

Efficacy of Citrus Postharvest Fungicides Applied in Water or Resin Solution Water Wax

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ABSTRACT

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Applications of benomyl, carbendazim, thiabendazole, or imazalil in water or resin solution water wax were compared in nonrecovery spray treatments of oranges for control of stem-end rot (SER) and green mold caused by *Diplodia natalensis* and *Penicillium digitatum*, respectively. Control of SER with the benzimidazole fungicides applied in water or wax was comparable until treatments were delayed after harvest by degreening. In such treatments, fungicides in water were more effective, but control with applications in wax was enhanced by doubling the fungicide concentration. Imazalil controlled SER less effectively than the benzimidazoles, particularly when applied in water wax to degreened fruit. Green mold was controlled less effectively with fungicide wax applications than with aqueous treatments when punctures in the rind were so small that the wax, being more viscous than water, did not enter such injuries as effectively as water. Movement of imazalil into healthy rind was hindered when the fungicide was applied in water wax. Imazalil was therefore less effective in wax than water in controlling infections of *P. digitatum* in injuries formed after fungicide treatment. All fungicides applied either in water or resin solution water wax equally penetrated rind injured abrasively with sandpaper.

Decay of citrus fruits is controlled by application of postharvest fungicides. These fungicides are usually applied as a nonrecovery fine mist spray to fruit rotating on brushes saturated with the material (1). This procedure minimizes problems with sanitation, pH control, and stability (4). Fungicides may be applied in water followed by a subsequent solvent or water wax application, or they may be incorporated into the water wax and applied in a single process (1). The water-based waxes, either resin solution or emulsion (12), are becoming more popular because solvent waxes manufactured from petroleum products have become more expensive and contribute to air pollution.

At comparable concentrations, fungicides are less effective when applied in water wax than in water (7,10,11,18,22). Nonavailability of the fungicide at the infection site because of encapsulation by the wax (22) and variable coverage with wax (17) have been suggested as reasons for reduced decay control. Efficacy of fungicides applied in wax reportedly is comparable to aqueous applications if fungicide concentrations are doubled (12). In this study, some postharvest citrus fungicides were applied in water or

resin solution water wax at the same or twice the aqueous rate to evaluate and identify factors affecting efficacy.

MATERIALS AND METHODS

Orange (*Citrus sinensis* (L.) Osbeck cv. Hamlin and Pineapple) fruit were degreened, washed, graded, and treated with fungicides as described previously (2). Efficacy of benomyl, carbendazim (MBC), thiabendazole (TBZ), and imazalil was evaluated by suspending these materials in water or resin solution water wax (Citrus Lustr 266, 3.8 centiposes, 15.5% solids, Decco Tilbelt, Pennwalt, Monrovia, CA 91016). Carbendazim was prepared from benomyl (Benlate) as described (8). Benomyl, MBC, thiabendazole, or imazalil were applied at rates (1×) of 600, 600, 1,000, or 1,000 mg/L, respectively, in water or water wax and at twice these rates (2×) in water wax.

Stem-end rot (SER), caused by *Diplodia natalensis* P. Evans, occurred naturally during storage of fruit at 29 C and 94–97% relative humidity (RH), which are the conditions favoring its rapid development. Each treatment was applied to 80 fruits. Green mold, caused by *Penicillium digitatum* Sacc., was induced with spores inoculated through punctures or abrasive injuries. Needles for forming punctures were dipped in a concentrated aqueous suspension of spores (30×10^6 /ml) dispersed with Triton X-100 before injuring the fruit once at the equator. Punctures were formed with 1- and 0.3-mm-diameter needles to a depth of 2 and 4 mm, respectively. A deeper injury was

required with the smaller puncture so that both sizes of injury caused 90–100% of the fruit in the non-fungicide-treated control to become infected with *P. digitatum*. Each treatment was applied to 50 fruits, and the results from two trials were averaged. The abrasive injuries, about 14 mm in diameter, were formed after fungicide treatment by rubbing fruit on 60-grit coarse sandpaper. Care was taken to ensure that each injury was formed on unused areas of the sandpaper sheet to prevent contamination of injured tissue with fungicides. Injuries to 50 fruits in each of the treatments were dusted with dry spores of *P. digitatum*. Fruit inoculated with *P. digitatum* were stored at 24 C and about 94% RH.

