Lack of Control of Citrus Canker by Induced Systemic Resistance Compounds

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ABSTRACT

Graham, J. H., and Leite, R. P., Jr. 2004. Lack of control of citrus canker by induced systemic resistance compounds. *Plant Dis.* 88:745-750.

Induced systemic resistance compounds (ISRs), acibenzolar-*S*-methyl (Actigard), and harpin protein (Messenger) were assayed in the greenhouse against *Xanthomonas axonopodis* pv. *citrumelo*, the cause of citrus bacterial spot (CBS), and *X. axonopodis* pv. *citri*, the cause of Asiatic citrus canker. Actigard and Messenger applied as foliar sprays 3 to 7 days before inoculation reduced numbers of lesions when either bacterium at 10^3 or 10^4 CFU/ml was injection-infiltrated into Swingle citrumelo leaves. Based on this activity, the ISRs were evaluated in southern Brazil in orchards of sweet oranges with low to moderate canker disease incidence in spray programs with and without copper oxychloride (COC) and copper hydroxide (CuOH). Actigard and Messenger were applied full season or in the first two or three sprays of a six-spray program in an attempt to reduce early canker disease on foliage and thereby reduce subsequent fruit infection and premature drop. Sprays of COC and CuOH were moderately to highly effective in reducing canker disease incidence and preventing premature fruit drop. Actigard or Messenger in combination with COC and CuOH, respectively, did not significantly reduce citrus canker incidence on foliage or fruit drop compared with Cu alone. The lack of additional control with ISRs means they cannot be recommended at this time to augment Cu programs for management of citrus canker.

Outbreaks of the leaf and fruit spotting disease Asiatic citrus canker, caused by *Xanthomonas axonopodis* pv. *citri*, continue to occur in metropolitan areas of Tampa, Miami, Orlando, and southwest Florida (6). Minor outbreaks scattered throughout commercial citrus in southern Florida persist despite aggressive effort by a citrus canker eradication program (25). The existence of a large reservoir of infected and exposed citrus in metropolitan Miami and the frequency of outbreaks in commercial citrus jeopardize the prospects for effective eradication (6,25). The situation raises concern within the citrus industry regarding management of citrus canker should the disease become permanently established in Florida.

Induced systemic resistance (ISR) is the process of resistance dependent on the host

plant's physical or chemical barriers, activated by biotic or abiotic inducing agents (12,13). ISR is not the same as systemic acquired resistance (SAR), which is regulated through a distinct signal transduction pathway. Salicylic acid is implicated in SAR regulation, whereas jasmonic acid is implicated in ISR regulation. Different antimicrobial proteins are induced in the two pathways, but the distinction of terminology is somewhat confused by cross-talk between the two pathways. The term ISR will be used here to include ISR and SAR inducers with activity against several pathogens. Compounds such as benzothiadiazoles and harpin protein are known inducers of plant resistance to diseases (2,3,32). The compounds acibenzolar-*S*-methyl, a benzothiadiazole, registered as Actigard in the United States (Syngenta Crop Protection), and harpin protein, a *hrp* gene product registered as Messenger (Eden Bioscience), are marketed for control of certain diseases (2,30). ISRs are not viewed as replacements for traditional fungicides and bactericides, but they may allow for reduction in the number and dosage of applications of contact materials (24,30).

Copper (Cu)-based bactericides are a standard control measure for citrus canker worldwide (14,16,28). Cu reduces bacterial populations on leaf surfaces, but multiple applications are needed to achieve adequate control on susceptible citrus hosts such as grapefruit and sweet orange (8,17,26,27,28). Cu-based spray programs

are targeted to the spring leaf flush to protect leaves from the one-half to full expansion stage over a period of 2 to 4 weeks (10,28). Fruit are susceptible as they grow from 2.0 to 6.0 cm in diameter for a period of 90 to 120 days, depending on citrus species (11). Protectant activity of Cu is diminished by windblown rain, which introduces bacteria directly into stomata (4,5). Therefore, Cu is used in combination with windbreaks in South America under weather conditions similar to Florida (7,15,16,20). After long-term use, Cu bactericides have other possible disadvantages, including resistance in xanthomonad populations (18,22) and accumulation in soils with potential phytotoxic and environmental effects (1). However, other contact bactericides including antibiotics are not as effective as Cu (17,19,22,31), and antibiotic resistance has developed within xanthomonad populations (21,23,29).

