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Chemical management of Fusarium wilt of watermelon

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ABSTRACT

Watermelon yield loss due to Fusarium wilt is increasing in the U.S., due in part to the emergence of the virulent race 2 of *Fusarium oxysporum* f. sp. *niveum*, and to the shift in production to triploid cultivars, which generally have less host resistance than previously grown diploid cultivars. One potential management strategy is the use of soil-applied fungicides to reduce Fusarium wilt. The U.S. national program, interregional project 4 (IR-4) supported multistate trials of soil-applied chemicals to manage Fusarium wilt of watermelon. Greenhouse trials were conducted in Maryland, Indiana and Georgia to test the efficacy of 14 chemicals on Fusarium wilt. Based on the performance of these chemicals in the greenhouse, six in Maryland and Delaware and eight in Indiana were selected for subsequent field evaluations. These chemicals were applied once, as a drench at planting, in field trials in Maryland, Indiana, and Delaware in 2008. The fungicides prothioconazole, acibenzolar-S-methyl, and thiophanate-methyl resulted in the greatest reduction in Fusarium wilt, and caused no phytotoxicity. In Maryland and Indiana in 2009, these chemicals were applied through the drip irrigation line alone and in combination, at 0, 2 and 4 weeks after planting. The experiment was repeated in 2010 in Maryland. Prothioconazole alone and in combination with acibenzolar-S-methyl or/and thiophanate-methyl resulted in the greatest decrease in the area under the disease progress curve (AUDPC) of Fusarium wilt of watermelon in Maryland in 2009. The same trend was observed in 2010 in Maryland where three of the prothioconazole treatments ranked the lowest of all treatments and prothioconazole in combination with thiophanate-methyl had significantly lower Fusarium wilt AUDPC compared to the non-treated control. All chemical applications except for acibenzolar-S-methyl in combination with prothioconazole reduced Fusarium wilt AUDPC in Indiana in 2009. Prothioconazole alone and prothioconazole in combination with thiophanate-methyl ranked lowest in Fusarium wilt AUDPC, although not significantly lower than most other treatments. These studies are the first to demonstrate that the soil-applied fungicides prothioconazole and thiophanate-methyl may provide an additional field management option for Fusarium wilt of watermelon.

1. Introduction

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (FON), is the most severe soilborne disease of watermelons worldwide. The first symptom that appears in infected plants is a loss of turgor pressure of the leaves and vines. Initially plants appear to recover at night and only one or a few runners are affected (Kleczewski and Egel, 2011). However, as the disease progresses, infected plants turn from dull green to yellow and eventually become necrotic (Zitter et al., 2010). The vascular system of infected plants becomes discolored as the fungus invades and as the watermelon host forms tyloses as a defense response (Egel and Martyn, 2007). Severity of Fusarium wilt is positively correlated with the degree of colonization by the fungus in the vascular system (Zhou and Everts, 2004a). Plants that do not die are stunted and produce fewer and smaller fruit. Fusarium wilt is difficult to

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manage because the pathogen can survive for long periods of time in soil as chlamydospores. In addition, the pathogen survives on many symptomless hosts (Gordon et al., 1989).

Until recently, Fusarium wilt of watermelon in the United States was primarily managed through host resistance to FON race 1, which was present in most diploid cultivars (Egel and Martyn, 2007). However, the increase in production of triploid (seedless) cultivars, most of which lack host resistance to FON race 1, has resulted in the resurgence of losses due to Fusarium wilt. The prevalence of race 2 of FON has also led to an increase in Fusarium wilt. FON race 2, which is more virulent than race 1, is able to overcome resistance in diploid cultivars (Martyn, 1985). FON race 2 has now been reported in Texas (Martyn, 1985), Oklahoma (Bruton et al., 1988), Florida (Martyn and Bruton, 1989), Maryland (Zhou and Everts, 2001, 2003), Delaware (Zhou and Everts, 2001, 2003), Georgia (Bruton et al., 2008), Indiana (Egel et al., 2005) and South Carolina (Keinath and DuBose, 2009). In some regions the prevalence of FON race 2 is very high. For example, surveys of watermelon production fields in Maryland and Delaware found that the highly virulent, race 2 of FON, was present in 24% of fields (Zhou and Everts, 2003). Also of concern is the recent report of a new more virulent race, race 3, which was identified in Maryland (Zhou et al., 2010). The increase in yield losses due to Fusarium wilt throughout the eastern U.S. has increased the need for alternative control practices. Potential alternatives include grafting, use of cultural practices, and biological and chemical controls. Grafting onto non-host root stock reduces Fusarium wilt and increases fruit size and number (Keinath and Hassell, 2014). Although grafting is common in Asia and other parts of the world (Cohen et al., 2007; Louws et al., 2010), it remains prohibitively expensive in the U.S. (Davis et al., 2008). Significant research efforts are underway on biological and cultural practices to manage Fusarium wilt. Use of the cover crops *Vicia villosa* (hairy vetch) or *Trifolium incarnatum* (crimson clover) prior to production of watermelon reduces Fusarium wilt in some environments (Himmelstein et al., 2014; Zhou and Everts, 2004b, 2006). However, additional practices are needed. Some biological products are labeled for use on Fusarium wilt, however results in trials to evaluate efficacy remain mixed (Himmelstein et al., 2014). Therefore, commercial use of biological products remains very low (Fravel et al., 2005; Zhou and Everts, 2006).

