hydrothermal treatments can delay ripening in mango (Yimyong et al., 2011) and reduce the incidence of fruit fly in litchie and longan (Armstrong and Follett, 2007). The HW treatments have been applied in

Table 5. Effects of hot water temperature variation in fruits inoculated with *Colletotrichum simmondsii* (UNBBCGU01) and in non-inoculated ones on anthracnose severity and fruit quality.

Hot water temperature (°C)	FWL (%) ^z	DL (mm) or NL ^y	Firmness (kg·cm ⁻²)	pH	TSS (°Brix) ^x	TA (%) ^w
Inoculation						
Control	21.9 b ^v	13.2 a	2.5 c	3.8 a	9.3 a	0.8 b
43	22.5 b	8.8 b	2.0 a	3.7 a	8.9 b	1.3 a
45	23.5 b	7.5 c	3.0 b	3.6 a	9.1 a	0.9 b
47	22.6 b	6.5 c	2.3 c	3.7 a	9.8 a	1.7 a
49	18.0 c	8.3 b	2.9 b	3.6 a	11.1 a	1.9 a
51	25.4 a	8.8 b	4.4 c	3.8 a	9.1 b	1.4 a
Non-inoculation						
Control	12.2 d	33.5 a	1.4 b	3.7 a	10.6 a	1.5 a
43	11.6 d	26.0 b	1.6 c	3.8 a	8.1 b	1.1 a
45	7.7 e	10.0 d	2.7 c	3.8 a	9.1 b	1.1 a
47	6.8 e	11.2 d	2.8 c	3.7 a	9.4 b	1.7 a
49	7.4 e	13.0 d	2.3 a	3.8 a	8.9 b	1.2 a
51	6.8 e	16.7 c	2.0 b	3.7 a	11.1 a	1.5 a

^zPercentage of fresh weight loss.

^yDiameter of anthracnose lesion (DL) was measured for inoculated fruits only. N number of lesions (NL) for non-inoculated ones only.

^xTotal soluble solids.

^wTitrate acidity.

^vMeans followed by the same letters in columns are not significantly different according to Scott-Knott test ($p \leq 0.05$).

Table 6. Effects of 1-MCP application in fruits inoculated with *Colletotrichum simmondsii* (UNBBCGU01) and in non-inoculated ones on anthracnose severity and fruit quality.

MCP (mg·L ⁻¹)	FWL (%) ^z	DL (mm) or NL ^y	Firmness (kg·cm ⁻²)	pH	TSS (°Brix) ^x	TA (%) ^w
Inoculation						
0 (control)	33.8 a ^v	6.6 a	2.2 b	4.3 a	4.6 b	1.6 a
4	29.6 a	4.7 b	5.2 a	4.1 a	5.9 a	1.7 a
10	19.5 c	5.5 a	5.3 a	4.2 a	4.9 a	1.5 a
16	26.6 b	3.9 b	3.4 b	4.1 a	5.1 a	1.3 a
Non-inoculation						
0	12.8 a	24.5 a	2.3 c	3.6 a	8.9 a	1.0 a
4	9.2 b	25.0 a	4.3 a	3.6 a	9.6 a	2.4 b
10	9.1 b	21.5 a	3.2 b	3.6 a	9.9 a	2.8 b
16	12.8 a	10.0 b	3.6 b	3.5 a	10.1 a	1.7 a

^zPercentage of fresh weight loss.

^yDiameter of anthracnose lesion (DL) was measured for inoculated fruits only. Number of lesions (NL) for non-inoculated ones only.

^xTotal soluble solids.

^wTitrate acidity.

^vMeans followed by the same letters in columns are not significantly different according to Scott-Knott test ($p \leq 0.05$).

tangerines to extend their storage time in quarantines (Carvalho et al., 2008). The reduction in fruit damage caused by *Botrytis cinerea* after 30 days of storage at 1°C was successful after grapes were immersed in 30% ethanol with hot water at 40°C and 50°C (Karabulut et al., 2004). Moreover this HW at 54°C for 3 min could decrease the fruit rot caused by *Chalara paradoxa* in pineapples. After that these fruits were stored for 21 days at 10°C and kept at room temperature for

48 h. This treatment had no effect on the physico-chemical characteristics of fruits (Wilson Wijeratnam et al., 2005).

All concentrations decreased DL compared with the control in inoculated fruits, with the 4 and 16 mg·L⁻¹ treatment being the most effective. The highest firmness was reached following treatment with 4 mg·L⁻¹ 1-MCP. In the second experiment (Table 6), using non-inoculated fruits, the 1-MCP treatment at 16 mg·L⁻¹ was the most effective in reducing the NL. All

Table 7. Effect of individual treatments and the combination of hot water at 47°C for 20 min (HW 47, 20), 1-MCP (10 mg·L⁻¹), K-phosphite at 1.0 mL·L⁻¹ (K-Ph 1.0), calcium chloride (CaCl₂) at 2.0 mL·L⁻¹, and acetyl salicylic acid (ASA) at 0.3 mL·L⁻¹ on anthracnose severity and fruit quality in fruits inoculated with *Colletotrichum simmondsii* (UNBBG01).