Several mechanisms for ISR may operate simultaneously to control the pathogen, thus reducing the risk of development of resistance (30). ISR activity potentially could be deployed early in the season to slow growth of the bacterium in rapidly developing leaves and complement the protectant activity of Cu. Testing of novel control measures against citrus canker in Florida is restricted to greenhouses under containment and to outdoor evaluation of limited scope in quarantine zones. To determine the value of ISRs, Actigard and Messenger were first tested in a greenhouse assay to establish efficacy against citrus bacterial spot, a surrogate xanthomonad disease caused by *X. axonopodis* pv. *citrumelo* (9), and then against citrus canker under containment. Based on activity in the greenhouse, orchard trials were conducted in Paraná State, Brazil, where citrus canker is endemic. In these trials, early and season-long sprays of ISRs in combination with Cu formulations were evaluated for control of fruit-blemishing and disease-induced fruit drop.

MATERIALS AND METHODS

Bacterial cultures. *X. axonopodis* pv. *citrumelo* strain F1 isolated from a citrus nursery in 1984 (9) and *X. axonopodis* pv. *citri* X02-007 isolated from residential citrus in Broward County in 2001 were maintained by serial transfer on nutrient agar (NA) and periodically reisolated from inoculations described below to maintain virulence. For greenhouse tests, bacteria were grown in nutrient broth and incubated

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This research was supported by the Florida Agricultural Experiment Station and a grant from the Florida Citrus Production Research Advisory Council (Project 013-07P) to JHG and by a grant from PADCT/MCT, Brazil (no. 64.99.032.02) to RPL Jr. The authors also appreciate support from Syngenta Crop Protection and Eden Bioscience. This manuscript is approved for publication as Journal Series No. R-09476.

Accepted for publication 16 March 2004.

on a shaker at 28 to 29°C for 24 h to produce cells in late log phase. The bacterial suspension was prepared by washing cells in 0.075 M phosphate buffer and adjusting the concentration spectrophotometrically (620 nm). Bacterial concentration was confirmed by diluting and plating the suspension on NA plates, incubating for 3 days, and counting CFU.

Greenhouse tests. Seeds of Swingle citrumelo (*Citrus paradisi* Macfad. × *Poncirus trifoliata* (L.) Raf.) were sown in 150-cm³ containers (Stuewe & Sons Inc., Corvallis, OR) containing Metro Mix 500 (The Scotts Co., Marysville, OH), and seedlings were fertilized weekly with Peter's 20-10-20 Peat Lite Special (The Scotts Co.). Nine-month-old seedlings of each rootstock were then transplanted into 4,000-cm³ containers and fertilized as before and supplemented with Osmocote (The Scotts Co.). Seedlings of uniform vigor and stem caliper (approximately 1 cm diameter) were cut back to allow a single dominant shoot to develop. When three to four leaves on the shoot reached three-fourths to full expansion, the entire plant was sprayed with test compounds using an airbrush at ~20 ± 5 ml per plant. From 3 to 7 days after spray applications, three to four leaves per plant were injection infiltrated with inoculum on the abaxial surface of the leaf at three to four sites on each side of the midvein of the expanding leaves until the water-soaked area reached a total diameter of 6 mm per site. Inoculations were performed from 900 to 1200 h when stomata were fully open. Inoculated shoots were immediately enclosed in plastic bags for 48 h. After removal of the bags, plants were rotated on the greenhouse bench twice weekly. After 14 days, lesions within each injection-infiltration site were counted under a hand lens (×10).

