Metalaxyl for Postharvest Control of Brown Rot of Citrus Fruit

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ABSTRACT

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Metalaxyl was effective in postharvest treatments of citrus fruit against *Phytophthora citrophthora*. It provided limited control of the fungus inside the infected peel and reduced fruit rot. When incorporated in the wax coating of the fruit, metalaxyl prevented the spread of *Phytophthora* mycelium to sound fruit and thereby eliminated contact infection, but it promoted development of *Penicillium digitatum* in infected fruits. A combination of metalaxyl and imazalil controlled both *P. citrophthora* and *Penicillium digitatum*.

The fungicides commonly used in citrus packinghouses against stem end rots and blue and green molds have no effect against brown rot fungus inside the fruit (2). Immersion of fruit in hot water provides limited postharvest control of the disease (3–5). *Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leonian is sensitive to many fungicides tested in vitro. However, coating infected fruit with wax that is chemically amended or with aqueous preparations does not prevent development of disease (2) or spread by contact with sound adjacent fruit (1–3).

Preliminary experiments in 1977 and 1978 showed that metalaxyl (Ridomil), a systemic fungicide recommended for the control of plant diseases caused by Oomycetes (8), was the first chemical compound incorporated in wax that was an effective in vivo treatment against *P. citrophthora*. The effects of metalaxyl as a postharvest fruit treatment against brown rot caused by *P. citrophthora* are summarized in this paper.

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MATERIALS AND METHODS

Fruit inoculation. The fruits used were oranges (*Citrus sinensis* (L.) Osbeck 'Shamouti' and 'Valencia' and *C. reticulata* Blanco × *C. sinensis* (L.) Osbeck 'Temple'), lemon (*C. limon* (L.) Burm.), and grapefruit (*C. paradisi* Macf. 'Marsh Seedless'). Unwounded fruits were inoculated by dipping them in a suspension of sporangia and zoospores of *P. citrophthora* in water, obtained by covering cultures of the fungus grown in petri dishes on potato-dextrose agar (PDA) with tap water for about 10 days at 25 C. Cultures were incubated 4–5 days until many sporangia, about 100 per microscope field (×10 objective) examined directly in the culture, had developed (6).

Treatments. Two days after incubation at 17 C, 25 inoculated fruits were hand-dipped in wax amended chemically. The wax formulations (water-based) containing 18% solid matter as used for coating export fruits were Tag (Makhtechim Chemical Works Ltd., Beersheva), Zivdar (Safe-Pack Products Ltd., Kfar Saba), and Britex (Brochar Chemicals Ltd., Petah-Tiqwa). In addition, a storage wax (Brochar Chemicals Ltd.) containing 1% solid matter for lemons in storage was used.

The fungicide metalaxyl at 100–2,000 μg a.i./ml was added to Tag, Zivdar, Britex, and the storage wax. Metalaxyl was incorporated with sodium ortho-phenylphenate (SOPP), 2-amino-butane (2-AB), 2-(4-thiazolyl)benzimidazole (TBZ), methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl), 1-[β-allyloxy-2,4-dichlorophenethyl] imidazole (imazalil), 2,4 dichlorophenoxy-acetic acid, and gibberellic acid.

Aqueous solutions of metalaxyl from 100 to 2,000 μg/ml alone or combined with 500 μg of TBZ, 500 μg of SOPP, and 100 μg of chlorine per milliliter were used before degreening to drench inoculated fruit.

Contact inoculation. For contact inoculation of *P. citrophthora* from infected to sound fruit in packed boxes in storage for 3 mo, three infected fruits were placed among 42 grapefruits, and six infected fruits were placed among 99 healthy oranges (standard packaging of 45 and 105 fruits, respectively) so that each healthy fruit was in contact with one or more rotten fruits.

Healthy fruits were disinfected by immersing them in a 0.5% SOPP solution at 36 C for 3 min and rinsing them with fresh tap water; fruits were then coated by dipping them in Britex wax containing TBZ, 4,000 μg/ml; imazalil, 2,000 μg/ml; metalaxyl, 1,000 or 2,000 μg/ml; and metalaxyl, 2,000 μg/ml, plus imazalil, 2,000 μg/ml.

Treated fruits were not wrapped. Spread of decay to sound fruit was recorded after 1, 2, and 3 mo of storage at 11 C.

Preharvest spray. Metalaxyl and Bordeaux mixture were compared as prophylactic treatments by spraying Temple oranges on the trees 9 days before harvest. The metalaxyl concentration was 100 g (25% a.i.) in 200 L of water per 1,000 m^2; and Bordeaux mixture with 1% copper sulfate was applied in 200 L of water per 1,000 m^2.

