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Fungicidal activity of fluopicolide for suppression of *Phytophthora capsici* on squash

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ABSTRACT

The efficacy of a new fungicide fluopicolide in suppression of Phytophthora blight caused by *Phytophthora capsici* was evaluated under laboratory and field conditions. Studies with 51 *P. capsici* isolates from vegetable crops in Georgia, USA, indicated that 5.9% of the isolates were resistant, 19.6% were intermediately sensitive, and 74.5% were sensitive to 100 μg ml^{-1} of mefenoxam based on *in vitro* mycelial growth. EC_{50} values of fluopicolide in inhibiting mycelial growth of 25 isolates, representing resistant, intermediately sensitive, and sensitive to mefenoxam, ranged from 0.05 to 0.35 μg ml^{-1} with an average of 0.2 μg ml^{-1} EC_{50} values of fluopicolide in suppressing zoospore germination and sporangium production of the 25 isolates ranged from 1.1 to 4.5 μg ml^{-1} and 0.3–9.0 μg ml^{-1}, respectively. Evaluation of a collection of 150 *P. capsici* isolates from vegetables and irrigation ponds found none of the isolates were resistant to 10 μg ml^{-1} of fluopicolide. Field experiments were conducted to determine the efficacy and application methods of fluopicolide for control of *P. capsici* on squash in spring 2007 and 2009. Fluopicolide applied through drip irrigation or as a foliar spray at 86.6 or 115.4 g ha^{-1} consistently provided significant disease reduction and increased squash yield. Results with fluopicolide were similar or slightly superior to mefenoxam applied at recommended rate. Fluopicolide applied at 57.7 g ha^{-1} did not provide consistent satisfactory disease suppression. The results indicated that fluopicolide was effective in suppression of different stages of the life cycle of *P. capsici* and could be a viable alternative to mefenoxam for managing Phytophthora blight in squash production.

1. Introduction

Phytophthora blight, caused by the oomycete pathogen *Phytophthora capsici*, is responsible for serious yield and quality losses in the production of squash (*Cucurbita pepo*) and a number of other vegetable crops in North America (Ristaino and Johnston, 1999; Sholberg et al., 2007). The pathogen attacks more than 50 species of plants and causes plant wilt, root rot, crown rot, seedling damping-off, leaf and stem blight, fruit rot and other disease symptoms (Erwin and Ribeiro, 1996; Tian and Babadoost, 2004). The disease is favored by wet and humid weather conditions that are common in the southeast, USA. *P. capsici* produces sporangia and zoospores in the life cycle for asexual reproduction under favorable environmental conditions. Both sporangia and zoospores may germinate and infect the plant; hence, practices that reduce sporangium formation, zoospore germination, or mycelial growth of the pathogen have the potential to suppress the disease.

Phytophthora blight is among the most difficult diseases to control due to the wide host range of the pathogen, the ability of long-term survival of the pathogen in the soil, and the development of resistance to chemical compounds (Erwin and Ribeiro, 1996; Hausbeck and Lamour, 2004; Ploetz et al., 2002). At present, a single method for adequate control does not exist, and management relies on modification of cultural practices, crop rotation, and vigilant use of selected fungicides (Ristaino and Johnston, 1999). Managing water to avoid excess moisture is an important means for reducing infection and spread of the pathogen. For instance, planting in well-drained soils with raised beds helps to reduce disease severity (Ristaino and Johnston, 1999), however, this practice does not prevent disease outbreaks when weather conditions are favorable for the pathogen. Use of resistant cultivars is a recommended strategy for reducing losses to plant diseases; unfortunately, reliable resistance to *P. capsici* is not available in commercial cultivars of squash and other vegetable crops. Management of Phytophthora blight has relied primarily on application of chemical fungicides and general-purpose fumigants such as methyl bromide and chloropicrin. However, use of these chemicals is limited due to the increasing environmental concerns and

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development of resistant pathogen populations. For instance, the widespread use of methyl bromide has substantially reduced damaging soilborne pathogen populations in many vegetable production areas, but this is expected to dramatically change as methyl bromide is phased out due to the detrimental influence of this compound on the ozone layer. Products containing mefenoxam have been the most widely used fungicides for control of *P. capsici*, but mefenoxam-resistant strains that challenge the usefulness of this compound have developed (Café-Filho and Ristaino, 2008; Gevens et al., 2007; Lamour and Hausbeck, 2000; Mathis et al., 1999). Identification of alternative compounds and monitoring pathogen resistance development is critical for more efficient management of the disease.