ISR and bacterial treatments in greenhouse tests. Greenhouse experiments were arranged in a completely randomized design with eight plants per spray

treatment, three to four inoculated leaves per plant, and six to eight inoculation sites per leaf. Depending on the test, Actigard (acibenzolar-S-methyl, CGA-245704, 50 WP) at 20 or 40 mg a.i./liter or Messenger (3% a.i. harpin protein) at 20, 40, or 60 mg a.i./liter was sprayed on plants. Initial tests with *X. axonopodis* pv. *citrumelo* to determine efficacy and rates of ISRs were conducted from April to November 2000 in a greenhouse at the University of Florida, Citrus Research and Education Center in Lake Alfred. Tests to confirm efficacy of ISRs against *X. axonopodis* pv. *citri* were conducted from April to November 2001 and 2002 in a quarantine greenhouse at the Department of Agriculture and Consumer Services, Division of Plant Industry in Gainesville, FL. At both locations, day/night temperatures were 27/23 ± 2°C.

Orchard trials. Based on the results of greenhouse activity against *X. axonopodis* pv. *citri*, Actigard and Messenger were evaluated in spray programs with and without copper oxychloride (COC, Cupravit Verde, 50% metallic Cu) and copper hydroxide (CuOH, Kocide 2000, 35% metallic Cu), respectively, in replicated trials located in northern Paraná State, Brazil. Experimental design for orchard experiments was a randomized block with five blocks of three measurement trees per block (15 trees per treatment) for 10 treatments in 2000–2001 trials and eight treatments in 2001–2002 trials.

2000–2001 trials. One trial was conducted in the spring at Paranavaí with 4-year-old IAPAR 73, a midseason orange variety (*C. sinensis* (L.) Osbeck), under low citrus canker disease incidence. A second trial was conducted in spring at Munhoz de Melo with 3-year-old Valencia, a late-season sweet orange, under moderate disease incidence. Tree spacing was 4.5 × 6.5 m (approximately 350 to 400 trees per hectare), and the 10 treatments were arranged in a randomized block design with five blocks of five trees per treatment (five trees in a row, 25 trees

per treatment). Sprays were applied on as many as six dates with a backpack sprayer calibrated to deliver approximately 2 liters of spray material per tree at incipient runoff from foliage. Treatments, spray timing, and dates of application are described in Table 1.

Foliar disease incidence (percentage of infected leaves) on the most recent mature foliage was assessed on eight limbs per tree (four limbs on each side of the tree) of the middle three trees in each five-tree block. In each orchard, two unsprayed checks were compared to evaluate the consistency of foliar disease evaluation and the uniformity of disease incidence in the plots. Readings of foliage were performed on 14 September, 14 November, 18 December, 17 January, 20 February, 16 March, 17 April, and 18 May. At Munhoz de Melo on each date, fruit on the ground under the middle three trees in each block was counted, and the presence or absence of canker on the fruit was recorded before removal. At harvest, 60 fruit per block (20 fruit from each quadrant of the middle three trees in each block) were picked to measure the incidence of citrus canker on fruit and quality of the juice (percentage of juice, acid, Brix) extracted from the fruit.

2001–2002 trials. Two trials were conducted in spring at Munhoz de Melo with two sweet orange varieties: site I was 4-year-old Valencia orange with moderate disease incidence, and site II was 4-year-old Pera, a midseason orange with moderate disease incidence. Experimental design and disease evaluations were the same as in 2000–2001 trials. In each site, there were eight treatments as described in Table 1.

Foliar disease and fruit drop were evaluated for site I on 5 September, 3 October, 30 October, 29 November, 27 December, 28 January, 26 February, 27 March, and 25 April, and for site II on 19 September, 15 October, 8 November, 4 December, 2 January, 29 January, 26 February, 26 March, and 24 April.