Treatment	FWL (%) ^v	DL (mm) ^v	Firmness (kg·cm ⁻²)	pH	TSS (°Brix) ^x	TA (%) ^w
Control	45.2 a ^v	20.1 a	1.0 c	4.2 a	5.9 b	1.1 b
K-Ph 1.0	34.8 b	10.6 c	1.6 c	3.7 b	8.4 b	1.3 b
CaCl ₂ 2.0	35.7 b	11.2 c	1.3 c	3.9 a	7.4 b	1.2 b
ASA 0.3	30.9 c	9.8 b	1.5 c	3.5 b	7.9 b	2.0 a
HW 47-20	38.4 b	5.2 d	1.9 c	3.6 b	7.1 b	1.2 b
MCP 10	26.4 c	7.2 c	4.8 a	3.9 a	10.9 a	2.0 a
ASA 0.3-CaCl ₂ 2.0	16.4 e	7.4 c	1.7 c	3.8 a	9.9 a	1.5 a
CaCl ₂ 2.0-ASA 0.3	14.9 e	7.2 c	1.3 c	3.9 a	9.1 a	1.1 b
HW 47, 20-ASA 0.3	9.8 e	6.4 c	1.3 c	4.0 a	9.6 a	1.2 b
ASA 0.3-HW 47, 20	21.4 d	10.1 b	1.0 c	3.9 a	8.9 b	1.2 b
HW 47, 20-CaCl ₂ 2.0	12.0 e	10.0 b	1.4 c	3.9 a	9.4 a	0.9 b
CaCl ₂ 2.0-HW 47, 20	22.8 d	10.1 b	1.1 c	3.8 a	7.9 b	1.2 b
HW 47, 20-MCP	14.0 e	4.3 d	1.6 c	3.8 a	10.1 a	1.1 b
MCP-HW 47, 20	15.4 e	5.5 d	1.8 c	4.0 a	9.9 a	0.9 b
HW 47, 20-(K-Ph 1.0)	15.7 e	2.7 d	1.5 c	4.0 a	8.6 b	1.1 b
(K-Ph 1.0)-HW 47, 20	15.9 e	2.4 d	1.3 c	4.0 a	7.9 b	1.0 b
MCP-(K-Ph 1.0)	14.6 e	4.7 d	1.0 c	3.9 a	8.9 b	1.1 b
(K-Ph 1.0)-MCP	14.9 e	4.5 d	1.3 c	3.8 a	9.4 a	1.0 b
MCP-CaCl ₂ 2.0	22.9 d	6.9 c	1.7 c	3.9 a	7.9 b	0.8 b
CaCl ₂ 2.0-MCP	28.5 c	9.3 b	1.1 c	3.9 a	8.6 b	0.8 b
MCP-ASA 0.3	18.0 d	6.4 c	1.8 c	3.9 a	6.6 b	0.8 b
ASA 0.3-MCP	18.0 d	7.3 c	1.7 c	3.8 a	7.6 b	1.1 b
(K-Ph 1.0)-CaCl ₂ 2.0	27.8 c	6.7 c	1.1 c	3.6 b	8.9 b	0.9 b
CaCl ₂ -(K-Ph 1.0)	30.2 c	4.9 d	0.7 c	4.1 a	9.9 a	0.9 b
(K-Ph 1.0)-ASA 0.3	21.2 d	4.6 d	2.5 b	3.6 b	10.9 a	1.2 b
HW 47, 20-MCP-(K-Ph 1.0)	25.4 c	7.2 c	3.6 a	3.8 a	9.6 a	0.7 b
HW 47, 20-MCP-CaCl ₂ 2.0	30.8 c	6.1 d	4.2 a	3.7 b	10.9 a	1.5 a
HW 47, 20-MCP-ASA 0.3	26.7 c	7.0 c	1.6 c	3.7 b	10.4 a	1.3 b
MCP- (K-Ph 1.0)-CaCl ₂ 2.0	32.6 c	7.2 c	2.9 b	3.6 b	11.9 a	1.3 b
MCP-CaCl ₂ 2.0-ASA 0.3	30.8 c	5.8 d	2.5 b	3.6 b	10.4 a	1.7 a
(K-Ph 1.0)-CaCl ₂ 2.0-ASA 0.3	30.0 c	3.7 d	0.9 c	3.5 b	9.1 a	1.8 a

^vDiameter of anthracnose lesion (DL) was measured for inoculated fruits only.^xTotal soluble solids.^wTitrate acidity.^vMeans followed by the same letters in columns are not significantly different according to Scott-Knott test ($p \leq 0.05$).

concentrations used were effective and the fruits were firmer following treatment with 1-MCP than they were without or with fruit submitted to treatments of at 4 mg·L⁻¹ 1-MCP being the firmest. In both assays, no other parameters (pH, TSS and TA) were affected by 1-MCP.

