Sensitivity of *Colletotrichum orbiculare* Isolates in Virginia Watermelon to Thiophanate-methyl, Pyraclostrobin, and Prothioconazole

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ABSTRACT

Fungicide resistance development is a major concern for growers due to the limited modes of action available and the limited number of applications that can be used in the field. Determining sensitivity to fungicides and assessing risk of resistance is vital to the development of future chemistries necessary to inhibit or control pathogens. One common simple *in vitro* method of measuring sensitivity to fungicides is measuring radial growth of a pathogen exposed to multiple doses of fungicides in fungicide-amended agar to determine EC_{50} which is the concentration of fungicide that provides 50% inhibition of the isolate as compared to a non-fungicide-amended control. *Colletotrichum orbiculare* (syn. *C. lagenaria*) is the causal organism for anthracnose of cucurbits**.** Thiophanate-methyl and pyraclostrobin are common fungicides used to manage anthracnose in cucurbit crops. Prothioconazole, is labeled for use in cucurbits but not specifically recommended for management of anthracnose; however, control of anthracnose with fungicides containing this active ingredient has been observed in the field. A mycelial growth assay was conducted using fourteen *C. orbiculare* isolates collected from watermelon (*Citrullus lanatus*) in Southampton County, Virginia. Isolates were incubated on fungicide-amended PDA at fungicide concentrations 0 – 100 µg/mL of each fungicide, and the diameter of fungal growth on fungicide-amended and non-amended media was measured and compared to determine percentage of growth reduction. There was little variation in the fungicide sensitivity profiles of the fourteen isolates examined. Overall they were highly sensitive to pyraclostrobin

(EC_{50} <0.1 µg/mL), and insensitive to thiophanate-methyl (EC_{50} > 1 µg/mL) and prothioconazole (EC_{50} > 100 µg/mL).

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Introduction

Fungicide resistance development is a major concern for growers due to the limited modes of action (MOA) available and the limited number of applications that can be used in the field. Fungicide resistance can develop when fungicides of the same MOA are used in a frequent rotation pattern. Following seasons of repeated use of similar MOA fungicides, the targeted population becomes resistant when the susceptible population is eliminated, leaving only isolates that are resistant (Brent & Hollomon, 2007a). Key risk factors for fungicide resistance include single-site MOA, exclusive use of a given fungicide on highly susceptible cultivars, climatic conditions, and cultural practices favoring disease (Staub & Sozzi, 1984).

Monitoring of field populations for sensitivity is an important step in developing use patterns of fungicides necessary to inhibit or control pathogens. Determining risk for resistance involves integrating several factors. Establishing a baseline of sensitivity can be accomplished through several methods in the laboratory. Baselines are a profile of the sensitivity of the target fungus to the fungicide that has been determined by using biological or molecular techniques to examine the response of previously unexposed fungal individuals or populations to the fungicide (Russell, 2004). One common simple *in vitro* method is measuring radial growth of a pathogen exposed to a range of fungicide doses in fungicide-amended agar to determine EC_{50} (also referred as ED_{50}) which is the concentration (dose) of fungicide that provides 50% inhibition of the isolate as compared to a non-fungicide-amended control (Brent & Hollomon, 2007b). Higher EC_{50} values indicate reduced sensitivity and possible resistance to the fungicide (Secor & Riveria, 2012). However, choosing a technique to determine sensitivity depends on the purpose of monitoring and the fungus/fungicide combination; not all fungicides inhibit spore germination

so alternative or additional procedures may be required and have been established (Staub & Sozzi, 1984).

Colletotrichum orbiculare (syn. *C. lagenaria*) is the causal organism for anthracnose of cucurbits including watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), pumpkin (*Cucurbita pepo*), squash (*Cucurbita maxima*) and gourd (*Lagenaria siceraria*) (Keinath, Wintermantel, & Zitter, 2017; Damm, Cannon, Liu, Barreto, Guatimosim, & Crous, 2013). Cucurbit anthracnose is a common foliar disease in humid regions that causes lesions on seedlings, leaves, petioles, stems, and fruit (Keinath et al, 2017). Characteristics of *C. orbiculare* include straight conidia with an obtuse apex, dark brown to black or greyish black cultures, and slow growth (Sutton, 1992). Black stromata with black setae and hyaline conidiophores are produced by the pathogen on the host; conidia sprout at tips of the conidiophores and accumulate in a salmon pink mass (Keinath et al, 2017). Combination of 2h minimal duration leaf wetness with 21 to 24°C temperatures provide optimal environmental conditions for rapid disease development and growth of *C. orbiculare* (Monroe, Santini, & Latin, 1997). *C. orbiculare* has the ability to overwinter on crop debris and develop slowly during the first cycle growing season when cooler temperatures are present (Peterson & Campbell, 2002).