While few studies exist on the use of fungicides for management of FON, some related *formae speciales* have been evaluated for sensitivity to fungicides. *In-vitro* evaluations of fungicides have been conducted on *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *gladioli*, and *F. oxysporum* f. sp. *lilii*. Prochloraz, bromuconazole, benomyl, and carbendazim significantly reduced mycelial growth of *F. oxysporum* f. sp. *lycopersici* in-vitro (Amini and Sidovich, 2010). However, fludioxonil and azoxystrobin did not. Chung et al. (2009) found that six isolates of *F. oxysporum* f. sp. *gladioli* and 13 isolates of *F. oxysporum* f. sp. *lilii* varied in their sensitivity to benomyl, thiophanate-methyl, carbendazim and thiabendazole. They attributed this variation to resistance development.

Additional studies have been conducted in the greenhouse. Reid et al. (2002) evaluated benomyl, fludioxonil, and thiabendazole on Fusarium crown and root rot (*F. oxysporum* f. sp. *asparagi* and *F. proliferatum*) on greenhouse grown asparagus. At high pathogen inoculum levels, all three fungicides reduced plant death compared to no treatment. Fludioxonil significantly reduced plant death compared to benomyl and thiabendazole was intermediate. These greenhouse evaluations indicated that chemical control may be a potential option for field-grown watermelons.

Following the resurgence of watermelon Fusarium wilt, the U.S. national program, interregional project 4 (IR-4) received several requests for the registration of fungicides for watermelon Fusarium wilt. However, without efficacy data agrichemical companies had little interest in registration of their products. The objectives of this study were to 1) conduct greenhouse screens of chemicals to reduce Fusarium wilt of watermelon and identify candidates for field evaluation and 2) evaluate the efficacy of chemicals in field production systems that are typical in the eastern, midwestern and southern United States.

2. Materials and methods

2.1. Greenhouse experiments

Greenhouse trials were conducted in the winter of 2007–2008 at the University of Maryland's Lower Eastern Shore Research and Education Center, Salisbury, MD; Southwest Purdue Agricultural Center, Vincennes, IN; and the University of Georgia, Tifton, GA. A nontreated control along with 13 fungicides, and the resistance inducing chemical, acibenzolar-S-methyl (Actigard, Syngenta Crop Protection, Inc., Greensboro, NC) were evaluated when applied at a single rate in soil that was inoculated with FON. In addition, Actigard was applied at a second rate (0.02 g/L) in Salisbury, MD. The experiments were conducted as a randomized complete block with four replications; an experimental unit was a pot filled with 1.5 L of 4:1:1 (v:v:v) of sand:peat:vermiculite. The watermelon cultivar 'Black Diamond' (Indiana) or 'Sugar Baby' (Maryland) was seeded six to a pot and thinned to three per pot approximately 7 days later. Fungicides were applied 150 mL per pot at the first true leaf stage. Fungicide rates were calculated based on the product label rate per hectare. Three days after fungicide application, 150 mL of a race 1 strain of FON was applied to each pot. In Indiana, an additional control treatment received water instead of fungicide, but was not inoculated with FON.

A race 1 strain of FON was stored on sterile filter paper at 4 °C. Four 1-cm disks from the leading edge of a FON colony on PDA were added aseptically to 100 mL of a liquid mineral salts medium (Esposito and Fletcher, 1961). The liquid medium was shaken at 150 rpm for 3 days to 2 weeks, depending on experiments. The predominately microconidial suspensions were filtered through three layers of cheesecloth and adjusted to 1×10^6 (Maryland) and 1×10^5 (Indiana and Georgia) conidia/mL with the aid of a hemacytometer. A 150 mL solution was inoculated to each pot by pouring evenly over the soil surface and 150 mL of water served as a control.

Plants were watered and fertilized as needed. Wilt severity, the percent of the foliage of each plant showing symptoms of Fusarium wilt, was rated starting 1 week after inoculation at 2-to-3-day intervals 12 times (Maryland) and 5 times (Indiana and Georgia) with the Horsfall–Barratt scale (Horsfall and Barratt, 1945). The experiment was conducted once at each location.

2.2. Field evaluation of a single application of chemical in 2008

The fungicides which resulted in the greatest reduction in Fusarium wilt in the three greenhouse trials were evaluated in field trials in the summer of 2008. Field experiments were conducted at the University of Maryland's Lower Eastern Shore Research and Education Center, Salisbury, MD; the University of Delaware's Research and Education Center, Georgetown, DE; and the Purdue University Southwest Agricultural Center, Vincennes, IN. All fields were naturally infested with FON. The fields in Maryland and Delaware were known to have a mixed population of both races 1 and 2 (Zhou and Everts, 2007). It was not known which races were present in Vincennes, IN.

The experiments were conducted as a randomized complete block design with four replications in Maryland and Delaware and six replications in Indiana. The open pollinated watermelon cultivar 'Sugar Baby' in Maryland and Delaware, or 'Black Diamond' in