All results of the decay studies were converted to percentage decay control to compare differences among fungicides and between methods of application. Percentage decay control was determined by subtracting the percentage decay in the treatment from that in the control, dividing the difference by the percentage decay in the control, and multiplying the quotient by 100.

Movement of water or water wax into injuries 1 or 0.3 mm in diameter was judged by adding 6 ml of red food coloring to each liter of water or water wax. The materials were then applied nonrecovery to six fruits after injuring each one at six locations on the equator. Injured tissue was subsequently removed from the rind and observed for any red discoloration within the punctured area.

Movement of fungicide into the rind was determined using grapefruit (*C. paradisi* Macf. cv. Marsh seedless), which was more easily handled than the thinner orange rind. Fifty microliters of 1× material in water or wax was applied to a circular area 9 mm in diameter marked on the equator of each of three fruits. Penetration of fungicides into injured rind was studied by rubbing the fruit against 220-grit sandpaper before applying the materials in water or water wax. Treated fruit were stored at 21 C and about 92% RH for 6 days, and surface fungicide residues were then removed with cheesecloth dampened with deionized water. A 5-mm-diameter plug of rind was removed from the center of the treated area by peeling off the rind and forcing a cork borer into the endocarp and through the exocarp to the cuticle. Some of the endocarp was removed, then the plug was mounted upright and frozen on the stage

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of a sliding freezing microtome. Eight sections, each 250 μm thick, were removed individually, starting with the rind surface and proceeding into the exocarp to a depth of 2 mm. The microtome blade was rinsed with deionized water and wiped dry with clean paper tissue between each section. Sections were kept separate and frozen overnight in glass petri dishes and bioassayed for fungicide by the method of Edgington et al (6).

Residues of benomyl, MBC, and thiabendazole were measured by incorporating spores of *P. expansum* Thom into the bioassay medium (Difco potato-dextrose agar). Spores of *P. digitatum* were added to the medium to detect imazalil. Five milliliters of warm media with spores was added to each dish (100 \times 15 mm), and the solidified agar in the dish was separated into three bands (10 mm wide) to assay the three replicate sections for fungicide (6). After placing sections on the agar, the plates were stored 20–24 hr at 4 C to permit diffusion of fungicide into the agar before initiation of fungal growth. Plates were transferred to 25 C for development of zones of inhibition within the next 2 days. Effect of pH on movement of imazalil into grapefruit rind was studied by buffering aqueous imazalil with veronal-HCl at pH 6.8 or 9.5.

The effect of water wax on fungicide movement was studied by adding fungicides to potato-dextrose agar after autoclaving. Prepared plates were stored at 25 C for 2 days to allow water condensed on the dish lid and on the surface of the agar to dry. Aluminum foil supports (15 \times 15 mm) (6) with a center hole 7 mm in diameter were used to

support squares (20 \times 20 mm) of dialyzer tubing. The tubing was floated on water and positioned over the foil support. The foil, overlain with the square of tubing, was picked up with tweezers, blotted on paper toweling to remove excess water, and placed on the agar surface. Fifteen microliters of water wax was placed on the tubing in the well formed by the 7-mm hole in the foil. Samples of wax, in amounts of 10 and 5 μl , were dry about 2 hr after each addition in an aseptic dust hood. Curling of the tubing was prevented by keeping the tubing around the edge of the foil support in contact with the agar during drying. The dried wax (of gel consistency) was easily broken when touched with a dissecting needle. Dry spores of *P. digitatum* dusted on the wax surface germinated overnight at 25 C.