In all experiments, we recorded the number of fruit rotted by *P. citrophthora*, average number of brown rot spots per fruit, occurrence of mycelial growth on the surface of rotten fruit, and development of other fungi during storage.

Residue determination. Samples of five whole fruits treated with metalaxyl at 1,000 or 2,000 μg/ml in contact inoculation experiments were extracted with acetone and diluted with water, and the active ingredient was partitioned into dichloromethane. After column chromatographic clean-up on alumina, residues were determined by gas-liquid chromatography (Becker Model 419) with a nitrogen-selective thermionic detector (Model 713) and a glass column (3 ft × 2 mm, i.d.) packed with 3% Carbowax 20M on Chromosorb WHP, 80–100 mesh. Temperatures were column oven, 170 C; injector, 240 C; and detector, 250 C.

Samples of untreated fruits were treated with known quantities of metalaxyl ranging from 0.2 to 1 μg/ml and then analyzed. The percent recovered was determined on six replicates to be 93%, with an absolute standard deviation of 7%. The limit of detection of the method was 0.1 μg/ml.

Table 1. Effect of metalaxyl with other fungicides in Britex wax on brown rot spots and mycelial growth of *Phytophthora citrophthora* in dip-inoculated citrus fruit during storage

Britex wax containing[a] (μg/ml)	Metalaxyl (μg/ml)	Waxing 2 days after inoculation: Infected fruits (%)	Spots per fruit[b]	Mycelial growth (%)
Temple oranges stored 23 days at 17 C				
TBZ (4,000)	0	100	4.1	70
	100	100	4.1	100
	500	100	3.2	0
	1,000	80	3.1	0
Benomyl (2,000)	0	70	2.1	40
	100	70	3.0	10
	500	70	1.9	0
	1,000	60	1.4	0
TBZ (3,500)	0	100	4.7	100
+ SOPP (1.5%)	100	100	4.8	90
	500	100	3.4	0
	1,000	100	4.0	0
TBZ (3,000)	0	90	3.3	80
+ 2-AB (2%)	100	70	2.9	10
	500	60	1.4	0
	1,000	70	1.2	0
Imazalil	0	80	3.3	70
(2,000)	100	100	3.9	20
	500	90	3.8	10
	1,000	90	3.5	0
Light green lemons[c] stored 30 days at 14 C				
2-AB, 1.5%	0	100	1.9	100
	500	90	0.1	0
	1,000	60	0.2	0
	2,000	10	0.1	0
2-AB, 1.5%	0	100	1.2	100
+ 2,4-D (500)	500	70	0.6	0
	1,000	60	0.3	0
	2,000	30	0.2	0
2-AB, 1.5%	0	100	1.0	100
+ 2,4-D (500)	500	60	1.0	30
+ GA_3 (50)	1,000	60	1.0	0
	2,000	30	0.1	0
TBZ (3,500)	0	90	2.1	90
+ SOPP, 1.0%	500	80	1.0	10
+ 2,4-D (500)	1,000	60	0.4	0
+ GA_3 (100)	2,000	40	0.1	0
Yellow lemons[d] stored 30 days at 14 C				
TBZ (5,000)	0	100	7.5	100
	500	100	3.8	50
	1,000	100	3.8	20
	2,000	100	2.1	0
TBZ (5,000)	0	100	6.7	100
+ GA_3 (50)	500	100	3.6	0
	1,000	100	3.2	0
	2,000	100	3.6	0

[a] 2-AB = 2-amino-butane, 2,4-D = 2,4-dichlorophenoxyacetic acid, GA_3 = gibberellic acid, TBZ = 2-(4-thiazolyl) benzimidazole, SOPP = sodium orthophenylphenate.
[b] Average for 25 fruits per trial.
[c] Coated with 1% storage wax.
[d] Coated with 18% Britex wax as for export.

RESULTS

Fungus development and mycelial growth. The incidence of rotten fruit and the average number of brown spots per fruit were inversely proportional to the concentration of metalaxyl incorporated in Tag, Zivdar, Britex, and storage wax. At 1,000 μg a.i./ml, metalaxyl reduced brown rot development 20–50% in inoculated fruit and reduced the average number of brown spots per fruit to 1.1–3.5, compared with 100% infection and 3.1–6.4 spots (average) in control fruit. Metalaxyl at 1,000 μg/ml inhibited surface growth of mycelium on rotten fruit, even in fruit treated 2 days after inoculation and examined 20 days after storage at 17 C. Metalaxyl at 500 and 100 μg/ml in the waxes was less effective in controlling *P. citrophthora* in inoculated Shamouti oranges.