Table 1. Spray treatments and schedules for orchard trials conducted in Paraná State, Brazil, in 2000–2001 and 2001–2002

2000–2001 at Paranavaí and Munhoz de Melo		2001–2002 at Munhoz de Melo sites I and II	
Treatment	Number and timing of spray applications ^a	Treatment	Number and timing of spray applications ^a
Unsprayed check-1; unsprayed check-2	...	Unsprayed check-1; unsprayed check-2	...
COC at 1.8 g/liter	All 6 times	COC at 1.8 g/liter	All 6 times
CuOH at 1.3 g/liter	All 6 times	CuOH at 1.3 g/liter	All 6 times
Actigard at 40 mg a.i./liter + COC	All 6 times	Actigard at 20 mg a.i./liter + COC and COC	1, 2 and 3, 4, 5, 6
Actigard + COC	1, 2, 4	Actigard + COC and COC	1, 2, 3 and 4, 5, 6
Actigard + COC and COC	1, 2, 4 and 3, 5, 6	Messenger at 62 mg a.i./liter + CuOH and CuOH	1, 2 and 3, 4, 5, 6
Messenger at 62 mg a.i./liter	All 6 times	Messenger + CuOH and CuOH	1, 2, 3 and 4, 5, 6
Messenger + CuOH	All 6 times	Messenger + CuOH and CuOH	1, 3, 5 and 2, 4
Messenger and CuOH	1, 3, 5 and 2, 4		

^a Spray timing and dates at Paranavaí were: (1) first flush, 15 September; (2) petal fall (PF), 19 October; (3) PF + 3 weeks, 14 November; (4) first summer flush (SF), 18 December; (5) SF + 4 weeks, 17 January; and (6) SF + 8 weeks, 17 February. Spray timing and dates at Munhoz de Melo were: (1) 22 September; (2) 19 October; (3) 14 November; (4) 18 December; (5) 16 January; and (6) 8 February.

^b Spray timing and date at site I were: (1) first flush, 6 September; (2) PF, 4 October; (3) PF + 2 weeks, 31 October; (4) SF, 30 November; (5) SF + 4 weeks, 3 January; and (6) SF + 8 weeks, 30 January. Spray dates at site II were: (1) 17 September; (2) 16 October; (3) 9 November; (4) 5 December; (5) 4 January; and (6) 31 January.

At harvest, fruit disease and fruit quality were evaluated on 60 fruit per block as in the 2000–2001 trials. Total fruit harvested from the middle three trees in each block was measured to calculate the yield and the percentage of total fruit that dropped due to citrus canker infection.

Statistical analysis. *Greenhouse tests.* Results of each greenhouse test were subjected to a factorial analysis of variance using SAS PROC GLM to test for effects of leaf, plant, and spray treatment on number of lesions per inoculation site. Since no interaction of leaf or plant on number of lesions per inoculation site was detected, a one-way ANOVA was performed on the number of lesions per site pooled across leaves and plants ($n = 21$ to 32). Means were separated using Student-Newman-Keuls multiple range test at $P < 0.05$.

Orchard trials. For each trial in each year, data for the area under the disease progress curve (AUDPC, calculated from seven or eight readings of foliar disease incidence), the incidence of fruit with canker, fruit drop, and juice quality parameters were subjected to a one-way ANOVA using SAS PROC GLM. Means ($n = 5$) were separated using Student-Newman-Keuls multiple range test at $P < 0.05$.

RESULTS

Greenhouse tests. Discrete lesions were easily counted using a $\times 10$ hand lens at 14 days after stomatal inoculation of Swingle citrumelo leaves at 10^3 or 10^4 CFU/ml. In two tests against *X. axonopodis* pv. *citrumelo*, a single application of Actigard at 40 mg a.i./liter reduced the number of CBS lesions by 55 to 80% (Table 2). Lesion reduction by Actigard at 40 mg/liter was similar at the two inoculum densities, but was less consistent at the 20-mg rate, so the higher concentration was chosen for further evaluation against *X. axonopodis* pv. *citri*.

Control of *X. axonopodis* pv. *citrumelo* by Messenger at concentrations from 20 to 60 mg a.i./liter was less consistent than control by Actigard (Table 2). In test 1, reduction of lesion number from 25 to 55% occurred at 40- and 60-mg rates at 10^3 or 10^4 CFU/ml. Messenger at all rates tested was ineffective in test 2. The 40-mg rate of Messenger was chosen for further evaluation of activity against citrus canker.