The 1-MCP could have a small effect in terms of alleviating the severity of anthracnose associated with the delay in fruit ripening during post-harvest stage, which is the main objective of 1-MCP treatment. Considering the safety, toxicity, and environmental profile of 1-MCP in relation to humans, animals, and the environment, the use of this compound is extremely favorable. Different results have reported that 1-MCP led to

a delay in fruit softening after 30 days of storage at ambient air temperature (20°C) in non-astringent persimmon and mango (Liu et al., 2010; Ramin, 2008). Peach fruits, which were very sensitive to ethylene, had delayed ripening leading to prolonged shelf life after the application of 1-MCP (Cuquel et al., 2006). 1-MCP delayed ripening, ethylene production, and the expression of ACC synthase (Tassoni et al., 2006); and it reduced the color value and loss of firmness in tomato (Tadesse et al., 2012). 1-MCP was a gaseous substance that prevented ethylene binding to its receptor causing delayed color development (both lycopene accumulation and chlorophyll degradation), softening, and ethylene production in fruits

Table 8. Effect of individual treatments and the combination of hot water at 47°C for 20 min (HW 47, 20), 1-MCP (10 mg·L⁻¹), K-phosphite at 1.0 mL·L⁻¹ (K-Ph 1.0), calcium chloride (CaCl₂) at 2.0 mL·L⁻¹, and acetyl salicylic acid (ASA) at 0.3 mL·L⁻¹ on number of lesions and fruit quality in non-inoculated fruits.

Treatment	FWL (%) ^z	NL ^y	Firmness (kg·cm ⁻²)	pH	TSS (°Brix) ^x	TA (%) ^w
Control	9.9 b ^v	63.7 a	1.0 e	4.1 b	10.6 a	0.9 b
K-Ph 1.0	7.4 b	37.5 b	1.1 e	3.7 c	7.4 b	1.5 a
CaCl ₂ 2.0	8.3 b	18.5 c	1.1 e	3.5 c	9.2 a	1.8 a
ASA 0.3	10.1 b	23.5 c	1.2 e	3.7 c	7.4 b	1.2 a
HW 47, 20	10.4 b	16.5 c	1.0 e	3.8 c	7.1 b	1.4 a
MCP 10	6.2 b	21.5 c	1.2 e	3.4 c	7.1 b	1.6 a
ASA 0.3-CaCl ₂ 2.0	7.9 b	30.0 c	0.8 e	3.8 c	10.6 a	1.2 a
CaCl ₂ 2.0-ASA 0.3	11.9 b	20.0 c	1.0 e	3.7 c	8.4 b	1.5 a
HW 47, 20-ASA 0.3	8.6 b	6.2 c	1.7 d	3.9 b	9.6 a	1.5 a
ASA 0.3-HW 47, 20	15.3 b	34.5 b	0.8 e	3.8 c	8.1 b	1.3 a
HW 47, 20-CaCl ₂ 2.0	9.4 b	25.0 c	1.8 d	4.0 b	10.4 a	1.0 b
CaCl ₂ 2.0-HW 47, 20	10.9 b	25.5 c	1.1 e	2.5 d	7.9 b	1.3 a
HW 47, 20-MCP	45.7 a	15.5 c	1.3 e	3.9 b	10.6 a	1.1 a
MCP-HW 47, 20	13.5 b	16.0 c	2.2 c	3.9 b	9.9 a	1.0 b
HW 47, 20-(K-Ph 1.0)	5.1 b	11.2 c	1.5 d	4.0 b	8.9 a	0.7 b
(K-Ph 1.0)-HW 47, 20	4.6 b	15.2 c	1.7 d	4.0 b	10.1 a	0.9 b
MCP-(K-Ph 1.0)	10.2 b	12.7 c	1.5 d	3.6 c	10.6 a	1.0 b
(K-Ph 1.0)-MCP	5.5 b	4.7 c	2.0 d	3.9 b	9.4 a	0.7 b
MCP-CaCl ₂ 2.0	5.4 b	80.0 a	1.7 d	4.1 b	8.9 a	0.8 b
CaCl ₂ 2.0-MCP	3.7 b	35.5 b	1.1 e	4.0 b	8.4 b	0.8 b
MCP-ASA 0.3	7.5 b	41.5 b	1.7 d	4.1 b	9.1 a	0.8 b
ASA 0.3-MCP	5.6 b	30.7	1.0 e	3.9 b	8.1 b	0.6 b
(K-Ph 1.0)-CaCl ₂ 2.0	6.9 b	23.7 c	1.2 e	4.0 b	9.1 a	0.7 b
CaCl ₂ 2.0-(K-Ph 1.0)	6.5 b	45.7 b	0.9 e	4.5 a	9.6 a	0.6 b
(K-Ph 1.0)-ASA 0.3	4.6 b	12.5 c	3.2 b	3.6 c	10.1 a	1.0 b
HW 47, 20-MCP-(K-Ph 1.0)	3.2 b	16.7 c	2.6 c	3.7 c	11.1 a	0.9 b
HW 47, 20-MCP-CaCl ₂ 2.0	4.8 b	17.7 c	2.7 c	3.7 c	11.1 a	1.0 b
HW 47, 20-MCP-ASA 0.3	5.8 b	32.2 c	4.0 a	3.8 c	12.1 a	1.2 a
MCP- (K-Ph 1.0)-CaCl ₂ 2.0	4.1 b	6.2	3.1 b	3.6 c	10.6 a	1.1 a
MCP-CaCl ₂ 2.0-ASA	4.6 b	14.5 c	2.3 c	3.6 c	10.6 a	1.2 a
(K-Ph 1.0)-CaCl ₂ 2.0-ASA 0.3	3.9 b	8.5 c	1.0 e	3.7 c	4.1 c	1.3 a

