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Suppression of greasy spot disease caused by *Mycosphaerella citri* Whiteside on grapefruit trees in an organic orchard using an aqueous organic mixture of composted cornmeal, humic acid, molasses, and fish oil versus vegetable oil☆

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ABSTRACT

Greasy spot disease of citrus, caused by the fungus *Mycosphaerella citri* Whiteside, afflicts citrus trees in all citrus-growing areas of the United States, eastern Mexico, Central America, and the Caribbean islands, causing premature defoliation, blemished fruit, and reduced tree vigor, yield, and fruit size. This three-year study investigated the effects of organic, nonconventional tactics using an aqueous organic mixture, steeped for 10 h, of composted cornmeal, humic acid, molasses, and fish oil and an aqueous suspension of vegetable oil, separately and in combination, for greasy spot suppression in an organic grapefruit, *Citrus paradisi* Macfad., orchard in the Lower Rio Grande Valley of Texas. The extent of greasy spot infection on each tree was consistently reduced by each of the treatments throughout much of every growing season. The organic mixture had a more consistent, though lesser, effect on greasy spot reduction when precipitation was relatively high in the spring than when spring rains were relatively light. In four of five field experiments conducted during this study, late season bare patches on the tree canopies were more extensive on the control trees than on treated trees. While fruit yield was not consistent in terms of treatment effects, the organic mixture and vegetable oil treatments each protected foliage from greasy spot infection to limited extents during years with wet springs. The greatest protection was observed during a year with a relatively dry spring.

1. Introduction

Greasy spot disease of citrus, caused by the loculoascomycete fungus *Mycosphaerella citri* Whiteside (Timmer et al., 1980; Mondal et al., 2004), afflicts citrus trees in all citrus-growing areas of Florida, Texas, eastern Mexico, Central America, and the Caribbean islands, with grapefruit, *Citrus paradisi* Macfad.; lemon, *C. limon* (L.) Burm.; and tangelo, *C. tangelo* J.W. Ingram & H.E. Moore, being the most susceptible (Timmer and Gottwald, 2000; Mondal and Timmer, 2006). Symptoms begin with pale yellow, slightly raised areas on the underside of leaves that turn brown or black with oily margins, while yellow spots appear on the upper leaf surfaces (Timmer and Gottwald, 2000; Mondal and Timmer, 2006). Left unchecked, greasy spot disease can result in premature defoliation which, in severe instances, may reach 100%, and fruit can be blemished with greasy spot rind blotch rendering it unsuitable for fresh market (Whiteside, 1982; Timmer and Gottwald, 2000; Mondal and Timmer, 2006). Infections can reduce tree vigor and yield, and fruit size (Timmer and Gottwald, 2000), although data on economic impact is sparse and largely anecdotal (Whiteside, 1977). In Florida, infections typically commence in summer, but symptoms become pronounced in the fall and winter (Whiteside, 1982). In south Texas, infections during wet summers can affect >80% of leaves on grapefruit trees by July or August (ATS, personal observation). The disease reproduces by pseudothecia, and the greatest source of inoculum is leaf litter on the orchard floor from which mature ascospores are released within 30–60 min of rain events; alternate wetting and drying perpetuates the releases and relative humidity (RH) favors survival and further spread particularly when it is >90% (Hidalgo et al., 1977; Timmer et al., 1980, 2000; Mondal and Timmer, 2003).

☆ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Greasy spot disease has been controlled using petroleum oils that conform to a specific range of distillation temperatures (Trammel and Simanton, 1966a,b; Whiteside, 1989), but efficacy has not always been reliable (Hidalgo et al., 1977) and where the disease is severe, or where rind blotch occurs, copper fungicides are often added (Whiteside, 1983, 1984). Copper fungicides, though effective (Whiteside, 1973, 1989), are associated with darkening of scars on grapefruit rinds and, if applied late, efficacy is substantially diminished (Whiteside, 1989). Only one midsummer copper fungicide application might be required if the timing is optimized, otherwise, as much as three sprays are necessary (Mondal and Timmer, 2005).

While certain oils and copper-based fungicides are considered to be organic, others are not. Some organic growers will apply copper-based fungicides in south Texas for greasy spot suppression on grapefruit, but prefer even "softer" tactics as alternatives to petroleum oil and copper-based fungicides. On some crops, compost steeped in water for a period of time to transfer soluble organic matter, beneficial microorganisms, and micro- and macronutrients into a solution called "compost extract" or "compost tea" (Scheuerell and Mahaffee, 2002, 2004, 2006; Hargreaves et al., 2008). Use of compost extract was initially promoted as a means of natural plant disease control (Hargreaves et al., 2008) by increasing microbial activity and diversity with populations of plant-beneficial and disease-antagonistic organisms in the soil and on plant foliage (Garbeva et al., 2004; Mazzola, 2004; Larkin, 2008). Depending on the compost used to "brew" it, compost extract has been reported shown to suppress incidence of fungal-based diseases, including gray mildew, *Cinerea botrytis*; late blight, *Phytophthora infestans*; downy mildew, *Plasmopara viticola*; powdery mildew, *Uncinula necator*; apple scab, *Venturia inequalis*; and *Fusarium* (Weltzein, 1989, 1990; Kai et al., 1990; Elad and Shtienberg, 1994; Cronin et al., 1996). The purpose of this study was to determine the effects of an aqueous mixture, steeped for 10 h, of composted cornmeal, humic acid, molasses, and fish oil (hereafter referred to as the "organic mixture") versus vegetable (non-petroleum) oil on greasy spot disease of grapefruit.

