Proc. Fla. State Hort. Soc. 116:375-378. 2003.

CONTROL OF GREEN MOLD ON FLORIDA CITRUS FRUIT USING BICARBONATE SALTS

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Additional index words. Diplodia natalensis, Penicillium digitatum, P. italicum, blue mold, potassium bicarbonate, postharvest handling, postharvest decay, sodium bicarbonate, stem-end rot

Abstract. **Carbonate and bicarbonate salts have been reported to be effective for green and blue mold control of citrus fruit. Evaluation of potassium bicarbonate (PBC) for the suppression of *Penicillium digitatum* mycelial growth and the control of green mold was conducted *in vivo* using both inoculated and naturally infected orange fruits and an aqueous packingline drip system. Test data showed that PBC actively suppressed the mycelial growth of *P. digitatum in vitro* with an ED_{50} of 0.08%. Using both inoculated and naturally infected 'Navel' oranges, the concentration of PBC greatly affected the efficacy for green mold control and a rate of 3.4% was demonstrated to be the best concentration to reduce green mold incidence under the test conditions. Choosing 3.4% PBC in two additional tests using 'Valencia' or 'Pineapple' oranges, PBC significantly ($P \leq 0.05$) reduced green mold incidence in two tests. However, in all tests PBC at 3.4% never significantly reduced stem-end rot caused mainly by *Diplodia natalensis*. PBC at 3.4% achieved a similar efficacy as imazalil at 0.1% for green mold control in all 4 tests, and it also performed similarly to thiabendazole (TBZ) at 0.1% for green mold control in three tests in which TBZ was used as a standard fungicide. When PBC was compared to sodium bicarbonate (SBC) for decay control, PBC and SBC at the same concentration of 3.4% performed similarly, both effectively controlled green mold, but not stem-end rot. No fruit damage was observed in any test. The information of this study suggests that bicarbonate salts such as PBC and SBC could be alternative chemicals for citrus green mold control under the current packing system in Florida.**

Green mold caused by *Penicillium digitatum* (Pers.:Fr) is one of the most important commercial postharvest citrus diseases in Florida (Brown and Miller, 1999) and other citrus producing areas (Eckert and Brown, 1986; Palou et al., 2001). The control of this decay in Florida is conducted through an integrated procedure with the application of fungicides (imazalil, thiabendazole and sodium *o*-phenylphenol) as the core component (Brown and Miller, 1999; Zhang, 2002). Due to increasingly stringent regulations, pathogen resistance development and public concerns about chemical residues in fruit and their products, alternative methods for decay control are obviously needed. Carbonate and bicarbonate salts such as sodium carbonate (SC), sodium bicarbonate (SBC) and potassium bicarbonate (PBC) have been reported to be effective for green and blue mold control when citrus fruit are immersed into solutions of carbonate or bicarbonate salts for a short time (Palou et al., 2001; Smilanick et al., 1997; Smilanick et al., 1999). Increasing the temperature of SC or SBC solutions to 45 °C enhanced the effectiveness of the carbonate and bicarbonate salts for green and blue mold control (Palou et al., 2001; Smilanick et al., 1999). Combination of SBC with a biocontrol agent based on the yeast *Candida oleophila* achieved better control of green mold than SBC or the biocontrol agent alone on grapefruit (Porat et al., 2002). In California, many packinghouses have a water soak tank as a part of the packingline system and use of SC solutions has become increasingly popular for citrus postharvest decay control (Smilanick et al., 1999). Citrus fruit immersion in 3% (wt/vol) SC at 35 °C for 30 s is a common commercial practice in California (Smilanick et al., 2003). However, very few packinghouses in Florida have water soak tanks as a part of their packinglines and thus carbonate or bicarbonate salts have not been used for decay control. A non-recovery spray or dripping system for fungicide and wax application is commonly used in Florida packing systems. There is therefore, a need to know if carbonate or bicarbonate salts can be applied through a dripping system for green mold and other decay control. Since the sodium content in discharged water might be a concern if SC or SBC was used, PBC has been chosen for evaluation. The objectives of this work were to (i) determine the efficacy of PBC at different concentrations for green mold control using a non-recovery dripping system at room temperature; and (ii) compare the effectiveness of PBC to SBC and the standard postharvest chemicals thiabendazole and imazalil for decay control under the test conditions.