The use of disease resistant cultivars, crop rotation, and early season planting are best recommended practices for disease management of cucurbit anthracnose. Fungicides are available and commonly used for control. Benzimidazoles, quinone outside inhibitors (QoI), dicarboximides, and demethylation inhibitors are groups of single-site MOA fungicides that are labeled for anthracnose control (Keinath et al, 2017). Thiophanate-methyl 70WP, a benzimidazole, at 0.5 lb/acre and pyraclostrobin 20WG, a QoI, at 12 to 16 oz/A are two fungicides recommended for anthracnose control. Foliar applications of these fungicides are

typically applied three times during the growing season for management of cucurbit anthracnose (Quesada-Ocampo, 2018). Prothioconazole, a triazolinthione, is not listed specifically as a recommendation for cucurbit anthracnose management, but is labeled for use on cucumber and watermelon in several mid-Atlantic states for control of numerous foliar diseases (Wyenandt, 2018). Prothioconazole has been noted to control cucurbit anthracnose in field observations (D. L. Langston, personal communication, October 31, 2017).

Thiophanate-methyl is a thiophanate fungicide of the methyl benzimidazole carbamate (MBC) group, designated Group 1 by the Fungicide Resistance Action Committee (FRAC) and listed as high risk for fungicide resistance (FRAC, 2018). MBCs bind to the β-tubilin subunits and inhibit nuclear division disrupting mitosis (Davidse, 1986). Fungicide resistance to MBCs has been determined for many fungal species and in most cases, is associated with point mutations in the TUB2 gene that alter amino acid sequences at the binding site, most commonly mutations at positions 198 and 200 (Ma & Michaildes, 2005).

Pyraclostrobin is a methoxy-carbamate fungicide from the QoI group which is designated as Group 11 by FRAC and also regarded as high risk for development of fungicide resistance (FRAC, 2018). The QoI fungicide MOA is the inhibition of the fungal mitochondrial respiration (Krämer, Schirmer, Jeschke, & Witschel, 2012a). These fungicides prevent mitochondrial respiration of fungi by binding to the cytochrome bc1enzyme complex (complex III) at the Qo site, which blocks electron transfer and terminates ATP synthesis (Bartlett, Clough, Godwin, Hall, Hamer, & Parr-Dobrzanski, 2002). A common mechanism of fungicide resistance to QoI fungicides is the amino acid substitution of glycine by alanine at position 143 (G143) of the cytochrome b protein (Ma, Felts, & Michailides, 2003).

Prothioconazole, a triazolinthione, is a systemic fungicide that controls various diseases in large and small acreage crops. Prothioconazole is a demethylase-inhibitor (DMI) fungicide of the sterol biosynthesis inhibitor (SBI) class 1 group designated Group 3 by FRAC and considered at medium risk of resistance development (FRAC, 2018). Prothioconazole MOA is inhibition of sterol biosynthesis which disrupts membrane structure (Krämer, Schirmer, Jeschke, & Witschel, 2012b). Common mechanisms of fungicide resistance to DMI fungicides are mutations in the target gene cyp51 or overexpression of the cyp51 gene, but it is suggested that other mechanisms may also be responsible (Ma, Proffer, Jacobs, & Sundin, 2006).

Review of Literature

Resistance to thiophanate-methyl has been studied and documented in several pathogens including *Fusarium* spp., *Botrytis cinerea*, and *Monilinia laxa* (Petkar, Langston, Buck, Stevenson, & Ji, 2017; Zhou, Lu, Zhang, Qi, Wang, & Zhang, 2017; Egüen, Melgarejo, & De Ca, 2016; Amiri, Zuniga, Mertely, & Peres, 2014). Resistance to pyraclostrobin was determined in studies of the pathogen *Alternaria alternata* observed in pistachio isolates from California orchards along with resistance to boscalid, a fungicide chemistry often used in rotation with pyraclostrobin (Avenot & Michailides, 2015). Results of studies with *Phomopsis vexans* in eggplant revealed pyraclostrobin displayed strong activity in the inhibition of the mycelial growth, conidium germination, and disease lesion development using isolates both sensitive and resistant to benzimidazoles (Zhang, Dai, Di Wang, & Zhang, 2016). In *in vitro* studies examining fungicide resistance of *Fusarium graminearum* in wheat, moderate resistance to prothioconazole was determined but isolates showed little resistance to thiophanate-methyl (Rekanović, Mihajlović, & Potočnik, 2010). Sensitivity studies conducted in 2007 using isolates from North Dakota sugar beet with *Cercospora beticola*, the causal organism of leaf spot in sugarbeet (*Beta*

