

Table 1. Effect of hot water and gamma radiation, alone or combined, on the development of green mold of grapefruit when applied 2, 24, 48, or 72 hr after inoculation.^z

Treatment	Percentage fruit with green mold rot ^y			
	2 hr	24 hr	48 hr	72 hr
Water (24-27°C; 5 min)	71.7	—	—	—
Hot Water (50°C; 5 min)	30.0*	16.7**	13.3**	35.0*
Radiation (250 Gy)	15.0**	56.7	51.7	51.7
Hot Water + Radiation	13.3**	10.0**	6.7**	28.3*

^zEach figure represents the mean of 3 replicates or a total of 60 fruit. Decay was determined after holding the fruit at 24°C for 9 days after inoculation.

^yMeans followed by a single or double asterisk are significantly different from the unheated water control at the 5% or 1% level, respectively, as determined by Dunnett's two-sided test (4).

of these tests. The reported synergism (2) was minimal at 250 Gy and was based on research (8) that showed an increase in the radiosensitivity of fungi as a result of the combination of heat with irradiation.

Nonrotted fruit from the various treatments showed no visual signs of internal or external injury and no off-

flavors. Additional work is needed to determine the possible effects of wrapping and prolonged storage on the effectiveness of hot water and radiation for decay control.

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EFFECTIVENESS OF POSTHARVEST FUNGICIDES FOR THE CONTROL OF CITRUS FRUIT DECAYS

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Abstract. Thiabendazole and benomyl were the most effective fungicides for the control of *Diplodia natalensis* Pole Evans stem-end rot, a major decay of early degreened fruit. This decay was also significantly reduced by imazalil and guazatine, but the level of control was not as consistent as that obtained with thiabendazole or benomyl. Control of *Diplodia* stem-end rot was not obtained with DF-100 or potassium sorbate. Sour rot, caused by *Geotrichum candidum* Lk. ex Pers., was controlled with guazatine and etaconazole but not with DF-100, Purogene, fenpropimorph or potassium sorbate. Green mold, caused by *Penicillium digitatum* Sacc., was effectively controlled with imazalil, guazatine and thiabendazole. However, efficacy of thiabendazole was greatly reduced in the presence of strains of *P. digitatum* with resistance to the fungicide. Potassium sorbate and DF-100 significantly reduced green mold only in some tests, but even then they were not as effective as imazalil or guazatine. Purogene did not control green mold and at high rates it significantly increased the decay. Of the fungicides tested, benomyl, thiabendazole, imazalil, and potassium sorbate are presently labeled and approved for application to citrus fruits to control postharvest decays.

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Postharvest decays frequently cause extensive losses of Florida citrus fruit before or after it reaches the consumer. A significant proportion of this loss can be prevented by applying effective fungicides to the fruit soon after harvest. A program to evaluate, identify, and aid in the registration of effective fungicides for decay control in citrus has been pursued at the Lake Alfred Center for many years. Efforts to develop new fungicides are necessary for several reasons. Improvements in efficacy, safety and expense may be realized. Use of registered fungicides can be discontinued at any time due to unforeseen health or environmental hazards. Furthermore, some pathogens, particularly *Penicillium*, can develop resistance to fungicides causing them to be ineffective.

The purpose of this report is to describe recent studies where the effectiveness of certain fungicides was evaluated for the control of 3 major decays of Florida citrus fruit, namely *Diplodia* stem-end rot, sour rot, and green mold.

Materials and Methods

In vitro evaluation of the fungicides was performed by incorporating the materials into sterilized Difco potato dextrose agar before pouring the media into petri dishes. After the media solidified, the plates were inoculated with the various fungi as described previously (10). Inhibition of growth was measured for each fungus at specific times after inoculation when substantial growth had occurred on the control plate.

The materials for the tests described in this study were obtained from the following sources: DuPont and Co.—benomyl (Benlate, 50% w.p.); FMC Corporation—thiabendazole (TBZ, 5% e.c.), Janssen Pharmaceutica—imazalil (68% e.c.); Ciba Geigy Co.—etaconazole (CGA 64251, 13.5% e.c.); Kenogard—guazatine (Panocline, 40% e.c.); Maag Agrochemicals—fenpropimorph (R014-3169, 25% e.c.); Sigma Chemical Co.—sorbic acid, potassium salt; Chemie Research and Manufacturing Co., Inc.—DF-100 (50% e.c.); and Bio Vet Corporation—Purogene (2% aqueous stabilized chlorine dioxide). Available chlorine dioxide was prepared by reacting sodium chlorite with muriatic acid (5).