Materials and Methods

Pathogen. A Penicillium digitatum strain (PD-9) was originally isolated from decayed citrus fruit. The pathogen was maintained in potato dextrose agar (PDA) slants in test tubes and stored at 4 °C. The fungal pathogenicity and virulence were maintained by inoculating citrus fruit with the fungus and re-isolating it every year.

Inhibition of P. digitatum mycelial growth by potassium bicarbonate (PBC) in vitro. PBC (Sigma, St. Louis, MO) was mixed with sterilized and non-solidified PDA at 0 to 0.2% before pouring the medium to plates (20 mL of PDA/plate). PDA discs (5-mm diameter) with *P. digitatum* mycelia were cut from the edge of fungal colonies (4 to 5 d old) and placed at the center of each plate. The plates were placed at 25 °C, and diameters of colonies were measured daily until the fungal colonies in controls reached the edge of plates. After the test was completed (5 d after starting), the fungal plugs from PDA with 0.2% PBC were transferred onto fresh PDA to evaluate the fungistatic or fungicidic activity of PBC against *P. digitatum* mycelia since the fungus did not grow on PDA containing 0.2% PBC.

Fruit. 'Valencia', 'Pineapple' and 'Navel' oranges were obtained from a local commercial packinghouse and used in various tests. Fruit were not treated with any fungicides preharvest or postharvest. Prior to each test, fruit were washed with a cleaner (Sooty Mold Kleen 278, Decco, Monrovia, Calif.), rinsed with water, dried and randomized the same day that fruit were brought to the research facility at Lake Alfred, Fla.

Fruit inoculation. If fruit inoculation was used, the inoculation process was conducted as follows: prior to the inocula-

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tion, *P. digitatum* PD-9 was recovered from PDA slants and grown on PDA at 25 °C for 7-10 d. Spores in a 10-cm PDA plate were harvested by adding about 8 mL of sterile water with 0.05% Triton X-100 and rubbing with a glass rod. The spore suspension was filtered through two layers of sterile cheese cloth to remove the mycelia and medium particles, and diluted with sterile water to an absorbance of 0.1 at 420 nm using a spectrophotometer. This density of the spore suspension was equivalent to approximately 10^6 spores/mL (Palou et al., 2001; Smilanick et al., 1999). Fruit were punctured with a probe with a 1 × 1 mm tip that had been dipped into *P. digitatum* spore suspension just before use. One inoculation site was established in each fruit and a minimum of 50 fruit were used in each replication of each treatment. Three to five replicates were used for each treatment in each experiment.

Fruit treatment with bicarbonate salts and fungicides. A simulated commercial fungicide application procedure was used for all tests. Inoculated or non-inoculated (natural infection) fruit were treated with the suspensions of PBC, sodium bicarbonate (SBC) (Sigma) or standard fungicides imazalil and thiabendazole (TBZ) by a non-recovery dripping system through a simulated commercial packingline. Fruit were rotated on brush beds and in contact with chemicals for 20 to 30 s during the treatment process. Treated fruit were then dried at 38-54°C for 1-2 min, and packed in 4/5 bushel fiberboard cartons. Treated fruit were incubated at 21°C with 95% relative humidity (RH) and decay incidence was recorded at weekly intervals for up to 4 weeks.

Four tests were conducted using different fruit varieties or fruit treatments. Test 1 was to determine the optimum concentration of PBC for green mold control using the non-recovery dipping system. 'Navel' oranges were dripped with PBC suspensions at concentrations of 0, 0.85, 1.70, 2.55, 3.4 and 4.25% twenty four hrs after fruit inoculation with *P. digitatum.* Imazalil and TBZ at 0.1% were used as standard fungicide controls. Test 2 was conducted the same as test 1 except that the 'Navel' fruit were not inoculated with *P. digitatum.* Based on the results of test 1 and 2, a rate of 3.4% PBC was determined optimal and chosen for further tests. In test 3, 'Valencia' oranges were used and fruit were not inoculated with the pathogen. In test 4, 'Pineapple' oranges were used and not inoculated, and the efficacy of PBC (3.4%) was compared to that of SBC (3.4%) for decay control.

