Effect of Ammonium Carbonate and Sodium Bicarbonate on Anthracnose of Papaya

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Colletotrichum gloeosporioides is the causal organism of anthracnose in *Carica papaya* L. (papaya, papaw). The effect of ammonium carbonate (3%) or sodium bicarbonate (2%) in aqueous solution or when incorporated into a wax formulation on anthracnose severity in inoculated or naturally infected fruits was examined. Both salts had significant effects, but that of ammonium carbonate was greater than that of sodium bicarbonate in controlling anthracnose. Ammonium carbonate (3%) incorporated into the wax formulation effectively reduced anthracnose incidence by 70% in naturally infected papaya and extended the storage life by maintaining the firmness, color and overall quality of the fruit in low temperature storage (13.5°C) and 95% r.h. for 21 days followed by 2 days under marketing conditions. The mode of action of ammonium carbonate on the control of anthracnose appears to be *via* complete inhibition of radial mycelial growth and conidia germination.
KEY WORDS: *Colletotrichum gloeosporioides*; anthracnose; ammonium carbonate; sodium bicarbonate; *Carica papaya*; papaya; papaw.

INTRODUCTION

Anthracnose disease caused by *Colletotrichum gloeosporioides* Penz. Sacc. is the major constraint in papaya production as well as in its export to overseas markets. Field inoculum of *C. gloeosporioides* in the form of conidia comes from dying infected petioles of the lower leaves. The conidia which are released into the atmosphere by rain splash are carried by air currents to developing fruits. If moist conditions exist for a few hours, the fungus develops appressoria from which infection threads penetrate the skin of the fruit and remain latent (5). The resulting incipient fungal infection becomes apparent only during the postharvest stage or ripening (21).

Anthracnose in papaya can be controlled by prochloraz or propiconazole (18). Hot water dip treatment at 43–49°C for 20 min was also found to control the disease (6). Double dip hot water treatment, which was developed to eradicate fruit fly, was also found to be effective in the control of anthracnose (2). Hot water dip treatment in combination with fungicides has also been studied in order to control anthracnose (7). However, in papaya fruits, hot water dip treatment affects the ripening process (14). Use of fungicides for extended periods may cause the emergence of fungicide-resistant strains of the fungus. In addition, the residues of fungicides present in the fruits are harmful to consumers.

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These facts have necessitated the development of non-hazardous methods for control of the disease. When developing such non-chemical control measures, feasibility and the cost of implementation, in addition to the effect on fruit quality, should be considered.

Carbonic acid salts such as bicarbonate are widely used in the food industry at levels of up to 2% for leavening, pH control, and taste and texture development (12). These chemicals also have broad-spectrum antimicrobial activity (13). The ability of bicarbonate salts to control postharvest pathogens has been demonstrated in citrus (3), carrot (15), bell peppers (10) and melons (1). Ammonium carbonate is classified as a Generally Regarded As Safe (GRAS) compound by the United States Food and Drug Administration (17) and is used as a dough strengthener, leavening agent, pH-control agent, and texturizer (17). The objective of this study was to examine the effectiveness of carbonic acid salts for the control of anthracnose in papaya fruit during storage.

MATERIALS AND METHODS

The fungus *C. gloeosporioides* was isolated from infected papaya fruits *(Carica papaya* L.). Pure cultures were maintained on potato dextrose agar (PDA) at 28°C. A mycelial disc of 1.2 cm diam obtained from a 4–5-day-old culture of the fungus on PDA was transferred to 100 ml PDA in a 250-ml conical flask and incubated at room temperature for 4–5 days. At the end of the incubation time, 30 ml of sterile water was added to each culture and the suspension was filtered through two layers of sterile muslin cloth. The concentration of the conidia in the filtered suspension was adjusted if required with sterile distilled water and used for the experiments described below. The conidia concentration was adjusted to 10^3 conidia ml^{-1} with the help of a haemocytometer.

Sodium bicarbonate, potassium bicarbonate, sodium carbonate, potassium carbonate and ammonium carbonate were used at concentrations ranging from 1% to 5%.

Effect of carbonic acid salts on radial mycelial growth of *C. gloeosporioides* A mycelial disc (1.2 cm diam) was taken from the peripheral region of a 5-day-old culture of *C. gloeosporioides* grown on PDA and transferred to the center of a 9-cm-diam PDA plate, which had been amended by incorporating the carbonic acid salt (aqueous solution) at the required concentration into the medium at 50°C before plating. The amended PDA was thereafter poured into a 9-cm-diam petri dish. Growth was assessed after 10 days by measuring the colony diameter along the two axes at right angles to each other (20). These experiments were repeated twice with ten replicates each time. For the control, sterile distilled water was used instead of carbonic acid salt solution. Since the pH of the carbonic acid salt-amended medium was 8.3–8.7, the pH of the controls was adjusted with 5 N NaOH (1,10). Radial growth reduction was calculated in relation to growth of the control, as follows:

$$\frac{C-T}{C} \times 100 = \%\ Inhibition\ of\ radial\ mycelial\ growth$$

where C is radial growth measurement of the pathogen in control and T is redial growth of the pathogen in the presence of a carbonic acid salt (19).

