Efficacy of Guazatine and Iminoctadine for Control of Postharvest Decays of Oranges

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ABSTRACT

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Guazatine is a random mixture of guanidated 1,8-diaminooctane and its higher oligomers, including dioctamethylenetriamine and trioctamethylenetetramine, while iminoctadine consists only of dioctamethylenetriamine. Iminoctadine was equal to or significantly better than guazatine in numerous tests to evaluate control of stem end rot caused by *Diplodia natalensis* and *Phomopsis citri*, green mold caused by *Penicillium digitatum*, and sour rot caused by *Geotrichum candidum*. Iminoctadine, unlike guazatine, controlled sporulation of *P. digitatum*, and spread of *G. candidum* during storage. All citrus decay pathogens, when tested in vitro, were more sensitive to iminoctadine than to guazatine. In comparisons with other fungicides, these two fungicides were less effective than benomyl for control of stem end rot, but were comparable to etaconazole for sour rot control and to imazalil and thiabendazole for control of green mold. Iminoctadine was comparable to imazalil for controlling soilage caused by *P. digitatum*.

Additional keywords: *Citrus sinensis*, fungicides, postharvest pathology

Guazatine (Panoctine, Kenopel) is a broad-spectrum, water-soluble fungicide that has been used commercially in postharvest applications to citrus fruits in Australia for several years (21). A residue tolerance of 5 μg/g has been established in Australia, Sweden, and the Federal Republic of Germany. Iminoctadine (Befran, DF-125) is applied in Japan to citrus trees before harvest to control decays that develop during storage (16). The two fungicides are chemically similar, except guazatine is a random mixture of oligomers of guanidated 1,8-diaminooctane and iminoctadine is entirely 1,1-iminiodi(octamethylene)-diguanidinium triacetate, one of the oligomers in the guazatine mixture. Efforts have been initiated to register guazatine for postharvest use on citrus fruit in the U.S.

Much of the previously published work has been conducted with guazatine (3,4,6,10–12,18–21). Guazatine effectively eradicated incipient infections of green and blue mold, including infection caused by isolates resistant to the benzimidazole fungicides. Residues of the fungicide on treated fruit did not protect against subsequent infections at new inoculation sites nor did high treatment levels (2,000 mg/L) inhibit sporulation of *Penicillium* on diseased fruits (4,6,11,12,21). Guazatine is particularly effective against sour rot (3,19,20), which is not effectively controlled by any of the fungicides currently registered in the U.S. for postharvest use on citrus (3,4). There is only one report on the effectiveness of iminoctadine for controlling postharvest rots (16). Green and blue mold were effectively controlled by this material, but preharvest applications did not control stem end rot caused by *Phomopsis citri* Fawcett as effectively as benomyl or thiophanate-methyl.

This study was specifically conducted to compare decay control properties of guazatine and iminoctadine and to evaluate the role of formulation in decay control efficacy. In some trials, activity was compared with that of other experimental and registered citrus postharvest fungicides.

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MATERIALS AND METHODS

The effect of guazatine and iminoctadine on growth of the citrus decay pathogens in vitro was studied by adding the materials to Difco potato-dextrose or cornmeal agar after autoclaving, but before cooling and pouring the media in culture plates. Disks of inoculum from actively growing cultures of each of the decay organisms were placed on the test media at four equidistant locations at the edge of the culture plate. The plates were incubated at 25 C and growth of each colony was measured from the edge of the inoculum disk to the center of the plate just before the colonies on the control plate coalesced. The concentration that reduced growth by 50% (ED_{50}) was determined by the linear relationship between the probit of the percentage of growth reduction and the logarithm of the fungicide concentration (2). Best fit of the data was determined by linear regression (1).

Tests were conducted with oranges (*Citrus sinensis* (L.) Osbeck, 'Hamlin', 'Pineapple', or 'Valencia') obtained from groves of the Citrus Research and Education Center, Lake Alfred or from a nearby commercial packinghouse. In some tests, fruit were degreened with 6 μl of ethylene/L of air at 29–30 C and 92–96% relative humidity for 60–70 hr. After degreening, or after harvest if not degreened, fruit were washed, graded for minimal surface blemishes and uniform size and color, and distributed randomly into the necessary treatment lots required for the test.

