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Evaluation of Fungicides to Control Citrus Black Spot on 'Valencia' Caused by *Guignardia citricarpa* in South Florida

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A field trial to evaluate the efficacy of fungicides and timing of spray regimes to control the recently introduced fungus, *Guignardia citricarpa*, causing citrus black spot (CBS) was initiated. The trial was established at a commercial site in a block of 'Valencia' that was identified with CBS in 2011. Fifteen treatments including ten products were applied at intervals starting after fruit harvest and continuing through September. Ratings on the number of the symptomatic fruit on the tree and the number of dropped fruit with symptoms were taken in Dec. 2011 and Feb. and Mar. 2012. In general, most of the data from fungicidal regimes were not statistically different from the untreated plants in preventing symptoms of CBS. Additional field trials are needed to more fully investigate timing of applications and products for efficacy in suppression of this disease.

Citrus black spot (CBS), an exotic fungal disease of citrus caused by *Guignardia citricarpa* Viala & Ravaz (1892) nom cons. was identified in Collier County, South Florida in Apr. 2009 (Schubert, 2012). The disease has been described worldwide in Australia (Benson, 1895; Kiely, 1948), South Africa (Kotze, 1981a), Uganda (Reeder et al., 2009), Asia (Fawcett, 1936; Wang et al., 2012), South America (Garran, 1996; Reis et al., 2006; Rodriguez, 1996), and Cuba (Hidalgo Gongora and Perez Vicente, 2010). Symptoms of the disease are lesions on the fruit that can be of several distinct types named according to their appearance on the fruit. Fruit lesions include hard-spot also called shot hole spots, false melanose or speckled blotch, freckle spot and virulent spot (Kotze, 1981a; Kotzé, 2000), and cracked spot (de Goes, 2000). Several lesion types may be found on a single fruit; however it seems that lesion type is related to the age of the fruit, the weather conditions, and some believe the spore type (ascospores or conidia) responsible for the infection. Premature fruit drop occurs when substantial infection occurs on or near the pedicle and Benson in 1895 described the coalescing of lesions "virulent spots" that lead to premature fruit drop (Kiely, 1948). Crop losses per season due exclusively to fruit drop prior to harvesting, particularly on late maturing varieties associated with CBS have been estimated at 71% in Ghana (Brentu et al., 2012) and up to 80% fruit loss in untreated groves in Australia and South America (Calavan, 1960). In 2010, United States citrus export was valued at $2.9 billion, with $380.8 million attributed to fresh oranges (Boriss, 2010) Florida is the largest producer of oranges (69%) in the U.S., producing 6.4 million tons in 2010, followed by California, Arizona, and Texas (Boriss, 2010). The potential economic losses due to the disease either through fruit drop or rejection for the fresh market could be estimated in the hundreds of millions of U.S. dollars.

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Copper products have been used as a fungicide against several fungal diseases on citrus throughout Florida's history (Driscoll, 2004). With the introduction and establishment of citrus canker caused by the bacterial pathogen, *Xanthomonas citri* subsp. *citri*, there has been an increase in the quantities and frequency of copper applications per season since 1995 (Driscoll, 2004). The use of copper in managing citrus canker is implemented in most if not all, affected Florida citrus groves. Additionally, it has been suggested that fruit be sprayed on a 21-d interval from the quarter-inch stage to fully grown depending on the citrus variety. Currently, only copper and strobilurins (azoxystrobin, pyraclostrobin, and trifloxystrobin) are labeled for control of CBS in Florida.

The purpose of this field trial was to establish methodology for conducting fungicide field trials on CBS, evaluate the timing and efficacy of fungicides for suppression of CBS on citrus.

Materials and Methods

The experiment was conducted in a commercial grove that was identified with CBS in 2009. In spring 2011, sites within the grove were scouted and rows of 'Valencia' were identified that had fairly even distribution of fruit with symptoms of black spot. Plots contained three trees with at least two trees serving as buffers between each plot. Trees that were unthrifty or declining were not included in trial plots. There were four replications per treatment arranged in a randomized complete-block design. Sprays were initiated in May after fruit were harvested. Fungicides and application regimes are presented in Table 1. Foliar applications were applied according to KAC standard protocol. The spray dates were: 18 May, 8 June, 5 July, 21 July, 15 Aug., 13 Sept., and 5 Oct. for a total of seven sprays. Ratings of CBS on fruit were taken as follows. Spray materials and application regimes are presented in Table 1. Disease was assessed on 21 Dec. 2011 by collecting fruit under the canopy on the ground of the middle

Table 1. The rate and application date of fungicides and products including the active ingredients and fungicide group codes for the treatments applied to 'Valencia' orange trees to manage citrus black spot in field trials.

