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Evaluation of soil applied systemic acquired resistance inducers integrated with copper bactericide sprays for control of citrus canker on bearing grapefruit trees

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A B S T R A C T

Soil application of systemic neonicotinoid insecticides and the commercial systemic acquired resistance (SAR) inducer, acibenzolar-S-methyl (ASM), provides season-long control of foliar infection by *Xanthomonas citri* subsp. *citri*, the causal agent of citrus canker. Reduction in leaf disease incidence with ASM is comparable to protection with 21-day interval foliar sprays of copper hydroxide (CH). Soil applications of ASM alone, rotated with the neonicotinoids imidacloprid (IMID), thiamethoxam (THIA), and clothianidin (CLOTH), or combined with foliar sprays of CH were compared for canker disease control on fruit of 5- to 7-year-old bearing 'Ruby Red' grapefruit trees in Southeast Florida. All treatments significantly reduced the incidence of canker lesions on fruit compared to the untreated check. Soil drenches of ASM and season-long rotations with IMID, THIA, and CLOTH were as effective for suppressing fruit canker as season-long foliar sprays with CH. SAR inducers combined with CH sprays provided optimum control of fruit canker when initiated before the onset of the susceptible foliar flush in the spring. Additional control of canker with soil-applied SAR inducers may enable reduction in the frequency of copper sprays and reduce disease loss from copper resistant Xcc strains where they are prevalent.

1. Introduction

Asiatic citrus canker, caused by the bacterium *Xanthomonas citri* subsp. *citri* (Xcc; syn. *X. axonopodis* pv. *citri*), causes significant economic loss of susceptible citrus cultivars grown in wet tropical or subtropical areas (Graham et al., 2004). The pathogen causes necrotic, erumpent lesions on leaves, stems, and fruits that may lead to defoliation, blemished fruit, premature fruit drop, twig dieback, and when uncontrolled, general tree decline.

Canker suppression on susceptible citrus cultivars is challenging with copper bactericides (Behlau et al., 2009; Leite and Mohan, 1990; Stein et al., 2007) because wind-blown rain introduces Xcc directly into stomata, by-passing the protective copper film on the plant surface (Graham et al., 2004). Recently in Florida, windbreaks have been deployed at the perimeter in 5–10 ha blocks of grapefruit (*Citrus paradisi* Macf.), the most important fresh fruit citrus grown in Florida (Bock et al., 2010; Graham et al., 2013). Even with windbreaks, frequent re-applications of copper are required to protect fruit that are continuously expanding over 90–120 days depending on the citrus cultivar (Behlau et al., 2009; Graham et al., 2016; Stall et al., 1982; Stein et al., 2007). More aggressive copper sprays to control epidemic citrus canker began after the Florida state-federal eradication program ended in 2006 (Dewdney and Graham, 2016). The rate of metallic copper used and the frequency of spray applications per season depend upon weather conditions and the desired period of protection (Behlau et al., 2009). Although the copper program in Florida has yet to be optimized, about 0.54–1.12 kg of metallic copper per hectare every 21 days is recommended to protect spring flush growth or fruit surfaces (Dewdney and Graham, 2016). Prolonged use of copper as fungicides has led to the accumulation of copper in Florida citrus soils with potential adverse environmental effects (Alva et al., 1995). Copper causes phytotoxicity in the fruit peel (Graham et al., 2008) and accumulates to toxic levels in citrus roots (Graham et al., 1986).

Systemic acquired resistance (SAR) is a natural plant defense that provides long-lasting protection against a broad spectrum of

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microorganisms (An and Mou, 2011; Fu and Dong, 2013). 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 (Zhang et al., 2010). SAR may be activated in the absence of pathogens by treating plants with chemical inducers (Gorlach et al., 1996). Acibenzolar-S-methyl (ASM, Actigard 50WP, Syngenta Crop Protection), a functional homolog of SA, is the most widely known commercially produced inducer of SAR (Tally et al., 1999). In a greenhouse trial, soil drenches of ASM, as well as neonicotinoids, induced a high and persistent up-regulation of pathogenesis-related (PR) gene expression that was correlated with reduction of canker lesions for up to 24 weeks after treatment (Francis et al., 2009).

In Florida citrus, neonicotinoid insecticides imidacloprid (IMID, Admire Pro, Bayer Crop Science), thiamethoxam (THIA, Platinum, Syngenta Crop Protection), and clothianidin (Belay, Valent USA Corp.) are applied as soil drenches to non-bearing trees for control of citrus leafminer (*Phyllocnistis citrella*) and the associated infection of leafminer galleries by Xcc (Rogers, 2012). Systemic neonicotinoids are essential for management of the psyllid vector *Diaphorina citri* of *Candidatus* Liberibacter asiaticus (Las), the agent associated with citrus huanglongbing (HLB; also known as citrus greening) (Gottwald and Graham, 2014). Psyllids and leafminers feed and reproduce on vigorous flushes of young trees with the potential for rapid increases in their populations and explosive increases in Las infection (Gottwald, 2010) and Xcc inoculum (Stein et al., 2007), respectively. The best management for these insects on young trees up to 3 m in height is a year-round program of soil-applied neonicotinoids (Rogers, 2012).