Inoculations with *Geotrichum candidum* Link ex Pers. or *Penicillium digitatum* Sacc. to evaluate decay control were made by injecting 5 μl of spore suspension (10^6/ml) (2,17) into the rind to a depth of 3 mm at the fruit equator. Spores of *G. candidum* were suspended in an aqueous solution of cycloheximide (10 μg/ml) to enhance infectivity (7).

To evaluate spread of *G. candidum* and nesting of sour rot in packed cartons, 0.1–0.2 ml of spores (10^6/ml) suspended in benomyl (100 μg/ml) were injected into the stem ends and stylar ends of each fruit with a hypodermic syringe. Four inoculated fruit were placed in the center of the top layer of fruit in a packed carton

subsequently stored 4 wk at 27 C and 92–96% relative humidity. After storage, percentage spread was determined by counting the number of fruit infected with *G. candidum* that grew from the original four inoculated fruit. A similar inoculation procedure was used to infect fruit with *P. digitatum* to evaluate sporulation control and soilage, except that with this organism the spores were suspended only in water. The amount of sporulation of *P. digitatum* on infected fruit was rated on a scale of 0–5, where 0 represented negligible sporulation and 5 represented dense sporulation over the entire surface of the fruit (7). Soilage was evaluated by randomly placing two inoculated fruit in each of four layers of fruit in a packed carton. After storage, the carton was dropped from a height of 84 cm to disperse the spores within the carton. Fruit were identified as soiled if the shine from the wax application was dulled by deposits of spores on the fruit surface. Part of the fruit surface was wiped with cheesecloth to provide contrast and a judgement on the effect of the spores on shine.

Treatments were applied to fruit within 2 hr of inoculation. The experimental fungicide formulations were guazatine (Kenopel 40E), iminoctadine (Befran 25S), and etaconazole (CGA 64251 13.5E). Registered formulations were benomyl (Benlate 50W), imazalil (Fungaflor 68E), thiabendazole (Freshgard 555 5E), and diphenyl. All fungicides, except diphenyl, were suspended in water and applied as a nonrecovery spray over rotating brushes (5). Fruit were then dried at 43–45 C and waxed with solvent wax, packed in 4/5 bushel fiberboard cartons, and placed in storage. Diphenyl was added during packing by placing fungicide-impregnated pads between the first and second and the third and fourth layers of fruit in the carton. Cartons with diphenyl were stored in a separate room to prevent the volatile fungicide from interfering with responses to the other treatments.

RESULTS

In vitro, all of the citrus decay pathogens were more sensitive to iminoctadine than to guazatine (Table 1). Response of benzimidazole-tolerant isolates of *P. digitatum* to guazatine or iminoctadine was similar to that of the wild type.

Comparative efficacy of guazatine and iminoctadine with other fungicides for controlling stem end rot in degreened and nondegreened oranges is shown in Table 2. Where degreened fruit were used, iminoctadine was more effective than guazatine in one test and equally effective in the other. In both tests with nondegreened fruit, iminoctadine-treated fruit had significantly less stem end rot than fruit treated with guazatine. Etaconazole was significantly less effective than iminoctadine in one of two tests in which it was included, but benomyl was more effective than either of these materials or guazatine in all tests.

Control of sour rot with various fungicides was compared in three separate tests using artificially inoculated fruit. Iminoctadine was more effective than guazatine in two of three tests, and more effective than etaconazole in one test (Table 2). Percentage spread of sour rot from infected to healthy fruit in packed cartons was significantly reduced from 4.8% in the control to 1.7% in fruit treated with guazatine at a rate of 2,000 μg/ml. Treatment with 1,000 μg/ml reduced spread to 3.0%, a value not significantly different from the control. Iminoctadine applied at 1,000 and 2,000 μg/ml reduced spread to 0.9 and 0.4%, respectively. This reduction in spread of sour rot was significantly different from the control and from comparable rates of guazatine. Iminoctadine was more effective than guazatine on fruit artificially infected with *P. digitatum*, but not on naturally infected fruit (Table 3). Control with iminoctadine was comparable or better than that obtained with either imazalil or thiabendazole on inoculated fruit. Iminoctadine inhibited sporulation of *P. digitatum* better than imazalil (Table 3). Sporulation was not affected by guazatine, and only slightly by thiabendazole. Control of sporulation significantly affected the extent of soilage in packed cartons of fruit (Table 4). In these tests, iminoctadine and imazalil were equally effective in preventing soilage, and they were more effective than two pads of diphenyl. Soilage was not reduced where only one diphenyl pad was used.