Stem-end rot and green mold developed from natural infections. The incidence of green mold in these tests was high because the Valencia fruit suffered freeze injury and the spore population of *Penicillium digitatum* produced on damaged, dropped and infected fruit was quite high in the grove. Fruit were inoculated with *Geotrichum candidum* to induce sour rot. Spores of this organism were adjusted to a concentration of 10^6 in 10 µg/ml of cycloheximide (6) and 5 µl of the suspension were injected to a depth of 3 mm into the rind at one site on the equator of each fruit.

Fungicide treatments were applied to oranges (*Citrus sinensis* (L.) Osb. cv. Hamlin and Valencia). The Hamlin oranges were degreened with 5-10 ppm ethylene at 84-86°F with 92-94% relative humidity for 50 to 72 hours. Fruit were washed, graded, and randomized into the various replicated lots. The fungicides were applied at specific rates in a nonrecovery spray to fruit rotating on horsehair brushes saturated with the fungicide. The fruit were dried at 120°F, waxed with a solvent wax, and packed in 2/5 bushel cartons. The Fruit were stored near 70°F at relative humidities of 85-90% for 1-3 weeks.

Results and Discussion

In vitro tests. Several of the fungicides were quite inhibitory to growth of some pathogens at a low concentration of 1 ppm (Table 1). Benomyl, thiabendazole, and imazalil, which are presently registered for postharvest treatment of fruit, exhibited superior activity against *Penicillium* (green mold) and *Diplodia* and *Phomopsis* (stem-end rot). Benomyl and thiabendazole were also quite effective in-reducing the growth of *Colletotrichum*, which causes anthrac-

nose in some cultivars. The experimental materials fenpropimorph, guazatine, and etaconazole effectively inhibited growth of *P. digitatum* at a concentration of 1 ppm, but except for fenpropimorph, they were less effective against *Diplodia* and *Phomopsis*. Guazatine and etaconazole were the only materials that significantly inhibited growth of *Geotrichum candidum* (sour rot).

Potassium sorbate, Purogene and DF-100 were much less inhibitory at 1 ppm than the other fungicides. However, use of higher concentrations of these less fungitoxic materials might improve their fungicidal effectiveness. *Phytophthora citrophthora* Sm. and Sm., the brown rot organism, was not inhibited by any of the fungicides applied at 1 ppm. The growth of *Alternaria citri* Ellis and Pierce (black rot) was inhibited best by fenpropimorph. Some of the materials showed no activity against this fungus.

Control of Diplodia stem-end rot. Benomyl and thiabendazole effectively and consistently controlled this organism in degreened oranges (Table 2). Significant control also was usually obtained with guazatine and imazalil, but the degree of control was not as good as that obtained with the benzimidazole fungicides. Effective control of *Diplodia* stem-end rot was not obtained with potassium sorbate, though it has controlled stem-end rot caused by *Phomopsis* in other studies (15). Low levels of DF-100 appeared to provide some control of stem-end rot in Test 1 but not in Test 3. The higher concentrations of DF-100 evaluated in Test 2, however, did not significantly reduce decay. There was some indication that propylene glycol, which comprises 25% of the DF-100 product, may possess some activity against *Diplodia*.

In previous studies (4), the effectiveness of guazatine and benomyl for the control of *Diplodia* was improved when the materials were applied before, rather than after, degreening. Similar improvement could be expected with the other materials used in these tests. Under commercial conditions, predegreening treatments are most easily applied in a drench application.

Control of sour rot. Sour rot was controlled with treatments of guazatine and etaconazole (Table 3). These experimental fungicides have proven effective in other studies (1, 2, 7, 13, 14). Guazatine has been used in Australia for several years, and is much more effective for sour rot control than sodium o-phenylphenate (14). However, availability of guazatine to Florida packers for decay

Table 1. Inhibition of different fungal pathogens on potato dextrose agar containing 1 µg/ml of certain fungicides.

Fungicide	Percent inhibition radial growth							
	<i>Penicillium digitatum</i> ^a	<i>Penicillium italicum</i>	<i>Phomopsis citri</i>	<i>Alternaria citri</i>	<i>Diplodia natalensis</i>	<i>Phytophthora citrophthora</i>	<i>Geotrichum candidum</i>	<i>Colletotrichum gloeosporioides</i>
Benomyl	100	100	100	0	100	0	0	100
Thiabendazole	100	100	100	0	70	0	0	100
Fenpropimorph	100	100	100	83	73	0	28	35
Imazalil	100	100	47	59	18	0	6	52
Guazatine	100	39	76	49	19	0	88	34
Etaconazole	100	100	63	59	70	0	92	66
Potassium sorbate	0	0	0	0	13	0	0	0
Purogene	0	0	0	0	0	0	9	0
DF-100	0	11	0	6	9	0	5	0

^aBenzimidazole sensitive strain.

Table 2. Comparison of fungicides applied to degreened Hamlin oranges for the control of stem-end rot caused by *Diplodia natalensis*.

