Soil Application of SAR Inducers Imidacloprid, Thiamethoxam, and Acibenzolar-*S*-Methyl for Citrus Canker Control in Young Grapefruit Trees

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Abstract

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Soil applications of inducers of systemic acquired resistance (SAR) imidacloprid, thiamethoxam, or acibenzolar-*S*-methyl, at various rates and application frequencies, were evaluated for control of citrus canker caused by *Xanthomonas citri* subsp. *citri* in a field trial of 3- and 4-year-old 'Ray Ruby' grapefruit trees in southeastern Florida. Reduction of foliar incidence of canker produced by one, two, or four soil applications of imidacloprid, thiamethoxam, and acibenzolar-*S*-methyl was compared with 11 foliar sprays of copper hydroxide and streptomycin applied at 21-day intervals. In the 2008 and 2009 crop seasons, canker incidence on each set of vegetative flushes was assessed as the percentage of the total leaves with lesions. By the end of the 2008 season, despite above-average rainfall and a tropical storm event, all treatments significantly reduced foliar incidence of citrus canker on the combined spring-summer-fall flushes. Sprays of copper hydroxide and streptomycin were effective for reducing canker incidence on shoot flushes produced throughout the season compared with the untreated control, whereas soil-applied SAR inducers reduced foliar disease depending on rate, frequency, and timing of application. Except for the treatment of four applications of acibenzolar-S-methyl at 0.2 g a.i. per tree or two applications of imidacloprid, SAR inducers were ineffective for reducing foliar disease on the flushes that were present during the tropical storm. In 2009, all treatments significantly reduced the incidence of foliar canker on the combined spring-summer-fall flushes but not all treatments of spring-summer flushes with SAR inducers were effective compared with the untreated control. Hence, depending on rate, frequency, and timing of application, soil-applied SAR inducers reduced incidence of canker on foliar flushes of young grapefruit trees under epidemic conditions.

Asiatic citrus canker, caused by the bacterial pathogen *Xanthomonas citri* subsp. *citri* (syn. *X. axonopodis* pv. *citri*), is a serious disease of commercial citrus cultivars and some citrus relatives (13). The pathogen causes distinctive necrotic, erumpent lesions on leaves, stems, and fruit. Severe infections can cause a range of symptoms from defoliation, blemished fruit, premature fruit drop, and twig dieback to general tree decline. Grapefruit (*Citrus paradisi* Macf.) is the most important fresh fruit citrus grown in Florida and the citrus cultivar most susceptible to canker (16). Canker has become endemic in grapefruit groves since a state-federal eradication program ended in January 2006 (11).

There are no highly effective canker disease suppression tactics for susceptible cultivars of citrus when the crop is grown in wet subtropical areas like Florida (18,19,26). Copper reduces bacterial populations on leaf surfaces but multiple applications are needed to achieve adequate control on susceptible citrus hosts (20,27,28). The protective activity of copper is diminished by wind-blown rain that introduces bacteria directly into stomata (10,12). Therefore, copper is used in conjunction with windbreaks in South America under weather conditions similar to those in Florida (3,16,19). Disadvantages for long-term use of copper bactericides include the selection for copper resistance in xanthomonad populations (4,22,25) and accumulation of copper in citrus soils with potential phytotoxic and adverse environmental effects (1). However, other contact bactericides are not as effective as copper because they lack sufficient residual activity to protect leaf and fruit surfaces for extended periods (16,20,23,26,31).

Systemic acquired resistance (SAR) is an innate plant defense that may confer long-lasting protection against a broad spectrum of microorganisms (6,33). Plants acquire an enhanced defensive capacity against subsequent pathogen attack as a result of induction by primary, limited pathogen infection. SAR requires the signal molecule salicylic acid (SA) and is associated with the accumulation of pathogenesis-related (PR) proteins, which are thought to contribute to resistance. SAR may be activated in the absence of pathogens by treatment of plants with chemical inducers (9). Acibenzolar-*S*-methyl (ASM; Actigard or Bion; Syngenta Crop Protection), a functional homolog of SA, is the most widely known commercially produced inducer of SAR (30).