As found for *X. axonopodis* pv. *citrumelo*, ISR activity of Actigard against *X. axonopodis* pv. *citri* was more consistent than that obtained with Messenger (Table 3). Actigard reduced canker lesion development to the same level as it reduced *X. axonopodis* pv. *citrumelo*. Messenger reduced lesions in one of the two tests and only at the lower inoculum density in this case. Consistency of Actigard for control of canker was further confirmed in three replicate tests in 2002 (data not shown).

Orchard trials. *2000–2001.* In each orchard, disease incidence was similar for

Table 2. Greenhouse evaluation of induced systemic resistance activity for different rates of Actigard and Messenger against *Xanthomonas axonopodis* pv. *citrumelo* injection-infiltrated at two inoculum concentrations into leaves of Swingle citrumelo^x

Treatment	Number of lesions per site			
	10^3 CFU/ml		10^4 CFU/ml	
	Test 1 ^y	Test 2	Test 1	Test 2
Untreated check	19.2 a ^z	12.8 a	76.1 a	48.0 a
Actigard 20 mg a.i./liter	2.2 b	12.2 a	62.7 a	39.0 a
Actigard 40 mg a.i./liter	8.7 b	3.8 b	39.1 b	39.0 a
Untreated check	5.5 a	6.4 a	42.9 a	44.2 a
Messenger 20 mg a.i./liter	4.3 ab	6.0 a	32.8 ab	43.6 a
Messenger 40 mg a.i./liter	4.1 ab	5.9 a	29.2 b	38.5 a
Messenger 60 mg a.i./liter	2.2 b	5.7 a	19.6 b	44.6 a

^x Lesions counted 14 days after inoculation.

^y Plants sprayed 3 (Actigard) or 7 (Messenger) days before inoculation.

^z For each compound, means ($n = 24$ to 32) within columns followed by unlike letters indicate significant differences at the $P < 0.05$ level according to Student-Newman-Keuls multiple range test.

Table 3. Greenhouse evaluation of induced systemic resistance activity for Messenger and Actigard against *Xanthomonas axonopodis* pv. *citri* injection-infiltrated at two inoculum concentrations into leaves of Swingle citrumelo^x

Treatment	Number of lesions per site			
	10^3 CFU/ml		10^4 CFU/ml	
	Test 1 ^y	Test 2	Test 1	Test 2
Untreated check	4.9 a ^z	3.6 a	37.4 b	22.2 a
Messenger	3.3 b	3.4 a	44.3 a	24.8 a
Actigard	3.0 b	2.4 b	25.3 c	17.1 a

^x Lesions counted 14 days after inoculation.

^y Plants sprayed with Actigard or Messenger at 40 mg a.i./liter at 3 or 7 days, respectively, before inoculation in each test.

^z Means ($n = 24$ to 32) within columns followed by unlike letters indicate significant differences at the $P < 0.05$ level according to Student-Newman-Keuls multiple range test.

Fig. 1. Foliar disease incidence in unsprayed checks for: **A**, Valencia and IAPAR sweet orange orchards in Munhoz de Melo and Paranavaí, respectively, in 2000–2001 and **B**, Valencia and Pera sweet orange orchards in Munhoz de Melo in 2001–2002, Paraná State, Brazil.

Table 4. Effects of foliar sprays of Actigard and Messenger with combinations of copper oxychloride and copper hydroxide for control of citrus canker on foliage and fruit in Paraná State, Brazil, 2000-2001, Valencia orange at Munhoz de Melo