vulgaris), determined prothioconazole sensitivity with average EC_{50} values of 0.765 µg/mL (Secor, Rivera, Khan, & Gudmestad, 2010).

Several studies have examined sensitivity and resistance risk of various *Colletotrichum* species. Isolates collected from creeping bentgrass in Mississippi and Alabama with symptoms of *Colletotrichum cereal*, the causal organism of turfgrass anthracnose, were examined for resistance to thiophanate-methyl; measurements of radial colony growth on amended PDA revealed resistance at a dose of 10 µg/mL (Young, Tomaso-Peterson, de la Cerda, & Wong, 2010). Isolates from Brazilian banana orchards with anthracnose symptoms from *Colletotrichum musae* were tested for resistance to thiophanate-methyl. Results showed 96% of isolates sensitive (EC_{50} <10 µg/ml) and 4% as moderately resistant (EC_{50} between 10 and 100 µg/ml); resistant isolates (EC_{50} >100 µg/ml) were not found (Vieira, Lima, Nascimento, Michereff, Reis, Doyle, & Camara, 2017). Researchers in Japan examined isolates from fruit crops, acacia, and tea with anthracnose from *Colletotrichum gloeosporioides* and *C. acutatum* for sensitivity and resistance to thiophanate-methyl and other fungicides; thiophanate-methyl doses of 0, 1, 10, and 100 µg/ml were used and revealed 5 highly resistant, 3 intermediately resistant, and 21 sensitive isolates based on determined EC_{50} values (Chung, Ishii, Nishimura, Fukaya, Yano, & Kajitani, 2006). In China, researchers observed sensitivity of *C. acutatum* isolates in chili (*Capsicum* spp.) to thiophanate-methyl and pyraclostrobin; inhibition of mycelial growth was <0.66 µg/mL for both fungicides (Gao, He, Li, Mu, Lin, & Liu, 2017). In Florida, *C. acutatum* isolates collected from strawberry fields between 1994 and 2011 showed sensitivity to azoxystrobin (EC_{50} = 0.30 µg/mL) and pyraclostrobin (EC_{50} = 0.014 µg/mL), but a majority of isolates collected between 2012 and 2015 were not inhibited when exposed to 100 µg/mL azoxystrobin and 10 µg/ml

pyraclostrobin indicating resistance development in the fungal population (Forcelini, Seijo, Amiri, & Peres, 2016).

Purpose of Project

The objectives of this project were to 1) research previous methodology in several *Colletotrichum* studies and determine best procedural methodology for conducting mycelial growth inhibition assays with *C. orbiculare*, and 2) examine sensitivity of *C. orbiculare* isolates from watermelon in Virginia to thiophanate-methyl, pyraclostrobin, and prothioconazole. Thiophanate-methyl and pyraclostrobin are two fungicides recommended for cucurbit anthracnose management and prothioconazole is a fungicide that has been noted to provide some control of *C. orbiculare* in field observations. This work will provide a basic framework for future studies on the fungicide sensitivity of *C. orbiculare* in the region.

Materials and Methods

Materials: For isolate growth, potato dextrose agar (PDA) was prepared by combining 500 mL purified water and 19.5 g PDA in 1L glass bottles and autoclaving for 30 min. Molten solution was cooled to 32°C and 500 μg streptomycin and 500 μg chloramphenicol were added to eliminate bacterial contamination and then poured into disposable sterile 100 mm x 15 mm Petri dishes.

For single-spore purification of isolates, ¼ strength water agar was prepared by combining 500 mL purified water and 7.5 g Bacto agar in 1L glass bottles and autoclaving 30 min. Molten solution was cooled to 32°C and 500 μg streptomycin and 500 μg chloramphenicol were added to eliminate bacterial contamination prior to pouring solution into Petri dishes.