Data analysis. Analysis of variance of data was performed using the Statistica program (StatSoft, Tulsa, Okla.). Percentage decay data were transformed to arcsine values before analysis. Treatment means were compared using the Duncan multiple range test ($P \leq 0.05$).

Results

Suppression of mycelial growth of P. digitatum by PBC. PBC actively suppressed the mycelial growth of *P. digitatum* when PBC was mixed with PDA at a concentration range of 0 to 0.2% (Fig. 1). An ED_{50} of PBC against *P. digitatum* was calculated as 0.08%. The concentration of PBC in PDA was negatively correlated with the radial growth of fungal mycelia (r = -0.9663, P = 0.0017). PBC at 0.2% in PDA completely suppressed the growth of *P. digitatum* mycelia, but did not kill the fungus since the fungus started to grow after the fungal mycelia on 0.2% PBC medium were transferred onto fresh PDA.

Effects of PBC concentrations on green mold control (test 1 and 2). When the concentrations of PBC at 0 to 4.25% were tested using both inoculated and non-inoculated 'Navel' oranges, the efficacy of PBC for green mold control was enhanced as the PBC concentration increased (Figs. 2 and 3). The concentration of PBC at 3.4% reduced decay by 82.1% using inoculated fruit, and a further increase of PBC concentration to 4.25% did not increase the decay control efficacy (Fig. 2). The trend of test results for naturally infected fruit was similar to that for inoculated fruit (Figs. 2 and 3). The rate of 3.4% PBC performed similarly when compared to standard postharvest chemicals, thiabendazole (TBZ) and imazalil, at the commercial rate of 0.1% for green mold control. No visible

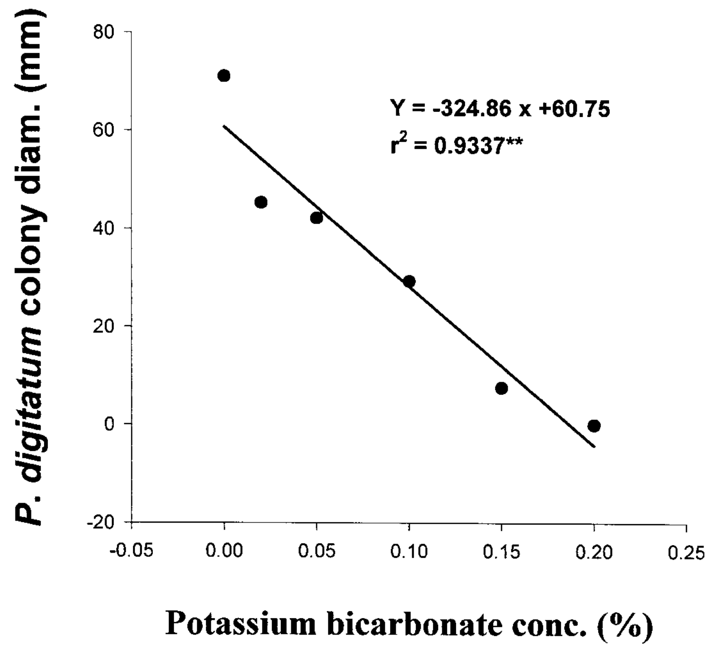

Fig. 1. Inhibition of Penicillium digitatum mycelial growth by potassium bicarbonate in potato dextrose agar. **Indicates significance at P = 0.01 level.