Treatment	AUDPC ^z	Fruit with canker (%)	Number of fruit dropped per tree due to canker	Fruit weight (g/fruit)
No spray check-1	32.2 a	69.0 a	21.5 abc	244 b
No spray check-2	32.3 a	71.3 a	22.8 ab	251 b
Copper hydroxide (CuOH) (6×)	19.8 b	48.3 bc	13.3 abc	255 ab
Copper oxychloride (COC) (6×)	15.9 b	35.0 c	5.5 bc	270 ab
Actigard + COC (6×)	18.4 b	38.9 c	6.8 bc	292 a
Actigard + COC (3×) and COC (3×)	16.3 b	33.5 c	7.3 bc	266 ab
Actigard + COC (3×)	18.6 b	51.2 bc	7.9 bc	268 ab
Messenger + CuOH (6×)	13.3 b	30.5 b	5.3 c	265 ab
Messenger (3×) + CuOH (2×)	27.5 a	64.7 ab	15.9 abc	267 ab
Messenger (6×)	30.5 a	72.4 a	27.3 a	265 ab

^z AUDPC = area under the disease progress curve for foliage.

Table 5. Effects of foliar sprays of Actigard and Messenger with combinations of copper oxychloride and copper hydroxide for control of citrus canker on foliage and fruit in Paraná State, Brazil, 2000-2001, IAPAR 73 at Paranavaí

Treatment	AUDPC ^x	Fruit with canker (%)	Fruit weight (g/fruit)	Juice content (%)	Fruit acid (%)	Fruit Brix (%)	Brix/acid
No spray check-1	2.03 ab ^y	0.04 a	99 b	53.7 a	0.85 b	10.3 b	12.10 a
No spray check-2	2.24 a	0.29 a	nt ^z	nt	nt	nt	nt
Copper hydroxide (CuOH) (6×)	0.69 bc	0.01 a	nt	nt	nt	nt	nt
Copper oxychloride (COC) (6×)	0.30 bc	0.01 a	105 b	45.5 b	0.91 b	10.8 a	11.79 a
Actigard + COC (6×)	0.46 bc	0.00 a	120 a	50.5 ab	0.85 b	10.8 a	12.67 a
Actigard + COC (3×) and COC (3×)	1.13 abc	0.02 a	nt	nt	nt	nt	nt
Actigard + COC (3×)	0.61 bc	0.02 a	nt	nt	nt	nt	nt
Messenger + CuOH (6×)	0.28 bc	0.00 a	101 b	47.3 b	0.83 b	10.5 ab	12.64 a
Messenger (3×) + CuOH (2×)	0.51 bc	0.01 a	nt	nt	nt	nt	nt
Messenger (6×)	0.17 c	0.00 a	129 a	46.1 b	1.24 a	9.4 c	7.56 b

^x AUDPC = area under the disease progress curve for foliage.

^y Means (n = 5) within columns followed by unlike letters indicate significant differences at the $P < 0.05$ according to Student-Newman-Keuls multiple range test.

^z nt = not tested.

the two unsprayed checks confirming the reproducibility of foliar disease evaluation and uniformity of disease pressure in the plots (Fig. 1A). At the Munhoz de Melo site, canker incidence increased through the season on untreated Valencia orange trees exposed in the spring to moderate disease pressure (Fig. 1A). Area under the disease progress curve (AUDPC) was reduced by all treatments that included COC and CuOH except the treatment of three Messenger plus two CuOH sprays (Table 4). This Cu program included one spray in the midspring (spray no. 2) and one in summer (spray no. 4) compared with other treatments that received three sprays early in the season or six sprays throughout the season of Cu formulations alone or in combination with Actigard or Messenger. The combination of Actigard or Messenger with Cu formulations did not reduce AUDPC compared with treatments with Cu alone. Six sprays of Messenger without CuOH were ineffective for control of foliar disease. The same relationship among treatments was observed for reduction of canker incidence on fruit and prevention of fruit drop due to canker infection (Table 4). As was the case for foliar disease, combination of ISRs with Cu formulations did not reduce fruit disease incidence and premature drop compared with treatments with Cu alone (Table 4). The treatments with Messenger alone and Messenger +

two CuOH sprays were the least effective for reducing canker incidence on fruit and prevention of fruit drop. Except for fruit weight, treatments did not affect fruit quality parameters (percent juice, Brix, acid, Brix/acid; data not shown) for the late-season Valencia fruit compared with the untreated checks (data not shown).