Isolates: Foliage and stem sections of watermelon (*Citrullus lanatus*) with symptoms of anthracnose were collected from a single site in Southampton County, Virginia. Fourteen isolates were obtained using methods similar to Cao et al, 2017. Pieces of symptomatic tissue were cut from the margin of lesions, soaked in 10% commercial bleach for one minute, rinsed in distilled water, and placed on sterile paper towels to dry. Surface-disinfested pieces were placed onto PDA medium. Isolates were incubated at room temperature (21 to 24°C) until fungal colonies had grown. Growing edges of fungal hyphae from the tissues were transferred to new PDA plates. An initial observation for morphological and cultural characterization of isolates was obtained by plating 5 mm disks taken from the edge of a 5-day old actively growing culture and placing two pieces each on 3 non-amended PDA plates incubated separately at 25°C in total dark, 12:12 hr dark/light photoperiods, and total fluorescent light conditions. Cultures were observed for mycelial growth diameter at five days; results revealed cultures grown for 5 days in total darkness were slightly larger in diameter. For single spore isolations, conidia were obtained from cultures using sterile wooden toothpicks and touched to one corner of 100 mm x 15 mm plates of ¼ water media. One drop of purified water was suspended onto initial point of introduction and then spread aseptically across surface of plate using a sterile glass spreader. Plates were incubated at 25°C for 12-16 hours in a dark growth chamber. Retrievable germinated single-spores were identified under a microscope and transferred to new PDA plates. Reserve isolates of dried mycelial growth were preserved on sterile filter paper and stored at -20°C. Active cultures were maintained for *in vitro* testing.

Chemicals and amended fungicide media: Technical grade thiophanate-methyl (Empirical Formula (Hill Notation) $C_{12}H_{14}N_4O_4S_2$; Pestanol™ analytical standard), pyraclostrobin (Methyl {2-[1-(4-chlorophenyl)-1*H*-pyrazol-3-

yloxymethyl]phenyl}methoxycarbamate; Pestanol™ analytical standard), and prothioconazole (2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione) were obtained from Sigma-Aldrich® Inc. Technical grade chemicals were used to eliminate possible interference of compounds present in commercial fungicide formulations.

Technical grade fungicides were dissolved in acetone to create stock solutions of serial tenfold dilutions of each fungicide from 0.001 to 100 µg/mL depending on fungicide observed. A stock solution of salicyhydroxamic acid (SHAM) was prepared by combining 400 mg SHAM to 4,000 µL methanol for use in pyraclostrobin amended fungicide and its untreated control; the use of SHAM is necessary with pyraclostrobin to inhibit the alternative oxidase respiration pathway (Møller, Bérczi, Plas, & Lambers, 1988).

Amended fungicide medium solutions were prepared by adding 500 µL fungicide stock solution concentration to 500 mL autoclaved molten PDA cooled to 55°C. 500 µg SHAM solution was added to 500 mL fungicide medium with pyraclostrobin. Molten solutions were poured into sterile disposable 60 mm x 50 mm Petri dishes. Due to observed smaller diameter growth of culture colonies, 60 mm x 50 mm Petri dishes were used to conserve on amount of medium necessary for the study.

Radial growth measurements: A test was conducted using a 5 mm plug from single-spore isolate growth placed on plates containing 1.0 µg/mL amended fungicide solution and untreated controls (PDA and PDA + SHAM) to determine appropriate µg/mL range for each fungicide. Separate tests were conducted for each fungicide using amended fungicide plates and one untreated control; four replicates were used with each of the 14 isolates. A core-borer was used to obtain a 5 mm single spore subculture from the original non-amended media and transferred inverted onto the center of the amended fungicide plate. Plates were placed in a dark

growth chamber and incubated at 25°C for 5 days. Radial growth was evaluated by making two perpendicular measurements across the colonies and averaging the diameters.

Data Analysis

Data were converted to a percent reduction of growth by comparing the average colony diameter data on amended media to the average colony diameter data on non-amended PDA agar medium; growth reduction data was evaluated to determine EC_{50} values for all isolates by plotting logarithmic fungicide concentrations vs. reduction of colony growth and locating the fungicide concentration point on the curve where growth is reduced by 50% (Secor et al, 2012). Analysis of variance was used to determine statistical means for percent of growth reduction using SAS© Vers. 9.4 for Windows software (SAS Institute, Cary, N.C.).