DISCUSSION

Control of decay with guazatine and iminoctadine was comparable to or better than the control obtained with the

Table 1. Concentrations of guazatine and iminoctadine that caused a 50% reduction in growth (ED_{50}) of citrus decay pathogens in vitro

Organism	ED_{50} (μg/ml)		Activity index[v]
	Guazatine	Iminoctadine	
Alternaria citri	1.320	0.061	22
Colletotrichum gloeosporioides	4.384	0.036	122
Diplodia natalensis	14.000	0.118	119
Geotrichum candidum	0.199	0.009	22
Penicillium digitatum[w]	0.020	0.001	20
P. digitatum[x]	0.068	0.001	68
P. digitatum[y]	0.034	0.003	11
P. digitatum[z]	0.016	0.004	4
P. italicum	8.100	0.255	32
Phomopsis citri	1.144	0.005	229
Phytophthora citrophthora	5.158	0.134	38
Phytophthora parasitica	4.628	0.829	6

[v] Activity index = ED_{50} guazatine/ED_{50} iminoctadine.
[w] Florida isolate sensitive to benzimidazole fungicides.
[x] Florida isolate tolerant to benomyl (2 μg/ml).
[y] California isolate tolerant to carbendazim (ED_{50} 36 μg/ml).
[z] California isolate tolerant to carbendazim (ED_{50} 1 μg/ml).

Table 2. Comparative efficacy of fungicides for the control of stem end rot and sour rot in oranges

Treatment	Rate (μg/ml)	Percent decay						
		Stem end rot[w]				Sour rot[x]		
		Test 1	Test 2	Test 3	Test 4	Test 1	Test 2	Test 3
Untreated	...	48.1 a[y]	26.9 a	32.4 a	11.6 a	77.5 a	24.2 a	26.9 a
Guazatine	1,000	40.5 ab	18.4 b	22.4 ab	13.8 a	49.5 b	12.1 b	14.7 b
Iminoctadine	1,000	14.3 c	15.7 b	9.0 c	3.4 b	5.0 d	8.9 b	4.9 c
Etaconazole	1,000	29.1 b	...[z]	14.8 bc	...	32.0 c	8.2 b	7.6 c
Benomyl	600	4.3 d	4.0 c	1.0 d	...	...	...	...

[w] Decay after storage for 3 wk (tests 1,2,3) at 21 C and 80–85% relative humidity, and after 2 wk (test 4) at 30 C and 92% relative humidity. Stem end rot was caused primarily by *Diplodia natalensis* except in test 3 where approximately 25% of it was caused by *Phomopsis citri*. Treatments were applied to 3 replications of 70 Pineapple oranges after degreening 70 hr (test 1), 9 replicates of 100 Valencia oranges after degreening 60 hr (test 2), 3 replicates of 70 Pineapple oranges (test 3), and 3 replicates of 80 Valencia oranges (test 4).
[x] Decay 1 wk after inoculation and storage at 21 C and 92–96% relative humidity. Treatments were applied to 4 replicates of 50 Hamlin oranges (test 1), 9 replicates of 45 Pineapple oranges (test 2), and 6 replicates of 75 Pineapple oranges (test 3).
[y] Mean separation in columns by Duncan's multiple range test, 5% level.
[z] No data.