At the Paranavaí site, initial disease incidence was low, and canker developed early and then declined thereafter (Fig. 1A). All spray treatments, except the three Actigard + COC plus three COC sprays program, significantly reduced AUDPC compared with untreated check-2 but not untreated check-1 (Table 5). Lack of statistical separation among treatments was due to low foliar disease at this site. Canker incidence was even less on fruit than on foliage, and fruit drop was not measurable. The relationship of treatments to reduction of incidence of canker on fruit was similar to that for foliage, but no significant differences in fruit disease were observed among treatments (Table 5). Canker was not detected on fruit for treatments with full season application ISRs: six sprays of Actigard + COC or six sprays of Messenger. These treatments also increased fruit weight but reduced percent juice of the midseason variety, IAPAR 73. Six sprays of Messenger delayed color-break (fruit remained green) and increased acid, reduced Brix and Brix/acid ratio of the juice.

2001–2002. In each orchard, disease incidence was similar for the two unsprayed checks, again confirming the reproducibility of foliar disease evaluation and uniformity of disease pressure in the plots (Fig. 1B). At Munhoz de Melo site I, Valencia orange trees in the same orchard as in the 2000-2001 trial had moderate canker disease early in the spring. Without treatment, disease increased, but after the rains stopped for several weeks in January and February, disease incidence declined to a low level and did not increase until the end of the season when rains resumed (Fig. 1B). In 2001–2002, ISRs were combined with the same Cu formulations as in 2000–2001 and ISR treatments made at the first through third spray timings. All treatments reduced AUDPC, incidence of fruit infection, and fruit drop due to canker compared with the untreated check treatments (Table 6). Treatments with combinations of two or three applications of Actigard or Messenger were no more effective than Cu formulations alone. No treatments significantly increased fruit yield, although as a group, treated trees had higher fruit numbers than unsprayed checks. None of the treatments affected the quality of late-season Valencia fruit at harvest (data not shown).

At Munhoz de Melo site II, Pera orange trees were exposed to slightly lower canker disease incidence than in site I, but the

Table 6. Effects of foliar sprays of Actigard and Messenger with formulations of copper oxychloride and copper hydroxide for control of citrus canker on foliage and fruit and fruit drop of Valencia orange (site I) and Pera orange (site II) in Munhoz de Melo, Paraná, Brazil, in 2001–2002

Site	AUDPC ^y		Fruit w/canker (%)		Fruit dropped due to canker infection (%)		Fruit dropped due to other causes (%)		Total fruit dropped (%)		Total fruit per tree	
	I	II	I	II	I	II	I	II	I	II	I	II
No spray check-1	6.84 a ^z	5.75 a	17.88 a	7.66 a	3.67 a	3.42 a	6.47 a	1.59 a	10.13 a	5.00 a	373 a	468 a
No spray check-2	8.28 a	5.32 a	13.56 a	6.04 a	2.75 a	2.91 a	8.43 a	1.59 a	11.17 a	4.50 a	324 a	457 a
Copper hydroxide (CuOH) (6x)	2.06 b	3.31 b	0.33 b	3.67 a	0.53 b	0.97 b	5.99 a	1.90 a	6.53 a	2.87 ab	432 a	519 a
Copper oxychloride (COC) (6x)	2.28 b	2.70 b	0.00 b	2.35 a	0.55 b	0.72 b	6.37 a	1.65 a	6.94 a	2.37 b	446 a	418 a
COC + Actigard (2x)	2.12 b	2.39 b	1.95 b	1.68 a	0.65 b	0.92 b	7.27 a	2.22 a	7.92 b	2.33 b	418 a	460 a
COC + Actigard (3x)	2.25 b	2.25 b	2.99 b	1.99 a	0.72 b	0.83 b	7.24 a	1.41 a	7.96 ab	3.05 ab	389 a	408 a
CuOH + Messenger (2x)	2.29 b	2.07 b	1.33 b	5.21 a	0.67 b	0.82 b	6.55 a	1.89 a	7.22 ab	2.70 ab	467 a	458 a
CuOH + Messenger (3x)	2.00 b	1.96 b	4.33 b	3.68 a	1.15 b	0.88 b	9.52 a	2.21 a	10.67 a	3.05 ab	380 a	442 a

^y AUDPC = area under the disease progress curve for foliage.