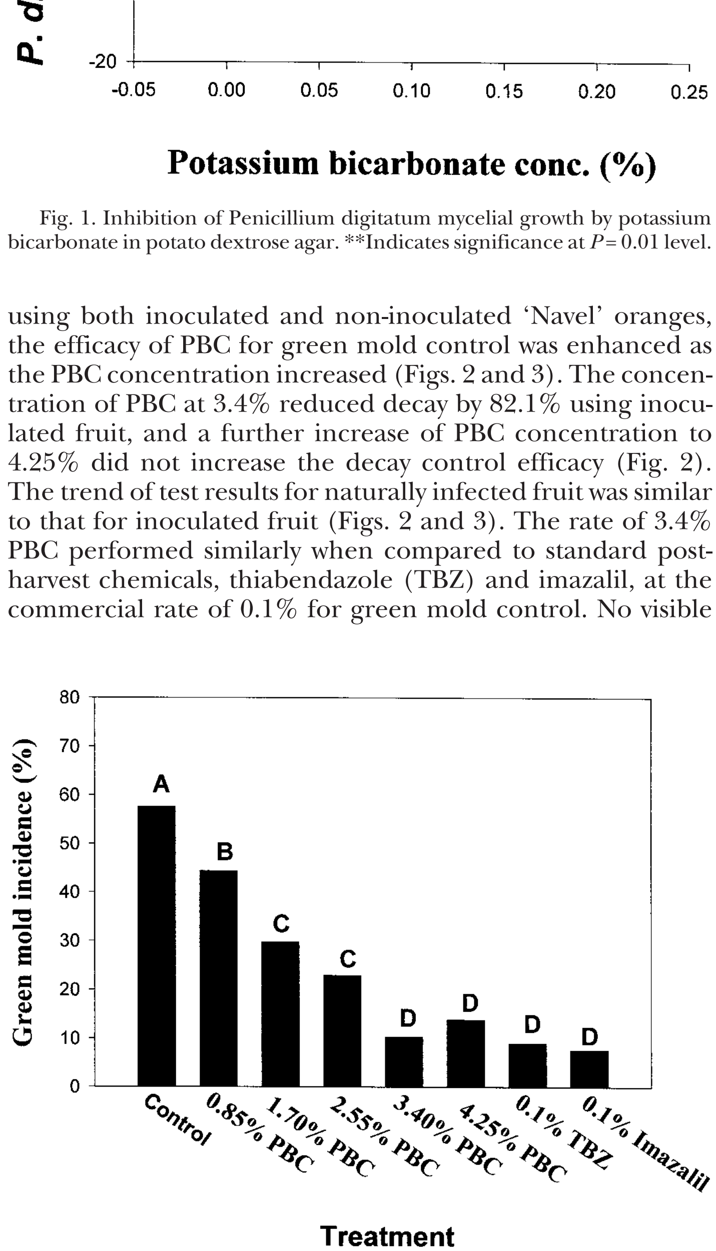

Fig. 2. Effects of potassium bicarbonate (PBC), thiabendazole (TBZ) and imazalil on green mold incidence using Navel oranges incoulated with *Penicillium digitatum.* Green mold incidence is cumulative data for one week at 21°C. Means with the same letter on each bar are not significantly different based on the Duncan multiple range test (P = 0.05).

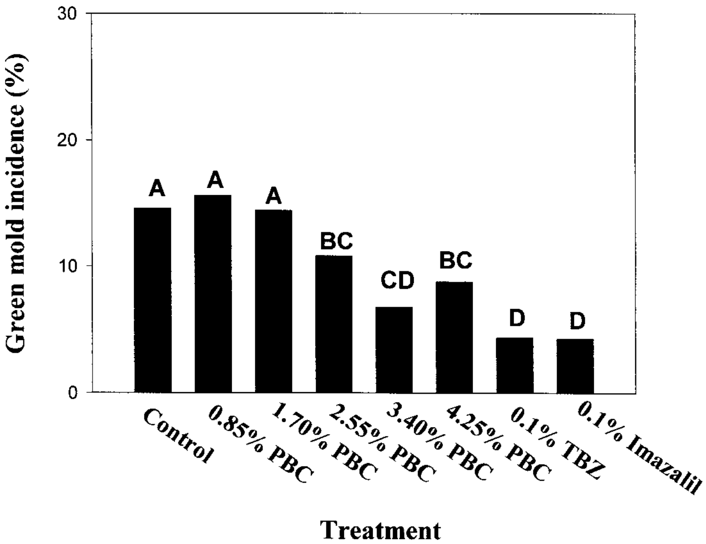

Fig. 3. Effects of potassium bicarbonate (PBC), thiabendazole (TBZ) and imazalil on green mold incidence using naturally infected Navel oranges. Green mold incidence represents cumulative data for 4 weeks at 21°C. Means with the same letter on each bar are not significantly different based on the Duncan multiple range test ($P = 0.05$).

fruit damage or phytotoxicity was observed from PBC treatment at all concentrations used.