Metalaxyl incorporated with other chemical compounds used in citrus packinghouses showed a similar effect in controlling *P. citrophthora* (Table 1). Inoculated Temple oranges treated with

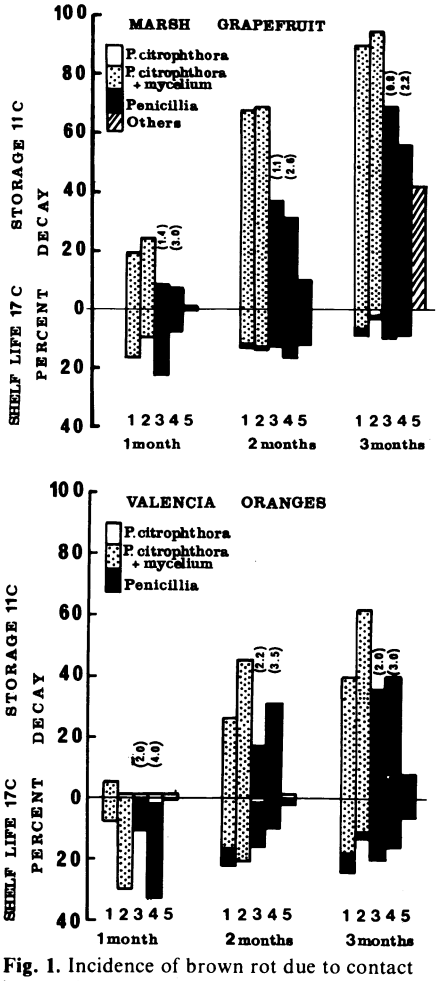

Fig. 1. Incidence of brown rot due to contact inoculation with *Phytophthora citrophthora* in grapefruit and oranges coated with Britex wax containing **(1)** 2-(4-thiazolyl) benzimidazole, 4,000 μg/ml; **(2)** imazalil, 2,000 μg/ml; **(3)** metalaxyl, 1,000 μg/ml; **(4)** metalaxyl, 2,000 μg/ml; **(5)** metalaxyl, 2,000 μg/ml, and imazalil, 2,000 μg/ml. Numbers in parentheses show residues of metalaxyl in milligrams per kilogram of fruit.

metalaxyl at 500–1,000 μg/ml were devoid of surface mycelial growth, but brown rot was only slightly reduced. Brown rot was reduced and mycelium was inhibited in mature, light green lemons treated with metalaxyl at 1,000–2,000 μg/ml and after 1-mo storage at 14 C (Table 1). Inoculated fruit treated with metalaxyl sustained a large number of infections by *Penicillium digitatum* (Pers. Sacc.). Inoculated fruits not treated with metalaxyl were covered with mycelia of *P. citrophthora* and later with *Penicillium italicum* Wehn. as a secondary infection.

Wax was not affected in pH, stickiness, and scaling, and coated fruits were not affected in shine or firmness after incorporation of metalaxyl alone or with other chemical compounds.

Aqueous drenches of metalaxyl with TBZ, SOPP, or chlorine were effective against brown rot (Table 2). The most effective drenches were metalaxyl alone at 500–1,000 μg/ml applied soon after inoculation of fruit.

Effect on contact infection. Fruit dipped in Britex wax containing metalaxyl at 1,000 or 2,000 μg/ml were free of brown rot (Fig. 1). When brown-rotted fruits were used as a source of contact infection, metalaxyl prevented development of aerial mycelium and thereby protected the sound adjacent fruit from possible infection.

Penicillium digitatum and *Penicillium italicum* mold decay developed on many metalaxyl-treated fruits, however, during storage and shelf life. Packed fruit treated with TBZ or imazalil, which do not control *P. citrophthora*, were rotted by *P. citrophthora* only and covered with its mycelium. A combination of metalaxyl and imazalil in the wax coating controlled *P. citrophthora*, and only a small amount of the fruit was decayed by Penicillia, as in Valencia oranges, or by Penicillia and other fungi, such as *Geotrichum candidum* Link. ex Pers. in Marsh grapefruit (Fig. 1).

Whole fruit treated with metalaxyl at 1,000 or 2,000 μg/ml had residues of 1.8 mg/kg in Marsh grapefruit and of 3.2 mg/kg in Valencia oranges, 1 day after treatment. After 3-mo storage, residues ranged between 0.8 and 2.2 mg/kg in Marsh grapefruit and 2.2 and 3.0 mg/kg in Valencia oranges (Fig. 1), after metalaxyl treatment at 1,000 and 2,000 μg/ml, respectively.