In previous studies (Graham and Myers, 2011, 2013), soil application of neonicotinoids IMID and THIA and ASM or rotations of ASM with IMID and THIA were highly effective for reducing foliar infection and canker-induced defoliation on non-bearing grapefruit trees. Furthermore, SAR inducers demonstrated potential to augment canker control with copper sprays. (Graham and Myers, 2013). For fruiting trees greater than 3 m in height, concerns about soil-applied neonicotinoids center on the dilution of the insect control activity as tree volume increases and the potential for detection of residues in the environment as rate of neonicotinoids increases (Graham and Vallad, 2011). For sustaining SAR induction, ASM provides a non-insecticidal option for sustaining SAR activity in trees greater than 3 m in height with low risk of movement through the root zone into the soil profile. Soil-applied ASM provides an option for use of systemic activity on fruiting trees to increase the efficacy of copper sprays programs (Graham and Myers, 2013).

The goals for the current research were to evaluate: 1) soil applications of ASM alone and rotated with neonicotinoids for SAR-induced control of canker on fruit of bearing grapefruit trees that exceeded the height and volume recommended for soil-applied neonicotinoids to still be effective for insect control, and 2) integration of SAR inducers with copper sprays to augment protectant with systemic activity.

2. Materials and methods

2.1. 'Ruby Red' grapefruit trials

From 2013 to 2015, trials were conducted with 5- to 7-year-old bearing 'Ruby Red' grapefruit trees located in Ashland, St. Lucie County, FL (27°29′09.70″N, 80°35′44.69″W). Tree spacing was 5.33 × 7.62 m (252 trees per ha). The experiment was a randomized complete block design for eight treatments in 2013 and 2014 (Table 1) and a modified set of seven treatments in 2015 (Table 2). Each treatment was replicated five times in blocks of five contiguous trees. Materials, application rates and dates of application for each treatment are listed in Tables 1 and 2. The untreated check (UTC) trees received a water-only spray treatment at each foliar spray time. Materials were mixed with municipal well water and applied as foliar sprays at 3.0 L per tree with a handgun sprayer at 1380 kPa air pressure. Soil-applied materials in 2.0 L of water per tree were drenched in the soil within 10–15 cm of the trunk. Treatments were initiated after the spring flush of foliage on April 15, 2013 and April 7, 2014, or before the spring flush on March 16, 2015.

Foliar insecticides abamectin (0.73 L/ha), fenpropathrin (1.16 L/ha), and dimethoate (0.22 L/ha) were applied throughout the season to protect new flush leaves from citrus leafminer and citrus psyllid to minimize the interaction with Xcc (Stein et al., 2007) and Las transmission (Rogers, 2012).

Monthly rainfall from 2013 to 2015 for St. Lucie West and Ft. Pierce [University of Florida/IFAS, Indian River Research and Education Center (UF-IRREC)] was obtained from the two Florida Automated Weather Network (http://fawn.ifas.ufl.edu/) stations located in St. Lucie Co. Monthly rainfall was compared to the average for the last 10 years at the UF-IRREC station (Table 3).

2.2. Disease evaluation

2.2.1. Fruit canker

On October 14, 2013, October 22, 2014, and October 13, 2015, incidence of fruit with canker lesions and the age of the lesions was assessed for 100 fruit harvested from the middle three trees (50 fruit per side) in each plot for each treatment. Lesions were classified as "old" if they were greater than 6 mm in diameter, coalescing with surrounding lesions, black in color, exuding gum, or had a prominent yellow halo; and "young" if lesions were smaller than 6 mm in diameter, brown in color, and were not coalescing with surrounding lesions. A fruit with both lesion ages present was considered to have old lesions with respect to calculation of the incidence of lesioned fruit.

2.3. Fruit fungal diseases

Incidence of lesions for common grapefruit fungal diseases (Agostini et al., 2003), citrus scab (*Elsinoe fawcetti*) and melanose (*Diaporthe citri*), was recorded on the same fruit assessed for canker incidence.

2.4. Statistical analysis

Data for incidence of canker-, scab-, and melanose-diseased fruit were subjected to a one-way ANOVA and the treatment means were separated using Student-Newman-Keuls multiple range test at $\alpha = 0.05$.

3. Results

3.1. 'Ruby Red' grapefruit

3.1.1. 2013 trial

Early season fruit infection (% incidence of old lesions) was substantial and similar to the incidence of later season infection (young lesions) because rainfall in April–May was relatively high compared to the 10-year average and remained above average throughout the season (Tables 3 and 4). Season-long soil drenches of SAR inducers were effective for reducing canker infection of 'Ruby Red' grapefruit compared to the UTC (Table 4). ASM drenched four times per season at the high rate (ASM-H) or rotations with THIA, IMID, or CLOTH were comparable to 10 sprays of CH for