Treatment	Rate (µg/ml)	Percent stem-end rot ^w		
		Test 1 ^z	Test 2 ^y	Test 3 ^x
Untreated	—	40.7 a ^v	60.5 a	22.0 a
DF-100	30	30.7 b	—	20.5 a
	60	30.0 b	—	20.5 a
	200	—	55.0 a	—
	2,000	—	55.5 a	—
Propylene glycol	150	—	—	21.5 a
	300	32.0 ab	—	—
	5,000	—	43.0 b	—
Potassium sorbate	20,000	—	—	17.0 a
Thiabendazole	1,000	3.3 d	11.0 d	7.0 b
Benomyl	600	3.3 d	7.0 d	0.0 b
Imazalil	1,000	11.3 cd	22.5 c	15.5 a
Guazatine	1,000	19.3 c	36.0 b	—

^xFruit were degreened 72 hours with ethylene before treatments were applied to 3 replicated lots each containing 50 fruit.

^yFruit were degreened 70 hours with ethylene before treatments were applied to 5 replicated lots each containing 50 fruit.

^zFruit were degreened 50 hours with ethylene before treatments were applied to 5 replicated lots each containing 40 fruit.

^wPercentage stem-end rot after 3 weeks of storage at 70°F.

^vMean separation in columns by Duncan's multiple range test, 5% level.

Table 3. Comparison of fungicides applied to inoculated Valencia oranges for the control of sour rot caused by *Geotrichum candidum*.

Treatment ^z	Rate (µg/ml)	Percent sour rot ^y	
		Test 1	Test 2
Untreated	—	75.0 a ^x	82.5 a
DF-100	2,000	76.7 a	—
	5,000	—	84.0 a
Purogene	1,000	—	80.5 a
Potassium sorbate	20,000	72.5 a	—
Fenpropimorph	1,000	—	76.5 a
Guazatine	1,000	46.7 b	37.0 b
Etaconazole	1,000	24.2 c	23.5 c

^xThe treatments were applied 4 hours after inoculation to 4 replicated lots each containing 30 fruit in Test 1 and 5 replicated lots each with 40 fruit in Test 2.

^yPercentage sour rot after 1 week (Test 1) and 6 days (Test 2) of storage at 70 F.

^zMean separation in columns by Duncan's multiple range test, 5% level.

control is still dependent upon additional toxicological data before the material can be registered. Thus, the industry must still rely upon sodium o-phenylphenate for sour rot control. Development of etaconazole for postharvest citrus applications is not being pursued at this time. Fenpropimorph, potassium sorbate, DF-100, and Purogene did not control sour rot. A recent study reported efficacy of potassium sorbate on sour rot (9); however, control has not been observed in studies at Lake Alfred.

Control of green mold. This decay was controlled most effectively in these tests by treatments of imazalil and guazatine (Table 4, 5). Thiabendazole was less effective in these tests than is usual in commercial packinghouses because of the presence in the storage room of inoculum, which was resistant to thiabendazole. Resistance of *P. digitatum* to the benzimidazole fungicides can reduce the effectiveness of these materials (3, 8, 11, 12, 16, 17). Imazalil and guazatine will control benzimidazole-resistant strains

Table 4. Comparison of fungicides applied to Valencia oranges for the control of green mold caused by *Penicillium digitatum*.

Treatment ^z	Rate (µg/ml)	Percent green mold ^y		
		Test 1		Test 2
		7 days	13 days	14 days
Untreated	—	21.0 cd ^w	41.0 cd	17.0 bcd
DF-100	2,000	15.0 de	38.5 de	13.0 cd
	5,000	12.5 de	28.0 e	8.5 de
Propylene glycol	2,500	—	—	19.0 bc
Glycerine	2,500	—	—	21.5 bc
Purogene	50	29.0 c	52.5 c	—
	500	51.5 b	69.5 b	—
	1,000	64.5 a	81.5 a	35.5 a
Chlorine dioxide	50	—	—	26.0 b
	100	—	—	24.0 b
	200	—	—	24.5 b
Potassium sorbate	20,000	12.5 de	43.0 cd	—
Thiabendazole ^x	1,000	9.0 ef	37.0 de	—
Imazalil	1,000	1.5 f	10.0 f	1.0 e
Guazatine	1,000	1.0 f	2.0 f	—

^zThe treatments were applied to 5 replicated lots each containing 40 fruit.

^yPercentage green mold after 7 and 13 days (Test 1) and 14 days (Test 2) of storage at 70 F.

^xStorage room contained spores of *Penicillium digitatum* with resistance to thiabendazole.

^wMean separation in columns by Duncan's multiple range test, 5% level.

Table 5. Comparison of fungicides applied to Valencia oranges for the control of mold caused by *Penicillium digitatum*.