Effect of preharvest spray. Preharvest spraying of Temple oranges was more effective with metalaxyl than with Bordeaux mixture (Table 3). The amount of decay due to brown rot in metalaxyl-sprayed Temple oranges after 2-mo storage at 5 C was 4.3%; in Bordeaux-sprayed fruit, 26.4%; and in unsprayed control fruit, 34.7%. Only the infected metalaxyl-sprayed fruit did not have any mycelium of *P. citrophthora* on the surface of the decayed fruit; however, the percentage of fruit decayed by *Penicillium digitatum* and *Penicillium italicum* was greater in the metalaxyl-sprayed fruit: 28.6 vs. 19.8% for the Bordeaux-sprayed fruit and 14.3% in the controls. An additional week of shelf life at 17 C increased the amount of decay due to brown rot and molds, especially in sprayed fruits.

Table 2. Effect of an aqueous metalaxyl drench on brown spots and mycelial growth of *Phytophthora citrophthora* in dip-inoculated citrus fruit during 7–9 days of storage at 17 C

Fruit	Water containing[a] (μg/ml)	Metalaxyl (μg/ml)	Drench 2 days after inoculation: Infected fruits (%)	Spots per fruit[b]	Mycelial growth[c]
Shamouti orange		0	20	0.5	–
		100	100	0	–
		500	0	0	–
		1,000	0	0	–
Lemon		0	20	0.2	–
		100	20	0.2	–
		500	0	0	–
		1,000	0	0	–
Shamouti orange	TBZ (500) + SOPP (500)	0	80	4.3	–
		100	80	3.2	–
		500	50	1.4	–
		1,000	60	1.5	–
Temple orange	TBZ (500) + SOPP (500)	0	90	3.6	+
		100	80	2.7	+
		500	40	0.7	–
		1,000	70	0.8	–
Shamouti orange	TBZ (500) + chlorine (100)	0	80	4.2	–
		100	40	1.4	–
		500	40	0.9	–
		1,000	30	0.4	–
Temple orange	TBZ (500) + chlorine (100)	0	90	3.8	+
		100	90	3.0	+
		500	90	1.9	–
		1,000	80	1.8	–
Lemon	TBZ (500) + chlorine (100)	0	100	6.1	–
		500	100	2.1	–
		1,000	70	1.5	–
		2,000	90	1.7	–

[a] TBZ = 2-(4-thiazolyl) benzimidazole, SOPP = sodium orthophenylphenate.
[b] Average for 25 fruits.
[c] + = growth, – = inhibition.

DISCUSSION

As a postharvest treatment of citrus fruit, metalaxyl was the only chemical incorporated in a wax coating that was effective against brown rot caused by *P. citrophthora*. Metalaxyl completely prevented fungus mycelium from growing on the surface of infected fruit. This is especially important in long-term storage of citrus fruit as it prevents contact infection and spread of the disease. The

Table 3. Effect of preharvest spray on brown rot caused by *Phytophthora citrophthora* in fruits during 5 C storage and 17 C shelf life[a]

Preharvest spray	30-day storage: *P. citrophthora* Infected fruits (%)	Spots per fruit[b]	Mycelial growth[c]	Penicillium rot (%)	60-day storage: *P. citrophthora* Infected fruit[b] (%)	Mycelial growth[c]	Penicillium rot (%)	1-week shelf life: *P. citrophthora* Infected fruit (%)	Mycelial growth[c]	Penicillium rot (%)
Control (unsprayed)	34.7	0.6	–	8.2	34.7	–	14.3	34.7	+	14.3
Bordeaux mixture (1% copper sulfate)	24.5	0.3	–	15.1	26.4	–	19.8	36.8	+	19.8
Metalaxyl 500 μg/ml[d]	1.0	0.1	–	13.7	4.3	–	28.2	6.8	–	36.8

[a] Spray on 2 February 1979, rain on 8 February 1979; harvest and inoculation on 11 February 1979.
[b] Average for 25 fruits per trial.
[c] + = growth, – = inhibition.
[d] 100 g in 200 L of water per 1,000 m^2.

small systemic effect of metalaxyl that we noted did not prevent growth of the fungus from inside infected fruit and gave only limited control of the disease; similar results were obtained with hot water treatment (3–5). Generally the deposit of wax and fungicide on fruit coated by hand-dipping is two to three times higher than that achieved by spray coating as practiced in citrus packinghouses.

In aqueous preparations, metalaxyl alone or combined with other chemicals, when applied as a drench, effectively controlled brown rot. Our results also showed that metalaxyl can be used as a preharvest spray to prevent brown rot development in harvested fruit in storage, similar to results of Solel et al (7).

Metalaxyl enhanced primary infections by *Penicillium digitatum* in fruit. We found a similar effect in in vitro experiments, when *Penicillium digitatum* grew better on PDA containing metalaxyl but showed only moderate growth on PDA without metalaxyl (*unpublished*).

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