RESULTS

Efficacy of the fungicides against *Diplodia* SER decreased as the degreening time was increased from 0 to 72 hr (Fig. 1). Levels of SER ranged from 60 to 80% in untreated controls degreened for 72 hr; however, benomyl and MBC still provided 100% control of decay when applied in water or wax to nondegreened fruit or fruit previously subjected to 24 hr of degreening. Thiabendazole in water or wax also prevented development of SER in nondegreened fruit, but less than perfect control was observed with wax treatments applied after 24 hr of degreening. At 48 or 72 hr, applications of the benzimidazole fungicides in water were more effective than equal concentrations in wax. Control with wax applications improved, however, if the fungicide concentrations were doubled. Average percentage control of the three benzimidazole fungicides in water (1X) vs. wax (2X) after 0, 24, 48, or 72 hr of degreening was 100 vs. 100, 100 vs. 97, 98 vs. 95, and 83 vs. 80, respectively. Water applications of imazalil were much more

effective against SER than wax treatments, though control with wax was improved by doubling the rate of imazalil. Imazalil, applied in either water or wax, was much less effective than the benzimidazoles for control of SER in degreened fruit (Fig. 1).

Control of green mold with fungicides in water or wax was influenced by the size of the punctures in the fruit rind required for infection by *P. digitatum* (Fig. 2). All treatments provided less decay control when infection originated in punctures 0.3 mm in diameter and 4 mm deep (mean of 41% decay control) compared with injuries 1 mm in diameter and 2 mm deep (mean of 87% decay control). With the exception of thiabendazole, however, control with fungicides in wax was comparable to control with fungicides in water (93 vs. 96%) where green mold originated in injuries 1 mm in diameter. In punctures 0.3 mm in diameter, the fungicides in wax did not control green mold as effectively as the aqueous fungicide treatments. Average control of green mold developing in 0.3-mm-diameter punctures with all fungicides in water was 56% compared to 32 and 35% in wax at 1 and 2X, respectively.

Wax penetrated punctures in the fruit rind less extensively than water, as shown by observations on the red staining of injured tissue from food coloring added to the wax or water. Staining was present in 75% of the 1-mm injuries and in only 44% of the 0.3-mm punctures when the dye was added to wax. Similar treatments in water caused staining of 97 and 75% of the 1- and 0.3-mm injuries, respectively.

As a measure of the ability of the various aqueous or wax fungicide formulations to penetrate the rind, inoculations with *P. digitatum* were made to injuries formed after fungicide application (Fig. 3). Control of green mold with benomyl, MBC, or thiabendazole was not affected by applying the materials in water wax. The best control was achieved with benomyl, less with

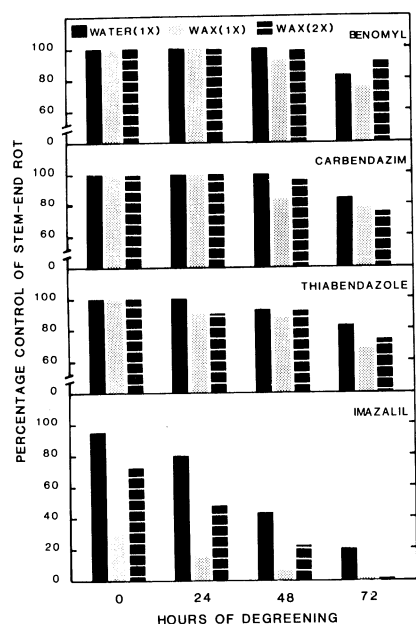


Fig. 1. Percentage control of stem-end rot caused by *Diplodia natalensis* with fungicides applied in water or wax to oranges after degreening them for 0, 24, 48, or 72 hr.

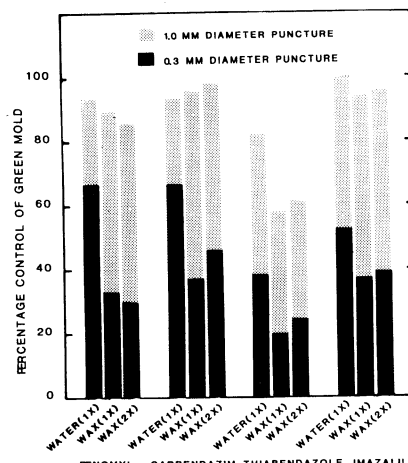


Fig. 2. Effect of size of injury (puncture) to orange rind on control of green mold caused by *Penicillium digitatum* with fungicides applied in water or wax.