ASM and other commercial inducers of resistance have been extensively evaluated as components for plant disease control in the field (32); however, their effectiveness for practical application in disease management has been questioned due to variability of control (34). Field studies showing promise for control of bacterial diseases have been conducted with foliar sprays of ASM either alone or in combination with copper on tomato and pepper (21,24). For citrus, foliar application of ASM was effective against citrus canker under greenhouse conditions but foliar sprays of ASM combined or alternated with copper oxychloride did not contribute to control of canker on sweet orange trees in field trials (14). Expression of the PR protein (β-1,3 glucanase) gene, PR-2, in citrus increased in response to ASM and isonicotinic acid (INA). However, PR-2 response and reduction of lesions after foliar sprays was sustained for only a few weeks (5). Likewise, ASM induced acidic PR-1 expression in tomato for 7 to 10 days (17), confirming why foliar applications at weekly intervals are required for field control of Xanthomonas leaf spot and Pseudomonas bacterial speck on tomato (21).

Soil application of the neonicotinoid imidacloprid (IMID) involves the use of this insecticide for citrus bacterial disease management through the control of the interaction of citrus leafminer (*Phyllocnistis citrella*) with *X. citri* subsp. *citri* or *X. alfalfae* pv. *citrumelonis*, the cause of citrus bacterial spot in Florida citrus nurseries (15,28). Reduction in the incidence of foliar lesions for weeks was not due to the prevention of exacerbation of citrus bacterial spot by leafminer because foliar insecticides were applied

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to control leafminer damage (15,28). Likewise, incidence of canker lesions on foliage of young sweet orange trees in the field was suppressed for months in Brazil, where foliar insecticides were applied to control leafminer (15). IMID breaks down in planta into 6-chloronicotinic acid, an analog of INA which induces SAR response (2,7,29). In a greenhouse pot trial, Francis et al. (8) confirmed that soil drenches of IMID as well as INA and ASM induced a high and persistent upregulation of PR-2 gene expression that was correlated with reduction of canker lesions for up to 24 weeks compared with 4 weeks for inoculated plants sprayed with ASM. Canker lesions on leaves of SAR-treated citrus seedlings were small, necrotic, and flat compared with pustular lesions on untreated inoculated plants. Population of *X. citri* subsp. *citri* per leaf was reduced 1 to 3 log units in plants treated with soil applications of ASM compared with untreated plants (8).

The purpose of this research was to validate the efficacy of soil-applied SARs for control of canker lesion development under citrus canker epidemic conditions in young grapefruit trees in southeastern Florida. In this study, we evaluated soil drenches of IMID, ASM, and INA for control of citrus canker on the foliage. Thiamethoxam (THIA), another soil-applied neonicotinoid widely used for insect control in Florida citrus, was also included for comparison.

Materials and Methods

Field site and treatments. In 2008, a field trial was conducted with 3-year-old nonbearing 'Ray Ruby' grapefruit trees planted in 2005 at Ft. Pierce, St. Lucie County, FL. Treatments were arranged in a randomized complete block design with 13 treatments replicated five times in blocks of five contiguous trees. Three foliar treatments were copper hydroxide at 0.57 g a.i. per tree per application (CH; Kocide 3000; 30% metallic Cu a.i.; DuPont), streptomycin at 0.80 g a.i. per tree per application (STREP; Fire-Wall 17WP; 17% a.i.; Agrosource, Inc.), and a mixture of CH and STREP at the same rates. Soil-applied treatments consisted of ASM (Actigard 50WG; 50% a.i.; Syngenta Crop Protection) at 0.1 and 0.2 g a.i. per tree in two or four applications, IMID (Admire Pro, 42.8% a.i.; Bayer Crop Science) at 1.48 g a.i. per tree in one application or 0.74 g a.i. per tree in two applications, THIA (Platinum, 21.6% a.i.; Syngenta Crop Protection) at 0.5 g a.i. per tree in one application or 0.25 g a.i. per tree in two applications, and INA at 1.9 g a.i per tree in one application. One treatment included a single soil application of IMID at 1.48 g a.i. per tree at the beginning of the season followed by CH sprays. The untreated control (UTC) trees received a water-only spray treatment at each foliar spray time. Products were mixed with water and applied as foliar sprays at 3.0 liters/tree with a handgun sprayer at 1,380 kPa of air pressure or applied as a soil drench at 0.230 liters/tree to the soil surface in a crescent within 10 to 15 cm of the trunk on the top side of the bed to minimize runoff. To facilitate infiltration, microsprinkler irrigation was applied for a few minutes to prewet the soil surface before drenching. The materials, application rates, and number and dates of application for each treatment are listed in Table 1. For the combination treatment of CH and STREP, the two materials were tank mixed prior to spray application. Treatments were initiated after the spring flush in March 2008. Foliar applications of the insecticides abamectin, fenpropathrin, and dimethoate were applied through the season to protect new flush leaves from citrus leafminer damage to minimize the interaction with *X. citri* subsp. *citri* (28).