Treatment ^z	Rate (µg/ml)	Percent green mold ^y			
		Test 1		Test 2	
		14 days	21 days	14 days	21 days
Untreated	—	21.9 a ^w	40.9 a	22.8 a	35.9 a
DF-100	5,000	14.7 b	37.5 a	10.6 b	18.4 c
Potassium sorbate	20,000	—	—	11.9 b	23.1 bc
Imazalil	1,000	0.6 c	4.1 c	1.9 c	3.4 d
Thiabendazole ^x	1,000	4.4 c	21.9 b	15.6 b	25.6 b
Guazatine	1,000	0.9 c	2.5 c	—	—

^zThe treatments were applied to 8 replicated lots each containing 40 fruit.

^yPercentage green mold after storage for 14 days and 21 days at 70 F.

^xStorage room contained spores of *Penicillium digitatum* with resistance to thiabendazole.

^wMean separation in columns by Duncan's multiple range test, 5% level.

of the fungus (3, 8, 16). Resistance of *P. digitatum* to imazalil under commercial conditions has not been reported, but resistance has developed to guazatine (17).

Potassium sorbate exhibited activity against green mold in some of the tests and has been effective in previous studies (15) and in the presence of benzimidazole-resistant inoculum (12). However, it is not as effective as imazalil or guazatine, or thiabendazole or benomyl in the absence of resistance to the latter materials.

Purogene is an aqueous solution of stabilized chlorine dioxide. The material is produced spontaneously when the solution is diluted and applied to materials which exhibit a chlorine dioxide demand. In these studies, Purogene did not control green mold at concentrations of 50, 500, or 1000 µg/ml (Table 4). In fact, for some unexplained reason concentrations of 500 and 1000 µg/ml significantly increased the incidence of mold. Treatments consisting of only the unstabilized chlorine dioxide at rates of 50, 100,

or 200 µg/ml did not significantly reduce the incidence of green mold.

Propylene glycol and glycerine, components of the DF-100 formulation, did not control green mold. DF-100, at a concentration of 5000 µg/ml, provided inconsistent control of green mold. In one test (Table 5, Test 2) after 21 days of storage, it did provide better control of green mold than thiabendazole in the presence of fungal resistance. However, in other tests (Table 4, 5) it was no better than thiabendazole even in the presence of resistance. The use of higher concentrations of DF-100 may prove more effective. However, an improved formulation would be required to prevent excessive foaming of the material which occurred when it was applied at 5000 µg/ml with a non-recovery spray over horsehair brushes.

Summary

The industry presently has fungicides that are quite effective for the control of the major postharvest decays, stem-end rot and green mold. Benomyl and thiabendazole are quite effective against the 2 stem-end rot pathogens. Green mold is also effectively controlled except in instances where the fungus has become resistant to the fungicides. Fortunately, imazalil, sodium o-phenylphenate, diphenyl, or potassium sorbate can be used to control benzimidazole resistant strains of the green mold organism. Of these 4 fungicides, imazalil is by far the most effective alternative to benomyl and thiabendazole where resistance is a problem, but its use is prohibited on fruit exported to Japan because the material is not approved for use on citrus fruit imported by that country. Sour rot, the other major decay of Florida citrus, is not effectively controlled by the approved postharvest fungicides. Sodium o-phenylphenate has some activity, but it has proven inadequate under high inoculum pressure. Registration of guazatine would provide the industry with an effective material for controlling sour rot. Use of the other fungicides evaluated in these tests for decay control is limited because of ineffectiveness or prevented at this time because of lack of registration.

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A QUANTITATIVE DETECTION METHOD FOR *PENICILLIUM DIGITATUM* AND *P. ITALICUM* IN CITRUS PACKINGHOUSES

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Abstract. Resistance of *Penicillium digitatum* Sacc. and *P. italicum* Wehmer to the benzimidazole fungicides, thiabendazole (TBZ) and benomyl has led to economic losses in the California and Florida fresh citrus industry. The standard air sampling technique of exposing benzimidazole-amended and nonamended potato dextrose agar petri plates for between 1 to 3 minutes is useful in determining spore presence and the level of resistance in lemon packinghouses. Because orange and grapefruit packinghouses generally have fewer *Penicil-*

lium spores present, the standard assay method which is qualitative may not accurately indicate a potential decay problem. A quantitative air sampling method was developed that has a high collection efficiency and high sampling volume. Sporeload can now be recorded as number of blue-greens per standard cubic foot of air.

In 1980, the contributing editor of Florida Grower & Rancher stated, "getting produce to the customer is as important as producing it, since that's the operation that brings returns to the grower" (3). Decayed citrus fruit results in market losses and many of these decays occur after harvest. Prevention and/or the reduction of postharvest