The fungicidal or fungistatic effect of the carbonic acid salts was tested by transferring the mycelial disc to freshly poured PDA and held at 28°C for 10 days (1). Growth was assessed at the end of the incubation period by measuring radial mycelial growth as mentioned above. This experiment was repeated twice.

Effect of carbonic acid salts on germination of *C. gloeosporioides* Cellulose membranes comprising 2 cm^2 pieces were cut from dialysis tubing (Medicell International Ltd., UK). The pieces were boiled once in distilled water for 5 min to remove the surface coating, after which they were washed with distilled water and placed on a glass slide in a petri dish (two pieces per petri dish) lined with a paper towel. This setup was sterilized by autoclaving for 15 min at 121°C; then the sterilized setup was allowed to reach room temperature (28°C). The conidia suspension (0.025 ml) prepared as described above was pipetted onto each membrane and samples were air-dried in the laboratory at 28°C for 30–60 min. The paper towel lining the dish was moistened with 4 ml of sterile water. The tested carbonic salt (0.1 ml) was introduced into the inoculated membranes at the required concentration and incubated at 28°C for 24 h (9). Inoculated membranes treated with sterile distilled water (0.1 ml) served as the control. The pH of the sterile distilled water used in the control was adjusted to the pH of the specific carbonic acid salt, as mentioned above. The cellulose membranes were removed from the petri dishes at the end of the incubation period and observed for conidial germination, using an Olympus CH2 light microscope at × 100 magnification (10). The lengths of the germ tubes were also measured. Random counts were made for 200 conidia per two fields at the evaluation period for each carbonic acid salt concentration. A conidium was considered to have germinated when the germ tube had exceeded half the length of the spore. Each treatment was replicated thrice and the experiment was repeated twice. A complete randomized design was adopted in both experiments described above. Data were analyzed by analysis of variance and means were separated by DMRT, P=0.05.

Effect of sodium bicarbonate or ammonium carbonate on inoculated papaya fruits Papaya fruits were picked from a commercial cultivation farm at Homagama, Sri Lanka. A set of 20 papaya fruits at color index 1 (11) was surface sterilized with 75% ethanol and wounded with a No. 2 cork borer (1.2 cm diam) to a depth of 3 mm on the fruit surface. The wound was inoculated with 0.1 ml of conidial suspension (10^3 conidia ml^{-1}) of the pathogen and incubated for 3 h at 28°C. At the end of 3 h, the inoculated wound of the fruit was treated with 0.1 ml of either sodium bicarbonate (2%) or ammonium carbonate (3%). Inoculated and sodium bicarbonate- or ammonium carbonate-treated fruits were placed in corrugated cardboard boxes and stored at 13.5°C and 95% r.h. for 10 days. At the end of the storage time, disease severity was assessed by measuring the length (in cm) of the lesion. The inoculated lesions were then cut and removed from the healthy margin of the fruit and homogenized in 0.1M acetate buffer pH 4.5 (w/v 2:1). The preparation was serially diluted and 0.1 ml of each dilution was plated on PDA by the spread plate method. The plates were incubated at 28°C for 5–7 days and observed for growth of *C. gloeosporioides*. A set of 20 fruits inoculated with the pathogen but treated with sterile water served as the control. This study was repeated twice with 20 replicates. A complete randomized design was adopted. Data were analyzed by DMRT, P=0.05.

Effect of sodium bicarbonate or ammonium carbonate on naturally infected papaya fruits A set of naturally infected fruits (20) was harvested at color index 1 (green with absence of yellow area) and dipped in 4 *l* of wax formulation (unpublished data from our laboratory), which contained 2% sodium bicarbonate or 3% ammonium carbonate. After dipping, the fruits were allowed to air-dry for 5 min at 28°C (1,10). The fruits were then lined with Styroform netting and placed vertically (stem-end facing down) in corrugated

cartons. Each carton had six fruits. The packed fruits were stored at 13.5°C and 95% r.h. for 21 days. Fruits dipped in sterile distilled water or fruits dipped in wax formulation without the two carbonic acid salts served as the control. This experiment was repeated twice with 20 replicates.