In 2009, the treatments were repeated in the same plot locations as for the 2008 trial with 4-year-old trees. The treatments were the same as in 2008, with the addition of a treatment with two applications of THIA (Table 1). Treatment application dates for 2009 are listed in Table 1.

Monthly rainfall in 2008 and 2009 was recorded at the nearby University of Florida/IFAS, Indian River Research and Education Center, Ft. Pierce and compared with the average for the last 10 years (Table 2).

Table 1. Treatments applied to 3- and 4-year-old 'Ray Ruby' grapefruit trees at Ft. Pierce, FL in 2008 and 2009

	Applications		Application dates	
Treatment[x]	Number[y]	Rate (g a.i.)[z]	2008	2009
UTC	…	…	…	…
ASM	2	0.1	3/3, 6/6	3/18, 6/15
ASM	2	0.2	3/3, 6/6	3/18, 6/15
ASM	4	0.1	3/3, 5/7, 7/7, 9/8	3/18, 5/13, 7/15, 9/16
ASM	4	0.2	3/3, 5/7, 7/7, 9/8	3/18, 5/13, 7/15, 9/16
IMID	1	1.48	3/3	3/18
IMID	2	0.74	3/3, 7/7	3/18, 7/15
INA	1	1.9	3/3	3/18
THIA	1	0.50	3/3	3/18
THIA	2	0.25	Not treated in 2008	3/18, 7/15
CH	11	0.57	3/7, 3/28, 4/18, 5/9, 5/30, 6/20, 7/11, 8/4, 8/26, 9/15, 10/6	3/23, 4/13, 5/4, 5/26, 6/15, 7/7, 7/27, 8/17, 9/8, 9/29, 10/13
STREP	11	0.80	3/7, 3/28, 4/18, 5/9, 5/30, 6/20, 7/11, 8/4, 8/26, 9/15, 10/6	3/23, 4/13, 5/4, 5/26, 6/15, 7/7, 7/27, 8/17, 9/8, 9/29, 10/13
STREP/CH	11	0.80/0.57	3/7, 3/28, 4/18, 5/9, 5/30, 6/20, 7/1, 8/4, 8/26, 9/15, 10/6	3/23, 4/13, 5/4, 5/26, 6/15, 7/7, 7/27, 8/17, 9/8, 9/29, 10/13
IMID/CH	1/11	1.48/0.57	3/3 (IMID) 3/7, 3/28, 4/18, 5/9, 5/30, 6/20, 7/11, 8/4, 8/26,9/15, 10/6	3/18 (IMID) 3/23, 4/13, 5/4, 5/26, 6/15, 7/7, 7/27, 8/17, 9/8, 9/29, 10/13

[x] UTC = untreated control, ASM = acibenzolar-*S*-methyl, IMID = imidacloprid, INA = isonicotinic acid, THIA = thiamethoxam, CH = copper hydroxide, and STREP = streptomycin.
[y] Number of applications.
[z] Amount of treatment per tree per application.

Table 2. Rainfall at Ft. Pierce, FL in 2008 and 2009

	Monthly rainfall (mm)								
Year	March	April	May	June	July	August	September	October	November
2008	66	0.7	88	8.6	219	429	117	121	29
2009	52	11	223	83	189	189	42	28	7
Average[z]	50	64	76	166	186	159	158	101	53

[z] Average monthly rainfall from 1998 to 2008 obtained from Florida Automated Weather Network at Indian River Research and Education Center, Ft. Pierce.