Effects of PBC on green mold and stem-end rot control on 'Valencia' oranges (test 3). When naturally infected 'Valencia' fruit were tested, PBC at 3.4% significantly ($P \leq 0.05$) reduced green mold incidence, but not stem-end rot incidence caused mainly by *Diplodia natalensis* compared with controls (Table 1). PBC at 3.4% achieved a similar efficacy for green mold control as both imazalil and TBZ at 0.1% did. However, both imazalil and TBZ significantly reduced the incidence of stem-end rot.

Comparison of PBC and SBC for decay control (test 4). When PBC and SBC at 3.4% were compared in a test to control postharvest decay using naturally infected 'Pineapple' oranges, PBC and SBC performed similarly (Table 2). Both PBC and SBC reduced green mold incidence by about 72%, but imazalil at a rate of 0.1% reduced green mold by 97%. Both PBC and SBC did not significantly reduced the incidence of stem-end rot, but imazalil at a rate of 0.1% significantly reduced it.

Table 1. Effects of potassium bicarbonate on green mold caused by *Penicillium digitatum* and stem-end rot caused mainly by *Diplodia natalensis* using non-inoculated Valencia orange and a non-recovery dripping system on a packingline.

	Comulative incidence of decay (%)[y]		
Treatment	Conc. (%)	Green mold	Stem-end rot
Control		10.6 A[z]	11.1 A[z]
Potassium Bicarbonate	3.4	3.3 B	10.9 A
Imazalil	0.1	4.2 B	4.2 B
Thiabendazole	0.1	2.8 B	3.5 B

[y]Treated fruit were stored 21°C for 4 weeks, and decay was recorded weekly.
[z]Means with the same letter in each column are not significantly different based on the Duncan multiple range test ($P = 0.05$).

Table 2. Effects of sodium and potassium bicarbonates on green mold caused by *Penicillium digitatum* and stem-end rot caused mainly by *Diplodia natalensis* using non-inoculated Pineapple oranges using a non-recovery dripping system on a packingline.

	Comulative incidence of decay (%)[y]		
Treatment	Conc. (%)	Green mold	Stem-end rot
Control		26.0 A[z]	12.9 A[z]
Potassium Bicarbonate	3.4	7.3 B	10.4 AB
Sodium Bicarbonate	3.4	7.4 B	9.8 AB
Imazalil	0.1	0.7 C	6.9 B

[y]Treated fruit were stored at 21°C for 4 weeks, and decay was recorded weekly.
[z]Means with the same letter in each column are not significantly different based on the Duncan multiple range test ($P = 0.05$).

Discussion

Carbonate and bicarbonate salts are widely used in food industry, and are generally recognized as safe compounds and have broad spectrum of antimicrobial properties (Fallik et al., 1997; Palou et al., 2001). They are also much cheaper compared with fungicides imazalil and TBZ. This suggests that the Florida fresh citrus industry and growers might receive economical benefits if carbonate or bicarbonate salts can be used to effectively control citrus postharvest diseases.

The current studies demonstrated that PBC or SBC effectively control green mold caused by *P. digitatum*, but not stem-end rot caused mainly by *D. natalensis* using a simulated commercial dripping application method. Carbonate and bicarbonate salts have been reported to be effective for green and blue mold control on citrus (Palou et al., 2001; Smilanick et al., 1997; Smilanick et al., 1999) using an immersion application method. Immersion of fruit in 3% sodium carbonate (SC) at 35°C for 30 s during the packing process is a common commercial practice in California packinghouses (Smilanick et al., 2003). Currently, carbonate or bicarbonate salts are not used in Florida packinghouses for decay control, possibly because packingline structures in Florida are different from those of California, and additional kinds of decays such as stem-end rot are present on Florida citrus. However, our current studies demonstrated that direct application of PBC or SBC through current fungicides application systems such as dripping without any changes to the existing packingline structures could effectively reduce green mold on Florida citrus fruit. The efficacy of PBC at 3.4% for green mold control using both inoculated and naturally infected fruit was similar to or slightly less than that of the currently registered fungicides imazalil and TBZ.