At completion of storage time the fruits were removed from cold storage and ripened with ethephon in special chambers (in 93 *l* volume cubicles, 1.6 ml ethephon was used with 20% head space) at 28°C. After ripening, fruits were held at 25°C for 2 days and assessed for fruit quality, according to the following parameters. Disease incidence was expressed as percentage of fruits showing anthracnose out of the total number of fruits in each carton. Disease severity was evaluated by measuring the lesion length (the disease symptom) (20). The skin color of the mid-side of each fruit was determined using a Minolta chromameter (9 mm aperture) and Commission International de l'Eclairage (CIE) color space (L* C* h*) (20). Overall quality was assessed according to the following score based on disease and color: 1–2 = fruits not marketable; 3 = poor quality, limited marketability; 4–5 = fair quality, marketable; 6-7 = good quality, marketable; 8-9 = excellent quality. Fruit firmness was measured with a Chatillon penetrometer (Chatillon and Sous, New York, NY), which was equipped with a 6 mm plunger capable of penetrating through the peel into the pulp. Ten fruits were tested each time, on opposite sides of each fruit.

RESULTS

All five carbonic acid salts tested in this study caused significant inhibition of radial mycelial growth of *C. gloeosporioides*. The inhibition increased with increasing concentrations of the salts. The most effective material was ammonium carbonate, followed by sodium bicarbonate. Ammonium carbonate gave complete inhibition at 2% concentration. Sodium bicarbonate at 2% gave 64% inhibition of radial mycelial growth (arcsine transformed data are given in Table 1). The effect of ammonium carbonate on mycelial growth was fungicidal.

TABLE 1. Effect of carbonic acid salts on inhibition of germination and of radial mycelial growth, and reduction in germ tube length

Treatments[z]	% Inhibition of germination	% Inhibition of radial mycelial growth (arcsine transformed data)	% Reduction in germ tube length
Control	0	0	0
AC 1%	90.00a[y]	84.58a	90.00a
AC 2%	90.00a	90.00b	90.00a
SBC 1%	53.73b	49.60c	80.02b
SBC 2%	64.90c	59.34d	85.56c
SC 1%	40.98d	39.23e	65.50d
SC 2%	35.97e	54.94f	74.66e
PC 1%	4.80f	26.99g	55.06f
PC 2%	24.88g	30.00h	55.06f
PBC 1%	30.00h	43.74i	64.90g
PBC 2%	40.00i	45.00j	74.77h

[z]Control, sterile distilled water; AC, ammonium carbonate; SBC, sodium bicarbonate; SC, sodium carbonate; PC, potassium carbonate; PBC, potassium bicarbonate.

[y]Within columns, means followed by the same letter do not differ significantly at P=0.05.

TABLE 2. Effect of ammonium carbonate (AC) or sodium bicarbonate (SBC) on anthracnose disease severity in inoculated papaya fruits and recovery of *Colletotrichum gloeosporioides* from the fruits

Treatment[z]	Disease severity (lesion length, cm)	Recovery of *C. gloeosporioides* (arcsine transformed data)
3% AC	1.00b[y]	11.97b
2% SBC	5.50c	51.71c
Control (sterile distilled water)	13.50a	90.00a

[z]Wounded papaya fruits were inoculated with 10^3 conidia ml^{-1} of *C. gloeosporioides*, followed by treatment at the wound site with SBC or AC, and incubation at 13.5°C and 95% r.h. for 10 days.
[y]Within columns, means followed by a different letter differ significantly at P=0.05.

TABLE 3. Effect of ammonium carbonate (AC) or sodium bicarbonate (SBC) on anthracnose disease incidence and severity in naturally infected papaya fruits

Treatment[z]	Anthracnose disease	
	Incidence[y] (arcsine transformed data)	Severity (lesion length, cm)
Wax + 3% AC	30.00b[x]	1.00b
Wax + 2% SBC	46.03c	3.80c
Wax	80.02d	5.00d
Control (sterile distilled water)	90.00a	12.00a

[z]Papaya fruits were dipped in wax formulation containing SBC or AC; effects were determined after 21 days at 13.5°C and 95% r.h.
[y]Disease incidence is expressed as the ratio of number of fruits with disease symptoms to the total number of fruits in a commercial pack.
[x]Within columns, means followed by a different letter differ significantly at P=0.05.