Disease evaluation. In 2008, disease on foliage was assessed as the percentage of canker-infected leaves on sets of leaf flushes: spring-first summer, spring-summer, and spring-summer-fall in June, October, and December, respectively. Canker incidence on each set of flushes was expressed as the percentage of the total number of leaves with canker lesions. In 2009, the number of canker-diseased leaves on flushes in spring-first summer, spring-summer, and spring-summer-fall was assessed in June, September, and November, respectively.

Statistical analysis. Data for incidence of diseased leaves were log-transformed and subjected to a three-way analysis of variance (ANOVA) to evaluate the main effects and interactions of trial year, disease evaluation time and chemical treatment using PROC GLM (SAS Institute). This analysis detected a significant year–treatment and evaluation time–treatment interaction. Therefore, a one-way ANOVA was performed and the treatment means were separated using Waller-Duncan multiple range test at $P < 0.05$.

Results

2008 trial. The rainfall until July was below average compared with the last 10 years (Table 2). All treatments significantly reduced incidence of canker on the spring-first summer flushes compared with the UTC (Table 3). On 20 to 22 August, 3 days of sustained windblown rain from Tropical Storm Fay increased canker incidence on the spring-summer flushes to levels 2.5 to 7 times higher than that observed on the earlier flushes with the same treatments (Table 3). Incidence of foliar canker on shoot flushes present during this period was reduced by foliar sprays of CH, STREP, and the combination of STREP with CH compared with the UTC. Among the soil-applied SAR inducers, only the treatments of four applications of ASM at 0.2 g a.i. or two applications of IMID significantly reduced foliar disease on the spring-summer flushes. On the spring-summer-fall flushes, canker incidence was significantly lower for all treatments compared with the UTC (Table 3). Disease control on trees sprayed 11 times with CH was significantly greater than for SAR treatments, except compared with four applications of 0.2 g of ASM. The disease incidence on the spring-summer-fall flushes for this soil-applied treatment was also statistically comparable with the other foliar treatments.

2009 trial. Despite above-average rainfall in May (Table 2), the spring-first summer flush was free of canker lesions in all treatments (Table 3). In contrast, untreated spring-summer flushes evaluated in September had 62% canker-diseased leaves. Foliar sprays of CH and IMID significantly reduced canker incidence on spring-summer flushes, whereas only SAR treatments of four applications of ASM at 0.2 g a.i., two applications of THIA, or one and two applications of IMID reduced foliar disease compared with the UTC. On the spring-summer-fall flushes, all treatments significantly reduced foliar disease compared with the UTC.

Discussion

Depending on rate, frequency, and timing of application, soil application of the SAR inducers IMID, THIA, and ASM reduced incidence of canker on foliar flushes of young grapefruit trees under epidemic conditions. During the period of highest disease pressure on the summer flushes, soil-applied SAR inducers failed to reduce foliar disease whereas effective disease control was maintained with foliar sprays of copper or streptomycin (3,16). However, by the end of each season, lower canker incidence on trees treated with SAR inducers was attained with one to four soil applications compared with 11 sprays of bactericides applied at a 21-day interval (13). Reduction of canker lesion incidence in the field by SAR inducers was predicted from greenhouse studies demonstrating a high level of control for up to 24 weeks compared with 4 weeks post application for foliar ASM (8). In previous field studies of tomato and pepper, foliar ASM was applied at weekly intervals for season-long control of bacterial leaf spots (21,24). In contrast, six sprays of foliar-applied ASM combined with copper failed to provide additional control of canker on sweet orange leaves and fruit compared with copper alone (14).

In contrast to the failure of foliar-applied ASM to reduce canker in previous greenhouse and field studies (5,14), soil-applied ASM was effective for reducing foliar disease incidence during seasonal canker epidemics, as demonstrated with artificially inoculated citrus plants (8). The longevity of activity produced by soil application may be attributed to gradual release and uptake of ASM into the roots, and the progressive translocation from root to actively growing shoots as previously demonstrated for IMID (29). Sustained supply of chemical inducers to expanding susceptible leaves may be further promoted through periodic soil applications, as demonstrated by the disease control effect of four applications of ASM at 0.2 g a.i. on spring-summer flushes in 2008 and 2009. Although the effectiveness of soil-applied SAR inducers was reduced by Tropical Storm Fay in 2008 and during the wetter conditions in summer of 2009, SAR inducers reduced disease incidence on the fall leaf flush that emerged after this period. An inoculum control effect of ASM, IMID, or INA is supported by the previous finding that, post infection, lesions on potted seedlings treated with soil drenches had 1 to 3 log units fewer bacteria than lesions from untreated plants (8).