Results

In an observation to determine µg/mL ranges for each of the three fungicides, measurements of average radial diameter growth at 5d were as follows: control (non-amended PDA) 2.1 cm; control w/SHAM 1.8 cm; and prothioconazole 1 µg/mL 1.95 cm. Thiophanate-methyl and pyraclostrobin at 1 µg/mL fungicide concentration showed no growth and plugs were blackened with no living culture observable. Based on results, fungicide concentration test ranges were determined as 0 (control), 0.0001, 0.001, 0.01, 0.1, and 1.0 µg/mL for thiophanate-methyl and pyraclostrobin w/SHAM; however due to researcher error during experimentation, 1.0 was unintentionally omitted from thiophanate-methyl observation. Test range for prothioconazole was determined as 0 (control), 1, 10, and 100 µg/mL. Following little to no growth reduction of isolates on thiophanate-methyl amended agar, a second test range of 0 (control), 0.125, 0.25, 0.5, 0.75, and 1 µg/mL amended fungicides was conducted.

For thiophanate-methyl, there was little growth reduction (0.9 – 5.1%) for any of the fungicide concentrations from ranges 0.0001 to 0.1 µg/mL. Similarly, little growth reduction was observed with prothioconazole (2.0 – 4.7%) with fungicide concentrations 1 to 100 (Table 1). For pyraclostrobin w/SHAM, growth reduction ranged from 4.1% to 67.2% with EC_{50} value > 0.01 and <0.1 µg/mL, respectively (Figure 1). Results for pyraclostrobin were further supported by analysis of isolates individually, but statistically there were no differences in sensitivity among isolates (P=0.23); growth reduction ranged from 20.6 – 41.5% at 0.01 µg/mL and from 54.6 – 63% at 0.1 µg/mL (Figure 2). Additional testing with thiophanate-methyl at 0 (control), 0.125, 0.5, 0.125, 0.5, 0.75 and 1.0 showed growth reduction from -1.3 to 1.2% only and growth on the fungicide amended media was not significantly different from the control (P=0.61) (Table 2).

Discussion

In this study, *C. orbiculare* isolates from a watermelon field in Virginia were assessed for their sensitivity to different fungicides that are used in the management of anthracnose. There was little variation in the fungicide sensitivity profiles of the fourteen isolates examined, and overall they were highly sensitive to pyraclostrobin (EC_{50} <0.1 µg/mL) and relatively insensitive to thiophanate-methyl (EC_{50} > 1 µg/mL) and prothioconazole (EC_{50} > 100 µg/mL). For anthracnose control in the field, fungicides applications are made up to three times per growing season. Pyraclostrobin is applied at 0.2 lb a.i./A, thiophanate-methyl at 0.35 lb a.i./A, and prothioconazole at 0.178 lb a.i./A. All of the active ingredients examined in this study are applied at similar rates in the field; however, the sensitivity of isolates to the fungicides in the lab assays was highly variable. Based on sensitivity of the isolates to active ingredients, the applied rates of pyraclostrobin are more than enough to control the disease.

This project was a review of studies to determine best procedural methods for future studies to examine the risk of fungicide resistance to chemistries used to combat cucurbit anthracnose by growers. Ten *Colletotrichum* sensitivity studies were reviewed for establishing methodology for the present study. Choice of medium used in fungicide-amended plates showed majority use of PDA with exceptions of one with ¼ PDA and one study utilizing yeast bacto agar; environmental conditions for mycelial growth included incubation period for growing isolates ranging 3 – 7d, no supplemental lighting with exception of one study using 12 hr:12 hr dark:light photoperiods, and an incubation temperature range of 22 – 28°C (Cao et al, 2017; Gao et al, 2017; Viera et al, 2017; Wong & Midland, 2017; Ishii, Zhen, Hu, Li, & Schnabel, 2016; Forcelini et al, 2016; Torres-Calzada, Tapla-Tussell, Martin-Mex, Nextican-Garcez, & Perez-Brito, 2015; Young et al, 2010; Chung et al, 2006; Mondel et al, 2005).