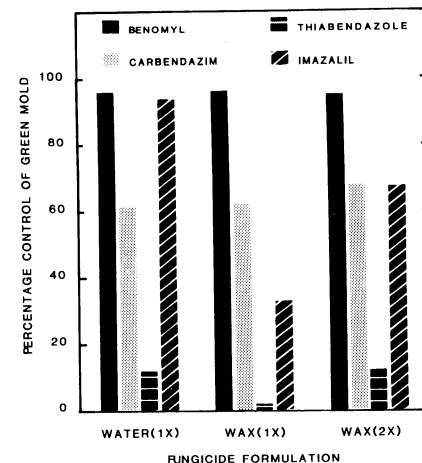


Fig. 3. Percentage control of green mold caused by *Penicillium digitatum* from infection through injuries formed after fungicide treatment.

MBC, and least with thiabendazole. Control of green mold with the water formulation of imazalil was comparable to the control achieved with benomyl;

however, treatments of imazalil in wax were less effective than water treatments. By using the 2× wax rate, control of green mold with imazalil was increased from 33 to 67%, but it still was not as good as the 96% control obtained with the aqueous application.

Differences were observed in the distribution of the fungicides in the 2 mm of tissue removed from the outer portion of the rind of grapefruit. Of the benzimidazole fungicides, benomyl penetrated 1 mm, MBC penetrated to a depth of 250 μm , and thiabendazole did not penetrate the rind. Similar movement of the benzimidazole fungicides into the rind occurred from applications in water or wax. Imazalil showed the best movement of all the fungicides. It was recovered from all eight sections of exocarp (2 mm) when applied in water or buffer at pH 6.8 or 9.5. When applied in water at pH 6.2, 14.6 μg of imazalil was recovered from the 24 sections of rind from three fruit; however, 15.5 μg was recovered at pH 6.8 and 11.1 μg at pH 9.5, representing a reduction in uptake of 28% at the higher pH. When applied in wax, imazalil was recovered only from the first two sections (500 μm) of exocarp. All fungicides prepared in water or wax and applied to injured surfaces were recovered from the eight sections taken from the 2 mm of rind.

Migration of fungicides through water wax was studied by germinating spores of *P. digitatum* on deposits of the wax. Water and nutrients necessary for spore germination readily diffused from the agar through dialyzer tubing into the wax. *P. digitatum* did not germinate when potato-dextrose agar was replaced with nonnutritive water agar. In the presence of fungicides, germination of spores was better on the tubing surface than on the agar because of some dilution of the fungicide by water that accompanied the tubing when it was transferred to the agar surface. In any event, germ tubes of *P. digitatum* were retarded and distorted by the benzimidazole fungicides at 0.1 $\mu\text{g}/\text{ml}$ and growth was inhibited over the range of 0.3–1 $\mu\text{g}/\text{ml}$, whether spores were deposited on the agar or on the wax (Fig. 4A). With imazalil, germination of spores on the agar was inhibited at a concentration of 0.3 $\mu\text{g}/\text{ml}$. On wax surfaces, germination and growth of *P. digitatum* occurred even at concentrations up to 1 $\mu\text{g}/\text{ml}$ of imazalil (Fig. 4B,C).

DISCUSSION

Because waxes are more viscous than water (16), residues of fungicides in wax may not be deposited in certain infection courts, such as in tiny rind injuries or beneath the sepals of the button (calyx and disk) attached to the fruit. Less control of green mold developing from infections through minute punctures was evident with wax treatments. Wax applications delayed by the degreening

process, which stimulates growth of *D. natalensis* from the button into the stem end of the fruit (3), were also less effective than comparable aqueous fungicide treatments for control of SER.

Another factor influencing potential efficacy is encapsulation or binding of the fungicide by the wax. This did not appear to be important with the benzimidazole fungicides; they retained their individual systemic properties whether applied in water or wax. In fact, these materials are more soluble in wax than water, a factor that might improve movement through the cuticle (21). In contrast, movement of imazalil was impaired by the resin solution wax. These waxes contain several alkali-soluble or resinlike materials that could bind the imazalil molecule (12). The alkali may partition more imazalil into the wax and leave less material in the aqueous phase to penetrate the fruit rind (20). Because alkaline aqueous solutions reduced rind residues, the high pH may have reduced the solubility of imazalil in epicuticular waxes and the cuticle (20). The fact that all fungicides in water or wax penetrated injured tissue, except in minute punctures, indicates the major role that the epicuticular waxes and the cuticle play in preventing penetration of postharvest fungicides into citrus rind. Less control with thiabendazole of green mold developing from punctures and from injuries formed after fungicide treatment may be attributed to the lack of systemic movement of this material on citrus (9,13,15,23) compared with benomyl (5,9,19,23), MBC (5,19), and imazalil (2,14).