Smilanick et al. (1997) demonstrated that the application rates of SC for green mold control using immersion method greatly influenced efficacy, whereas the influence of application temperature or immersion period on SC efficacy were small, and they found that 4% and 6% SC performed better than 2% SC. Palou et al. (2001) also reported that 3 or 4% SC performed better than 2% SC for the control of blue mold caused by *Penicillium italicum*. However, Palou et al. (2001) also described that temperature of SC solutions influenced effectiveness of SC for the control of blue mold more than concentration or immersion period. Under the current test conditions, the concentration of PBC greatly influenced efficacy of PBC for green mold control, and at ambient temperature 3.4% PBC was demonstrated to be the best concentration for effective control of green mold using both inoculated (24 h prior to treatment) and naturally infected fruit, and an increase of PBC concentration to 4.25% did not increase the efficacy. This appears to be consistent with the previous reports with SC or SBC for green and blue mold control using immersion test methods (Smilanick et al., 1997; Palou et al. 2001). The control of blue mold and sour rot caused by *Geotrichum citri-aurantii* by PBC has not been tested in the current studies, and should be studied in the future.

PBC was chosen for tests in the current studies primarily because the sodium content in discharged water might be a problem if SC or SBC is used in Florida packinghouses. However, in the current studies, a test for comparison of PBC to SBC for decay control using naturally infected 'Pineapple' oranges demonstrated that PBC and SBC at 3.4% performed similarly for decay control. Both PBC and SBC effectively reduced green mold, but not stem-end rot. However, Smilanick et al. (1999) compared the efficacy of SC, SBC, PBC and ammonium bicarbonate for green mold control using inoculated lemon fruit with an immersion treatment method, and showed that SC and SBC performed significantly better than PBC or ammonium bicarbonate. This disparity might be due to the different test methods and procedures used.

In California, commercial application of SC or SBC immerses fruit in 3% SC solutions for 30 s at 35 °C, and then rinses with water during the packing process. In the current studies, fruit were dripped with PBC or SBC solutions on the packingline, and treated fruit were not rinsed with water, which is similar to the commercial fungicide application procedure in Florida. Since fruit were not rinsed after treatments with PBC or SBC, their residues should be higher on the fruit surface and in injury sites compared to rinsed fruit. However, no fruit damage or phytotoxicity was observed from all tests conducted.

Carbonate and bicarbonate salts were fungistatic rather than fungicidic against *P. digitatum* and *P. italicum* (Palou et al., 2001; Smilanick et al., 1999). Our *in vitro* test data also indicated the fungistatic activity of PBC against *P. digitatum.* PBC has also been reported to be fungistatic rather than fungicidic against *Botrytis cirerea* and *Alternaria alternata* and to be effective for the control of postharvest decays caused by *B. cinerea* and *A. alternata* in sweet bell pepper fruits when peppers were dipped into 1 or 2% PBC solutions for 2 min (Fallik et al., 1997).

In our studies, PBC and SBC have been shown to be effective for green mold control, but did not have any effect on stem-end rot control. However, it is important to effectively control stem-end rot since it is one of the major postharvest diseases on Florida citrus (Brown and Miller, 1999; Zhang and Ritenour, 2002). To effectively control both green mold and stem-end rot on Florida citrus, fruit could be drenched with TBZ before ethylene degreening treatment for stem-end rot control and then dripped with PBC or SBC at 3.4% on the packingline for green mold control before packing. This could be an alternative approach to TBZ or imazalil for green mold control under the current packingline system in Florida. However, the optimum application conditions and combinations with different waxes for effective control of green and blue mold using carbonate and bicarbonate salts need to be further studied.

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