Trt. no.[z]	Product name	Active ingredient (a.i.) and % a.i. (FRAC code)[y]	Rate per acre	Application date[x]
1	Untreated control	---	---	---
2	Kocide 3000 DF	Copper hydroxide 30% (M1)	2.5 lb/acre	A
3	Gem 500SC	Trifoxystrobin (11)	2.0 fl oz/acre	A,C
	Kocide 3000 DF	Copper hydroxide 30% (M1)	2.5 lb/acre	B,D,E,F,G
4	Gem 500SC	Trifoxystrobin (11)	2.0 fl oz/acre	A,C,E,G
	Kocide 3000 DF	Copper hydroxide 30% (M1)	2.5 lb/acre	B,D,F
5	Gem 500SC	Trifoxystrobin (11)	2.0 fl oz/acre	A,C,E,G
	Enable 2FL	Fenbuconazole 23.5% (3)	8.0 fl oz/acre	B,D,F
	435 Citrus Oil	Mineral oil 99% (NC)	1% v:v	B,D,F
6	Enable 2FL	Fenbuconazole 23.5% (3)	8.0 fl oz/acre	A,B,C,D,E,F,G
	435 Citrus Oil	Mineral oil 99% (NC)	1% v:v	A,B,C,D,E,F
7	Enable 2FL	Fenbuconazole 23.5% (3)	8.0 fl oz/acre	A,C,E,G
	435 Citrus Oil	Mineral oil 99% (NC)	1% v:v	A,C,E,G
	Kocide 3000 DF	Copper hydroxide 30% (M1)	2.5 lb/acre	A,B,C,D,E,F,G
8	Enable 2FL	Fenbuconazole 23.5% (3)	8.0 fl oz/acre	A,C,E,G
	435 Citrus Oil	Mineral oil 99% (NC)	1% v:v	A,C,E,G
	Kocide 3000 DF	Copper hydroxide 30% (M1)	2.5 lb/acre	B,D,F
9	Kocide 3000 DF	Copper hydroxide 30% (M1)	3.5 lb/acre	A,C,E
	Quadris Top	Azoxystrobin 18.2% (11) + Difenoconazole 11.4% (3)	15.4 fl oz/acre	B,D,F,G
10	Serenade Max WP	*Bacillus subtilis* QST 713 (14.6%) NC	1 lb/acre	A,B,C,D,E,F,G
11	Serenade Max WP	*Bacillus subtilis* QST 713 (14.6%) NC	1 lb/acre	A,B,C,D,E,F,G
	Kocide 3000 DF	Copper hydroxide 30% (M1)	1 lb/acre	A,B,C,D,E,F,G
12	Serenade ASO	*Bacillus subtilis* QST 713 (13.96%) NC	2 qt/acre	A,B,C,D,E,F,G
	Kocide 3000 DF	Copper hydroxide 30% (M1)	1 lb/acre	A,B,C,D,E,F,G
13	Headline SC	Pyraclostrobin 23.3% (11)	9.0 oz/acre	A,B,C,D,E,F,G
14	Pristine	Pyraclostrobin 12.8% (11) + Boscalid 25.2% (7)	16 oz/acre	A,B,C,D,E,F,G
15	Magna-Bon CS2005	Copper sulfate pentahydrate 19.8% (M1)	100 ppm	A,B,C,D,E,F,G
16	Kocide 3000 DF	Copper hydroxide 30% (M1)	2.5 lb/acre	A,B,C,D,E,F,G
	Compound A	Non-fungicide[w]	1% v:v	A,B,C,D,E,F,G