2. Materials and methods

This study was conducted in Southmost, an area bordered by the Rio Grande in subtropical Cameron County, Texas, and it involved two field experiments in a ≈18-ha, orchard of organically grown mature Rio Red grapefruit trees. Trees were spaced 5 m apart within rows and 8 m apart between rows, and flood irrigation occurred once each month. Weeds were controlled manually in February of each year on the orchard rows where the study occurred, otherwise cultural practices involving the orchard floor did not occur (hence leaf litter is assumed to have been the same between all of the trees used in the study).

The first experiment involved a total of 67 adjacent trees in four rows (0.25 ha), 37 of them nontreated, non-sampled buffer trees. Half of the 30 remaining trees were randomly assigned to be treated with the organic mixture, and the other fifteen trees were nontreated controls. Trees with obvious pre-existing problems, such as heavy limb dieback or mechanical pruning, were excluded, serving as some of the buffer trees.

The organic mixture (routinely used for greasy spot control by some organic grapefruit growers in the Lower Rio Grande Valley) was made using 2.3 kg composted cornmeal, 0.91 kg humic acid, 38 L molasses, and 38 L fish oil in an aerated, 758-L vat of water, steeped for 10 h. It was applied at the beginning of each month, May to November, unless rain and mud delayed access to the orchard. Spray dates in 2009 were 28 May, 3 June, 2 July, 5 August, 3 September, and 4 October; in 2010 spray dates were 5 May, 2 June, 20 July, 3 and 31 August, and 5 October; and in 2011, 29 April, 3 May, 4 June, 1 July, 3 August, 4 September, and 3 October. The organic mixture was applied using a tractor-pulled 1894-L Rears orchard sprayer (Midland Tractor, Madera, California) with 12 #3 flat fan nozzles on each side (total of 24 nozzles) operating at 7 kg/cm^2 and moving at 1.6 km/h. Buffer trees were opposite sprayed trees on both adjacent rows to avoid accidentally treating control trees by the high pressure sprayer overshooting the target tree.

Analysis for phenolic deposition (from the organic mixture applications) on leaf surfaces of 10 leaves, each leaf randomly selected from 4 m and lower (a stepladder was used but the uppermost 25% of the canopy was not sampled) on the canopies of 10 different trees in the control and in the organic mixture treatment, was conducted on 3 June 2009, one day after spraying the organic mixture. The individual leaves were sealed in a Whirl-Pak (Nasco, Salida, California) bag and the bags were stored on ice in a cooler. In the laboratory, the leaves were placed in 50 ml of 0.25 M $CaSO_4$ in a 250-ml Erlenmeyer flask. Each flask was stoppered and shaken vigorously for 15 min. A pipette was used to transfer 5 ml of the organic mixture to a test tube, mixed with 0.75 ml of 1.9 M Na_2CO_3 and 0.25 ml of Folin-Ciocalteau reagent, and allowed to stand at room temperature for 1 h. A Spectonic Genesys 5 spectrophotometer (Milton Roy Instruments, Nyland, Pennsylvania) was used to quantify absorbance at 750 nm, for which the standard line was prepared with tannic acid in the 0–0.05 mg/ml range.

Microbial populations on the leaf surfaces were analyzed on 10 leaves from separate control and the organic mixture-treated trees one day after application. Each leaf was aseptically placed in a Whirl-Pak bag kept out of the sun and in a cooler at ~21 °C for transport. In the laboratory, each leaf was placed in 50 ml of sterile physiological saline solution and shaken vigorously for 10 min. Ten-fold serial dilutions to 10^{-5} were made on organic mixture agar, and incubated at 25 °C for 5 d, then microorganisms were counted under a microscope. The organic mixture agar was prepared by adding 10 g of nonaqueous organic mixture to one liter of water, shaking the mixture vigorously for 5 min, allowing to stand for 3 h and straining the solids using a #40 mesh screen (60 mesh/cm^2). One liter of the resulting concoction was mixed with 0.25 strength trypticase soy agar base and 15 g of agar.

Incidences of greasy spot disease and melanose leaf disease [caused by a fungus, *Diaporthe citri* (Fawc.) Wolf], and presence of citrus mealybug, *Planococcus citri* (Risso), were determined by randomly examining 20 leaves on the north side and 20 leaves on the south side of each tree (the sides facing the alleys between rows) and averaging the two values. Leaves were sampled, using a stepladder, from all of the canopy excluding the topmost 10% which was not accessible because of the rounded shape of the canopies. In 2009, sampling occurred on 16 June (pre-treatment), 30 June, 14 and 28 July, 7 and 20 August, 1, 9, and 29 September, 14 and 28 October, and 10 November, in 2010 on 3 May and 3 June (both were pre-treatment dates), 23 June, 21 July, 18 August, 15 and 30 September, and 15 October, and in 2011 on 28 April, 11 and 28 May, 3, 23, and 30 June, 8 and 22 July, 5 and 19 August, 2, 16, and 30 September, and 15 October. Treatment differences were calculated for each date by subtracting the mean number of infected leaves in the organic mixture treatment from the mean number of infected leaves in the control. On 22 October 2009, 15 October 2010, and 24 October 2011, the percentage of each tree canopy that appeared yellow from greasy spot infection was estimated by two researchers, each conducting the assessment at different times of the same day, from the north and south sides of the tree and averaged between researchers and sides of the trees. On 4 November 2009, 22 October 2010, and 4 November 2011, the north and south sides of each experimental tree canopy was examined to visually estimate the percentage of canopy consisting of bare branches where