With exception of pyraclostrobin at the 0.1 µg/mL amended concentration, results revealed growth reduction percentages ≤5.1 % with thiophanate-methyl and ≤2.7% with prothioconazole-amended medium. In the test of observation of mycelial growth using a single plug on 1 µg/mL fungicide amended medium, no growth or remaining live culture was shown with exposure to thiophanate-methyl; thus a decision to use lower concentrations of thiophanate-methyl was made. In thiophanate-methyl testing that followed, growth inhibition at 0.0001, 0.001, 0.01, and 0.1 µg/mL concentration was 0.9, 2.3, 2.2, and 5.1% respectively, demonstrating a trend of increasing growth reduction. Even though this increase in growth reduction was not repeated when isolates were tested again on thiophanate-methyl amended fungicide between 0.1 and 1.0 concentrations, based on the previous trend observed and in studies reviewed of sensitivity to thiophanate-methyl, higher concentrations up to 100 µg/mL should be tested in the future.

The objective of this project was to examine the sensitivity of a field population of *C. orbiculare* isolates to various fungicide active ingredients and to provide a basic framework for future studies on the fungicide sensitivity of *C. orbiculare* in the region. The methodology used for this objective was adequate, but higher concentrations of two of the fungicides, thiophanate-methyl and prothioconazole, should have been used. For future studies, it would be useful to have a baseline isolate or one that has been identified as highly sensitive to the different fungicides in previous studies. In baselines studies, isolates are collected from areas and crops that have not been treated with the fungicide of interest in the current or previous seasons, including fungicide active ingredients that show cross resistance (Russell, 2004). History of fungicide applications for the isolates used in the present study was unknown, and if cucurbits had been grown in this field in past seasons, it is highly likely that the fungal population had previous exposure to fungicides.

The limited number of isolates obtained for this study were from a single site, but it can be concluded that this population of *C. orbiculare* is highly sensitive to pyraclostrobin and relatively insensitive to thiophanate-methyl and prothioconazole. Future studies are needed to determine variation in sensitivity among different fungal populations. A limited number of samples from a limited number of plants from a single location have little chance of being representative of the entire population (Russell, 2004).

While the method used in this study worked given the objective, *in vitro* evaluation of mycelial growth inhibition is only one procedure used to evaluate sensitivity of pathogens to a fungicide. Additional procedures such as spore germination and germ tube elongation assays are often used. Mycelial growth inhibition assays are not always completely effective alone to assess the interaction between fungicide and fungal population as well. For example, as QoI fungicides

are powerful inhibitors of spore germination so an assay based on spore germination may be a more appropriate method for determining sensitivity of fungi (Mondal, Bhatia, Shilts, & Timmer, 2005). Furthermore, *in vivo* testing by observing the pathogen and fungicide interaction in greenhouse or field studies, can be utilized to examine sensitivity and fungicide resistance risk for populations (Russell, 2004).

It is recommended at the conclusion of this evaluation that future studies of *C. orbiculare* for sensitivity and resistance to prescribed fungicides use the methodology described herein for observing mycelial growth inhibition and utilize isolates obtained from several sources for more thorough representation of fungal populations throughout the region.

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APPENDIX A: Instrumentation

- Consolidated Stills & Sterilizers Mark II autoclave, model SSR-2A-PB
- Motic BA410T-LED biological microscope
- Thermo Scientific Barnstead MicroPure UV/UF water purification system,
- Thermo Scientific Forma environmental chamber, model 3940
- Labconko Logic+ biological safety cabinet
- Ohaus weighing scale
- Thermo Scientific Super-Nuova multi-place stirrer and stirring hot plates

APPENDIX B: Supporting documents

Table 1. Percent growth reduction of C. orbiculare isolates on thiophanate-methyl, pyraclostrobin/SHAM, and prothioconazole fungicide amended agar.

	----------% growth reduction[1]----------								
µg/mL:	0.0001	0.001	0.01	0.1	1	10	100	p(*f*)[2]	LSD
Fungicide									
Thiophanate-methyl	0.9b	2.3b	2.2b	5.1a	- -	- -	- -	0.006	2.63
Pyraclostrobin + SHAM	4.1e	8.5d	31.5c	59.3b	67.2a	- -	- -	0.0001	2.31
Prothioconazole	- -.	- -	- -	- -	2.0bc	4.7a	2.7ab	0.004	2.56

[1] Percent growth reduction calculated with comparison to growth of non-fungicide amended control. (- -) = not tested at given concentration.

[2] Means followed by the same letter(s) in a row are not significantly different according to Fisher's Protected LSD (P=0.05).

Table 2. Percent growth reduction of C. orbiculare isolates on thiophanate-methyl amended agar.

	----------% growth reduction[1]----------						
µg/mL:	0.125	0.25	0.50	0.75	1.0	p(*f*)	LSD[2]
Fungicide							
Thiophanate-methyl	0.8	1.2	-1.3	0.8	1.2	0.61	N.S.