Table 3. Comparative efficacy of fungicides applied to Valencia oranges for the control of green mold and inhibition of sporulation of *Penicillium digitatum*

Treatment	Rate (μg/ml)	Percent green mold[w]			Sporulation index[x]
		Naturally infected	Inoculated		
		Test 1	Test 2	Test 3	
Untreated	···	9.1 a[y]	75.0 a	56.7 a	5.0 a
Guazatine	1,000	0.8 b	48.0 b	43.8 b	4.9 ab
Iminoctadine	1,000	0.0 b	29.7 c	24.0 c	0.2 d
Imazalil	1,000	···[z]	41.7 b	29.9 c	2.3 c
Thiabendazole	1,000	···	46.0 b	40.7 b	4.7 b

[w] Percent green mold after 3 wk storage at 21 C and 92–96% relative humidity (test 1), and after storage at 24 C and 92–96% relative humidity for 1 wk (tests 2, 3). Treatments were applied to 3 replicates of 80 fruit (test 1), 3 replicates of 50 fruit (test 2), and 6 replicates of 50 fruit (test 3).
[x] Treatments were applied to 3 replicates of 10 fruit. Inoculated and treated fruit were held at 24 C and 92–96% relative humidity and evaluated for sporulation after 8 days by assigning each fruit a rating of 0 (negligible sporulation) to 5 (heavy sporulation).
[y] Mean separation in columns by Duncan's multiple range test, 5% level.
[z] No data.

Table 4. Efficacy of fungicides for reducing soilage of healthy Valencia oranges by spores of *Penicillium digitatum* dislodged from infected fruit in the same carton

Treatment	Rate (μg/ml)	Percent soilage[w]	
		Test 1	Test 2
Untreated	···	86.6 a[x]	89.8 a
Imazalil	1,000	···[y]	31.5 c
Imazalil	2,000	···	6.9 d
Iminoctadine	1,000	2.1 b	30.1 c
Iminoctadine	2,000	2.1 b	4.6 d
Diphenyl	1 pad[z]	···	87.5 a
Diphenyl	2 pads[z]	···	70.0 b

[w] Percentage of the 72 healthy fruit in a packed carton that were soiled by spores of *Penicillium digitatum* produced on 8 inoculated and infected fruit randomly placed within the same carton. Cartons were dropped a distance of 84 cm to disperse the spores within the carton after storage at 24 C and 92–96% relative humidity for 9 days. Each treatment was applied to 3 replicated cartons.
[x] Mean separation in columns by Duncan's multiple range test, 5% level.
[y] No data.
[z] Each pad contained 2.35 grams of diphenyl.

other experimental and registered fungicides tested, except that benomyl was more effective than either guazatine or iminoctadine for controlling stem end rot. Even though iminoctadine is a component of the guazatine mixture, ED_{50} values were consistently less than those of guazatine. Furthermore, iminoctadine often exhibited significantly better decay control than guazatine. The one oligomer selected for the iminoctadine formulation may be the most biologically active component of the mixture present in guazatine. The rate of this active component is probably too low in guazatine to allow control comparable to that achieved when using the active component alone as formulated in iminoctadine. Iminoctadine may be more active also because it is the smallest oligomer of the mixture. A smaller and less complex molecule of similar chemistry may be more easily absorbed by the fruit and/or the decay fungi and may be bound less tightly in a medium such as potato-dextrose agar (14,15), and thus exhibit higher activity in vitro. Better movement of iminoctadine than guazatine into the rind may more effectively suppress the growth and subsequent sporulation of *P. digitatum* on the fruit surface. A similar type of action has been reported for benomyl (9) and imazalil (5). Better control of the spread of *G. candidum* with iminoctadine could also be explained through systemic action.

Large deposits of fungicide on fruit surfaces can also retard surface mycelial growth and sporulation of *P. digitatum*. This occurs with benomyl or thiabendazole, a relatively nonsystemic fungicide on citrus (13), when applied to citrus fruit at high concentrations in resin solution or emulsion waxes (8). In the absence of systemicity, surface deposits of either iminoctadine or guazatine applied at equal concentrations presumably would be similar unless differences in the inert ingredients of the formulations had a significant effect on the deposits. If similar surface deposits exist, better sporulation control would be due to the greater toxicity of iminoctadine or due to differential sensitivity of the sporulation of *P. digitatum* to the two fungicides. Additional studies will be required to determine if differences in systemicity, mode of action, or toxicity account for the differences in decay control, sporulation, and spread of postharvest decays observed in these studies.

Decay and sporulation control properties of iminoctadine appear significantly better than those of guazatine. Registration of iminoctadine should be pursued even where guazatine is already registered.

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