^zPercentage of fresh weight loss.

^yNumber of lesions (NL) was measured for non-inoculated fruits only.

^xTotal soluble solids.

^wTitration acidity.

^vMeans followed by the same letters in columns are not significantly different according to Scott-Knott test ($p \leq 0.05$).

(Guillén et al., 2007).

Analysis of DL showed that all treatments differed from the control in the inoculated fruits. The smaller DL was mostly observed in treatments which included HW, where those with HW in association with phosphite produced the smallest lesion size. The largest DL was observed in treatments HW-ASA and CaCl₂-HW. All treatments had different effects on FWL compared with the control, in particular, those which involved HW treatment, led to the lowest FWL. The poorest performances in this parameter were observed following treatments which included the ASA, where the HW-ASA

performed the worst (Table 7).

Treatments with triple combination including the HW led to the highest values of firmness, with treatment (HW-MCP-CaCl₂) giving the firmest fruits. Concerning the TSS, treatments in both the double and triple combination included the phosphite resulted in higher TSS than in the control, with treatment (MCP-phosphite-CaCl₂) producing the highest value (Table 7). In the analysis of the pH, there values varied in comparison to the control and treatment with MCP only produced the most acidic fruit. The TA showed that the triple combination that included HW and phosphite differed from

the control, expressing high values (Table 7).

In fruits without inoculation, the NL observed following treatment (MCP-CaCl₂) did not differ from the control, and fruits in MCP-phosphite had the lowest NL. Only treatment (HW-MCP) differed from the control in terms of FWL, which showed the best results. In the analysis of firmness and TSS, not all treatments were different from the control, and triple treatment (HW-MCP-ASA) had the strongest effect (Table 8). Most of these had no effect on pH, but the CaCl₂-HW produced the lowest values. The highest TA was obtained with single application of AAS and MCP. In general, it is noted that inclusion of HW in the treatment leads to more effective results, particularly when used in combination with MCP and phosphite (Table 8).

Although the HW treatments had shown efficacy, they did not provide residual protection to fruits, allowing new infections by pathogens. Thus, the efficiency of HW treatment could be improved by using in combination with other treatments. This synergism provided an additional benefit over either of the treatments given alone, and in some countries the maintenance of fruits exposed to HW for a long time become expensive in terms of energy consumption. In apples, the benefit of synergism between HW treatment combined with calcium sprays was observed on fruit firmness, decay, peel color, and ascorbic acid content (Sharma et al., 2013). Phosphite might also be associated with other methods leading to increased efficiency, which might be a viable alternative to conventional fungicides for disease control (Brackmann et al., 2008). The use of phosphite did not usually alter the physico-chemical quality of fruits (Moreira and May-de Mio, 2009). In banana (*Musa* spp.) HW at 53°C for 15 and 20 min, and prochloraz (100, 125, and 250 mg·L⁻¹) reduced the incidence of anthracnose infection (Sponholz et al., 2004). A similar reduction was observed using hydrothermal treatment at 47°C in combination with a resistance inducer [*Saccharomyces cerevisiae*, Agro-Mos®, Agro-Mos, Improcrop Brasil, Curitiba-PR-Brazil] (Soares Pessoa et al., 2007). Furthermore, hydrothermal treatment (50°C for 1 min) in combination with calcium lactate (1.5%) in lettuce could inhibit enzymatic browning and reduce product respiration (Martin-Diana et al., 2005).

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