^z Means (n = 5) within columns followed by unlike letters indicate significant differences at $P < 0.05$ according to Student-Newman-Keuls multiple range test.

pattern of disease progress was similar (Fig. 1B). All treatments reduced AUDPC and canker-caused fruit drop compared with the untreated check treatments (Table 6). Incidence of fruit infection was numerically lower on treated trees, but the low incidence and high variability did not allow statistical separation of the treatments from the untreated checks. Treatments with combinations of two or three applications of Actigard or Messenger were no more effective than Cu formulations alone. None of the treatments affected fruit yield or quality at harvest (data not shown).

DISCUSSION

In the greenhouse assays, reduction of lesions by Actigard (acibenzolar-*S*-methyl) and Messenger (harpin protein) was presumed to be due to ISR and not to direct antimicrobial activity of the compounds. Although induction of plant antimicrobial compounds was not assayed in the present study, ISR activity was likely because: (i) the compounds were applied well in advance to induce systemic activity prior to inoculation, and (ii) bacteria were infiltrated through stomata to minimize direct contact with the compounds on the leaf surface. Bacterial infiltration into the leaf mesophyll produced discrete lesions at the point of stomatal infection which permitted quantitative assessment of ISR activity against *X. axonopodis* pv. *citrumelo* and *X. axonopodis* pv. *citri*. ISR compounds at 20 to 40 mg a.i./liter controlled citrus bacterial spot and canker in greenhouse assays, but the activity of Actigard appeared to be more consistent than that of Messenger. Actigard reduced lesions at concentrations effective for field control of *Xanthomonas* spot of bell pepper (24).

Based on greenhouse activity against citrus bacterial diseases, Actigard and Messenger were applied with Cu in an attempt to reduce bacterial multiplication in the leaf and complement contact activity early in the season. In four orchard trials, ISRs with or without Cu formulations did

not reduce citrus canker incidence on foliage or fruit or reduce fruit drop in susceptible sweet oranges compared with Cu alone. As previously demonstrated in Brazil and Argentina (7,15,16,20), Cu alone was moderately to highly effective for reducing foliar disease, fruit infection, and premature fruit drop on susceptible sweet orange varieties depending on the disease pressure.

A potential side effect of ISRs on fruit was detected for the midseason orange, IAPAR 73, but not for late-season Valencia orange. ISRs increased fruit weight, a desirable characteristic for IAPAR 73, which produces undersized fruit for the fresh market. However, Messenger also retarded fruit maturation and delayed color-break (fruit remained green), which could delay harvest of this variety.

Foliage and fruit disease control was best achieved with six sprays of either Cu formulation, but programs that reduced or delayed Cu sprays were less effective. This finding confirms that Cu coverage is critical during the early stages of foliage and fruit development because immature tissues are most susceptible (26,28). It is also well established from research in South America (16,28) and Japan (14) that control of early infection of fruit greatly reduces premature fruit drop. In the 2001–2002 trials, fruit drop was reduced from 3.2% (unsprayed) to 0.62 and 0.85% (treatments with Cu applied full season) for Valencia and Pera orange, respectively. For sweet oranges that are principally used for juice processing, prevention of fruit drop is much more important than reduction of fruit blemishes.

ACKNOWLEDGMENTS

We thank L. C. Teixeira and the support staff at the Instituto Agronômico do Paraná for their assistance in conducting orchard trials in Brazil. We also recognize the technical support of D. L. Drouillard and D. B. Bright at the Citrus Research and Education Center, Lake Alfred, FL, and X. Sun and M. Nielsen at the quarantine facilities at Florida Department of Agriculture and Consumer Services, Division of Plant Industry in Gainesville.

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