Fungicides at twice the rate in wax were usually more effective than the single rate. Fungicide treatments in wax at double the aqueous rate do not require twice the amount of fungicide to treat a comparable quantity of fruit (12). Less wax than water is lost from the brushes during application; therefore, more fruit can be treated with wax than with a comparable amount of water. For this reason and for the increased efficacy, the higher rate of fungicide should be used in wax applications.

Although some loss in decay control efficacy was observed with the benzimidazole wax combinations, there are certain instances where such applications may be more suitable than separate fungicide and wax treatments. The single fungicide-wax operation is obviously less expensive and more simple than separate treatments. Also, some packinghouses follow aqueous fungicide treatments with polisher-drier brushes before waxing to enhance shine, and this removes much of the fungicide residue on the fruit surface (4). Higher residues and better decay control would be expected by applying the fungicide in the wax in such situations. Efficacy of imazalil in wax, however, was more seriously

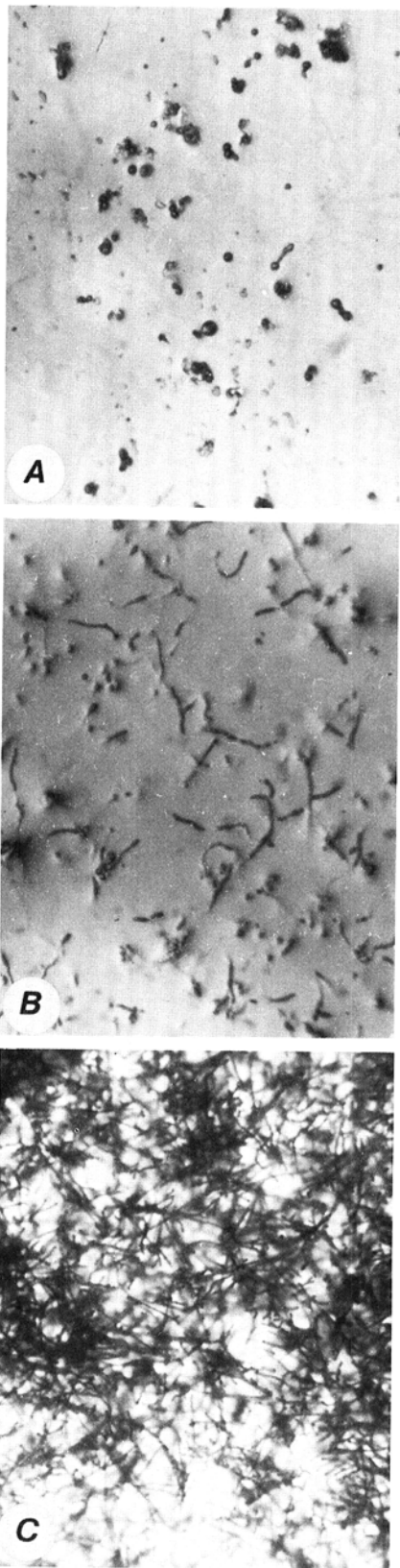


Fig. 4. Growth of *Penicillium digitatum* on wax ($\times 150$). (A) Spores inhibited by 1 $\mu\text{g}/\text{ml}$ of benomyl after 48 hr. (B) Spores germinating in the presence of 0.5 $\mu\text{g}/\text{ml}$ of imazalil after 16 hr. (C) Hyphae formed in the presence of 1 $\mu\text{g}/\text{ml}$ of imazalil after 48 hr.

impaired than the benzimidazoles, particularly in applications to degreened fruit. The benefits of a single treatment process would not appear to outweigh the loss in decay control activity. For that reason, imazalil should be applied in water for control of SER and green mold, unless other wax formulations (12) are found not to interfere with imazalil activity.

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