[z]Treatment number.
[y]FRAC (Fungicide Resistance Action Committee) code (fungicide group): numbers (1–44) and letters (M, NC, U, P) are used to distinguish the fungicide mode of actions groups. All fungicides within the same group (same number or letter) indicate same active ingredient or similar mode of action.
[x]Spray application date codes are: A = 18 May; B = 8 June; C = 5 July; D = 21 July: E =15 Aug.; F = 13 Sept.; G = 5 Oct.
[w]Product labeled for use on citrus but does not have any fungicidal properties.

three trees within plot and fruit were counted for presence or absence of black spot lesions. On 15 Feb. 2012, fruit on trees were assessed by holding a square meter frame to both sides of three center trees in plots and counting clean fruit and fruit with lesions of CBS. A final rating of fruit raked from beneath trees was taken on 21 Mar. 12 and the number of CBS vs. clean was determined.

All data were analyzed using ANOVA and means separated by LSD in these tables. Data were also analyzed by GLM, before and after transformations, and also to decrease the significance of reps. Only non-transformed data is shown except for the total number of fruit dropped on 21 Mar., which showed significant differences after the square root arc sine transformation of the number of fruit.

Results and Discussion

This trial was the first field trial conducted by University of Florida and KAC together on the recently detected exotic CBS disease on citrus. The data collection was formulated on the reports from other countries that CBS causes significant drop of infected fruit (Brentu et al., 2012; Kotze, 1981a, 1981b). In the two ratings taken several months apart of fruit recovered under trees in test, no statistical differences were detected among treatments for the percentage of fruit exhibiting CBS symptoms (Table 2). Furthermore, the data collected from the fruit on the tree were not significantly different amongst treatments. No fungicides spray regimes were shown to prevent CBS lesions on fruit significantly compared to the untreated trees. Fungicides in this trial were copper compounds, QoI (quinone outside inhibitors), mineral oils, biological agents, DMI (demethylation inhibitors), and SDHI (succinate dehydrogenase inhibitors) that represented FRAC groups M1, 11, NC, NC, 3 and 7, respectively. All of the products are labeled on citrus but not all of them are labeled in the control of CBS. There is a pressing need to determine the most effective timing for application of the various products for the subtropical environment of Florida. Future trials will be conducted to evaluate the efficacy of the various fungicides in spray regimes similar to those conducted in this trial.

The lack of identifying effective compounds and/or spray regimes may have been due to one or more factors. One reason might have been that the data collected was not representative of

Table 2. Disease assessment of citrus black spot (CBS) in field experiment using various spray treatments to suppress disease symptoms on 'Valencia' fruit.

	Fruit (%) with symptoms of CBS		
	on ground	per m²	on ground
Trt. no.[z]	21 Dec. 2011	15 Feb. 2012	21 Mar. 2012
1	16.5	24.3	88.7
2	34.8	49.4	87.7
3	34.3	47.1	93.5
4	17.8	20.0	79.1
5	26.4	34.0	87.4
6	38.3	30.1	84.1
7	41.6	40.8	84.9
8	25.4	28.3	76.4
9	18.1	22.8	81.2
10	47.5	49.7	90.3
11	37.9	42.6	94.5
12	18.7	24.3	84.2
13	22.7	24.3	73.2
14	16.1	39.3	73.1
15	23.9	28.3	87.0
16	56.3	50.4	88.7
P =	0.2661	0.8413	0.7869

[z]Products and application dates for treatments are presented in Table 1.
[y]Data were analyzed by ANOVA and means separated by LSD at P values indicated within same column. No significant differences were detected.

the actual damage to fruit on trees receiving the various treatments. Another factor may have been that CBS has been observed to aggregate (Sposito et al., 2007), particularly on trees exhibiting stress (Kotze, 1981a), as opposed to an even distribution. The amount of inoculum within the test plot was probably uneven resulting in differences amongst the four treatment repetitions as reflected in the statistical analysis. Finally, the infection of trees within the plot by other diseases such as huanglongbing (or greening) and/or citrus canker may have caused fruit drop independent of CBS symptoms. Future trials will examine the field site for patterns of aggregation of CBS and also monitor the incidence and severity of other diseases within test plots. Additional data points, including more evaluations of fruit on the tree for symptoms of CBS, will be taken.

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