Intense use of foliar-applied ASM can result in reduction in growth and yield of tomato and pepper (21,24), a phenomenon referred to as "yield drag". In greenhouse trials with citrus, ASM caused mild leaf chlorosis but the symptoms were temporary (8)

Table 3. Comparison of soil-applied systemic-acquired resistance treatments with contact spray treatments on the percentage of canker-infected leaves for three sets of flushes on 3- and 4-year-old 'Ray Ruby' grapefruit tree at Ft. Pierce, FL in 2008 and 2009[y]

		Spring-first summer flushes (%)		Spring-summer flushes (%)		Spring-summer-fall flushes (%)	
Treatment[z]	Applications	2008	2009	2008	2009	2008	2009
UTC	…	34.1 a	0.0	84.7 a	61.5 a	70.4 a	71.7 a
ASM 0.1 g	2	15.4 bc	0.0	62.6 abcd	27.4 bc	43.6 b	31.2 bcd
ASM 0.2 g	2	17.5 bc	0.0	59.6 abcd	35.2 abc	47.8 b	34.1 bcd
ASM 0.1 g	4	18.3 b	0.0	55.7 abcd	47.8 ab	33.6 bcd	46.6 b
ASM 0.2 g	4	8.7 bc	0.0	46.8 bcde	15.8 bc	22.1 cde	12.8 cd
IMID	1	11.9 bc	0.0	69.7 abc	29.5 bc	46.2 b	28.1 bcd
IMID	2	13.0 bc	0.0	44.4 bcde	21.1 bc	38.2 bc	20.8 cd
INA	1	10.9 bc	0.0	59.2 abcd	34.8 abc	36.1 bcd	38.7 bc
THIA	1	10.7 bc	0.0	76.7 ab	19.1 bc	35.8 bcd	18.1 cd
THIA	2	NT	0.0	NT	38.6 abc	NT	36.3 bcd
CH	11	4.8 c	0.0	15.2 e	22.8 bc	12.2 e	15.1 cd
STREP	11	9.4 bc	0.0	34.9 cde	11.8 c	18.2 de	11.1 d
STREP/CH	11	7.6 bc	0.0	27.9 de	15.1 bc	28.0 bcde	13.6 cd
IMID/CH	1/11	5.2 c	0.0	34.9 cde	9.0 c	27.8 bcde	10.0 d

[y] Flushes were evaluated in June, October, and December in 2008 and June, September, and November in 2009. Values are the means of five replicate plots of five trees. Different letters indicate significant differences at $P < 0.05\%$ according to Waller-Duncan multiple range test; NT = not treated in 2008.

[z] UTC = untreated control, ASM = acibenzolar-*S*-methyl, IMID = imidacloprid, INA = isonicotinic acid, THIA = thiamethoxam, CH = copper hydroxide, and STREP = streptomycin.

and no such symptoms were observed in the field trials. Stunting and yield reductions in certain plant species and cultivars have been attributed to the physiological cost of constitutive induction of plant defense (34). To optimize the use of SAR and to avoid phytotoxicity, the rate or frequency of SAR applications have been reduced or integrated with other bactericides such as copper (24). In this study, somewhat greater (though not significant) canker control was observed in the 2009 trial by combining the systemic control activity from a single soil application of IMID with contact activity of CH. This suggests that integration of systemic with contact activity may be more effective for protection of leaves from infection, as previously demonstrated (24).

Copper-resistant strains of *X. citri* subsp. *citri* have been identified in citrus groves in Argentina (4). Louws et al. (21) demonstrated that ASM is particularly useful for disease management of bacterial speck and bacterial spot where copper-resistant strains predominated. Therefore, soil-applied SAR inducers could be employed for copper-resistance management by reducing the rate and frequency of copper bactericide applications for highly susceptible young trees.

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