[1] Percent growth reduction calculated with comparison to growth of non-fungicide amended control.

[2] N.S. = non-significant

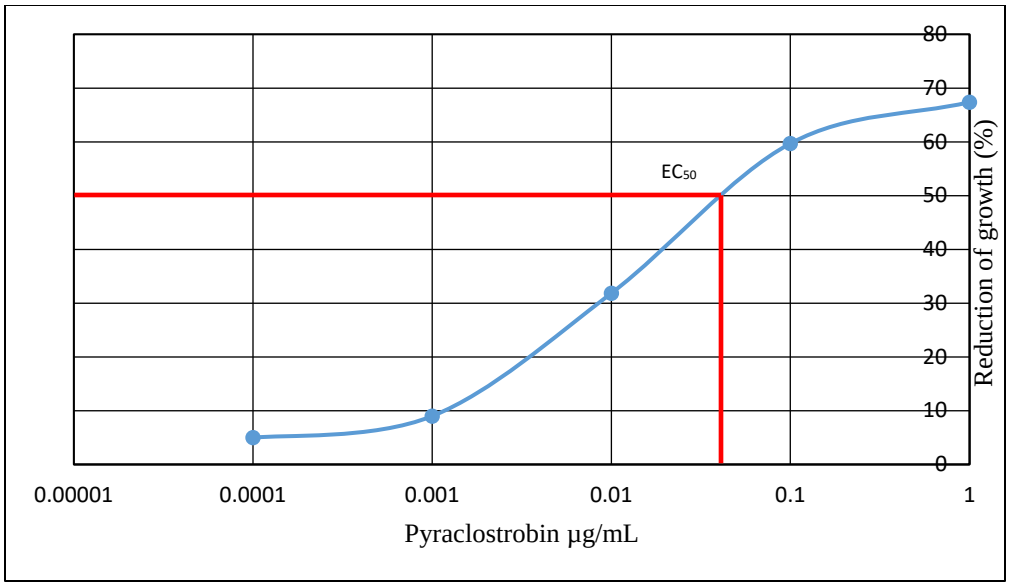

Figure 1. Growth reduction of C. orbiculare on PDA amended with different concentrations of pyraclostrobin.

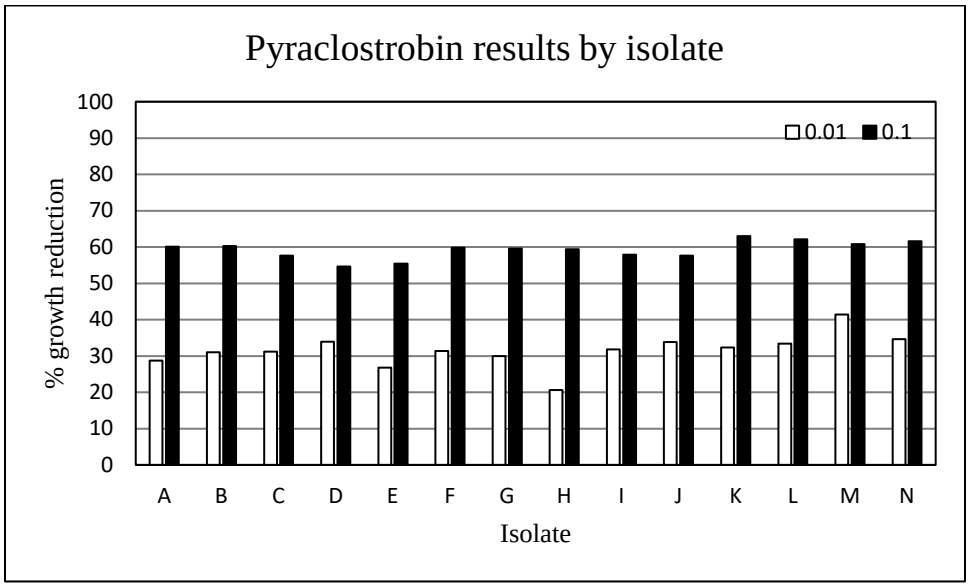

Figure 2. Variation in sensitivity to pyraclostrobin among fourteen C. orbiculare isolates from a single watermelon field in Virginia. White bars = 0.01 µg/mL and black bars = 0.1 µg/mL pyraclostrobin.

Disclaimer

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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