Significant ($P \leqslant 0.05$) inhibition of conidia germination was observed with increasing concentrations of four carbonic acid salts. Ammonium carbonate at 1% caused complete inhibition of germination (Table 1), and sodium bicarbonate at 2% gave 74% inhibition of germination (arcsine transformed data are given in Table 1). Germ tube length was significantly ($P \leqslant 0.05$) reduced with increasing concentration of the carbonic acid salts (Table 1). The percent reduction in germ tube length was significantly higher in the presence of 2% sodium bicarbonate than sodium carbonate, potassium carbonate or potassium bicarbonate. Anthracnose severity in inoculated fruits was significantly ($P \leqslant 0.05$) reduced in fruits treated with ammonium carbonate at 3% or sodium bicarbonate

TABLE 4. Effect of ammonium carbonate (AC) or sodium bicarbonate (SBC) on quality parameters in naturally infected papaya fruits (data averages of 20 fruits)

Treatments	Firmness (N)	Hue angle (H°)[z]	Overall quality[y]
Wax formulation	0.93a[x]	91.1b	3b
Wax formulation + AC	1.20b	87.3c	7c
Wax formulation + SBC	0.97c	88.8d	5d
Control (sterile distilled water)	0.92a	92.5a	1a

[z]Angle attributed to colors classed as red (0°), yellow (90°), green (180°), blue (270°), or an intermediate between any adjacent pair of colors.
[y]Overall quality assessed on a score of 1 to 9, where 1-2 = fruits not marketable; 3 = poor quality, limited marketability; 4-5 = fair quality, marketable; 6-7 = good quality, marketable; 8-9 = excellent quality.
[x]Within columns, means followed by the same letter do not differ significantly at P=0.05 (DMRT).

at 2%, compared with the control fruits (Table 2). The recovery of *C. gloeosporioides* from fruits treated with ammonium carbonate (3%) was significantly lower ($P \leqslant 0.05$) than in control fruits. The lowest recovery of *C. gloeosporioides* was also from fruits treated with 3% ammonium carbonate (Table 2).

Anthracnose incidence and severity in naturally infected fruits were significantly ($P \leqslant 0.05$) reduced in fruits waxed with wax formulation containing 2% sodium bicarbonate or 3% ammonium carbonate when compared with the fruits dipped in wax formulation without salts or only in sterile distilled water (Table 3). Application of wax formulation containing 3% ammonium carbonate resulted in only ~25% (arcsine transformed data are given in Table 3) anthracnose incidence after 21 days in low temperature storage. With sodium bicarbonate it was nearly 53% (arcsine transformed data are given in Table 3). Fruit firmness was significantly higher ($P \leqslant 0.05$) in fruits treated with a wax formulation containing sodium bicarbonate (2%) or ammonium carbonate (3%) than in fruits treated with a wax formulation alone or dipped in sterile distilled water. The strongest effect was obtained with a wax formulation containing 3% ammonium carbonate (Table 4). The H° angle was high, towards orangish yellow, in fruits dipped in sterile distilled water and greenish yellow in fruits waxed with wax formulation containing 3% ammonium carbonate (Table 4). The overall quality was significantly ($P \leqslant 0.05$) higher in fruits treated with a wax formulation containing 2% sodium bicarbonate or 3% ammonium carbonate in comparison with the two other treatments (Table 4). Fruits treated with a wax formulation containing 3% ammonium carbonate also had a higher score for overall quality and 70% of the fruits were ranked as marketable.

DISCUSSION

Carbonic acid salts had significant effects on both *C. gloeosporioides* and anthracnose disease, with ammonium carbonate being the most effective. The direct and indirect effects of carbonic acid salts on microorganisms have been shown previously (8,16). The mode of action of these salts is postulated to be due to the reduction in fungal turgor pressure resulting in the collapse and shrinkage of hyphae causing subsequent inhibition of sporulation (10). Further, the high pH values caused by ammonium carbonate (pH 8.7) and sodium bicarbonate (pH 8.5) are also detrimental to the fungi (8,16). Ammonium carbonate very effectively inhibited mycelial growth and conidial germination. As a result, ammonium carbonate in aqueous solution or wax formulations was able to maintain fruit quality in healthy fruits by preventing infection. Similar protection has been reported for sodium bicarbonate for citrus (3) and melon (1) fruits.

Anthracnose is a latent infection, with the fungus entering the fruit by direct penetration (5). During transportation and storage, anthracnose can spread rapidly from such infected fruits to healthy fruits by direct contact (5). Therefore, the presence of the wax formulation on the fruit surface would prevent disease transmission by acting as a physical barrier. The wax formulation has, in addition, fungicidal effects. Coating with wax is also known to reduce weight loss and shrinkage, retard ripening by modifying the internal atmosphere, and lengthen the storage life of fruits (4).

The concentration of ammonium carbonate used in this study was that recommended by the food industry (17). High concentrations of sodium bicarbonate (above 2%) were not used, due to the phytotoxicity reported to arise from changes in the chemical composition of the cuticular wax (1). It is therefore concluded that wax formulation having 3% ammonium

carbonate should be developed commercially to extend the storage life of papaya (color index 1) at 13.5°C and 95% r.h. for 21 days + 2 days under marketing conditions, by preventing the occurrence of anthracnose.

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