The objectives of this study were to determine: 1) the frequency of resistance of *P. capsici* isolated on vegetable crops in Georgia to the current chemical fungicide standard mefenoxam, and 2) fungicidal activity of a new fungicide fluopicolide in suppressing different stages of the life cycle of *P. capsici*. In addition, different application rates and methods of fluopicolide were evaluated for control of Phytophthora blight on squash under field conditions in Georgia. Fluopicolide ($C_{14}H_8Cl_3F_3N_2O$) belongs to the benzamide class and the pyridine class and was recently registered for control of oomycete pathogens (Valent U.S. Corporation, Walnut Creek, California, USA). Identifying a new effective fungicide for suppression of *P. capsici* has the potential to develop a practical alternative to mefenoxam to be used as an effective component in integrated management of Phytophthora blight on vegetables.

2. Materials and methods

2.1. Chemical compounds and P. capsici isolates

Fluopicolide (technical grade) and Presidio (a.i. fluopicolide) were provided by Valent U.S. Corporation and Ridomil Gold (a.i. mefenoxam) was obtained from Syngenta Crop Protection (Greensboro, North Carolina, USA). A collection of 89 *P. capsici* isolates was isolated from symptomatic vegetable crops, including squash, bell pepper, cucumber, zucchini, watermelon, cantaloupe, and pumpkin, in commercial fields in Georgia in 2007–2008 (Ji et al., unpublished). Additionally, 61 *P. capsici* isolates obtained from irrigation ponds in southern Georgia were included in the study (Wang et al., 2009). Single zoospore isolates of the strains were grown on V8 juice agar plates (100 ml V8 juice, 1.0 g $CaCO_3$, 16 g agar, 950 ml distilled water) and stored at 15 °C.

2.2. Determine frequency of resistance of P. capsici populations to mefenoxam

Fifty-one of the 89 *P. capsici* isolates from vegetable crops were tested for sensitivity to mefenoxam. The cultures were grown on V8 juice agar at 25 °C in the dark for 5 days. An agar plug (7 mm in diameter) taken from the edge of the colony was placed at the center of a V8 juice agar plate amended with mefenoxam at a final concentration of 0, 5, 10, and 100 μg ml^{-1} (i.e. 0, 10.5, 21, and 210 μg ml^{-1} of Ridomil Gold). The mefenoxam concentration range (0–100 μg ml^{-1}) has been generally used for determining resistance of *P. capsici* to this compound (Keinath, 2007). Ridomil Gold (a.i. mefenoxam) dissolved in sterile distilled water (SDW) was used to amend the agar medium. Duplicate plates were used for each concentration and the plates were incubated at 25 °C in the dark as described previously (Keinath, 2007). Colony diameter was measured in two perpendicular directions 4 days after incubation and averaged for analysis (Koné et al., 2009). The diameter of the agar plug was subtracted from the total colony diameter for calculating actual diameter of colony. The relative growth rate of *P. capsici* on mefenoxam amended and non-amended control plates was used to determine sensitivity to the fungicide (sensitive: <30% of the control, i.e. colony diameter on mefenoxam amended plates was less than 30% of colony diameter on non-amended control plates; intermediate sensitive: 30–90% of the control; resistance: >90% of the control) (Lamour and Hausbeck, 2000). The experiments were conducted twice under similar conditions.

2.3. Effect of fluopicolide on mycelial growth

Twenty-five isolates from vegetable crops, representing 3 resistant, 7 intermediately sensitive, and 15 sensitive to mefenoxam, were evaluated in the study. *P. capsici* isolates were grown on V8 juice agar at 25 °C in the dark for 5 days. An agar plug (7 mm in diameter) taken from the edge of the colony was placed at the center of a V8 juice agar plate amended with fluopicolide at a final concentration of 0, 0.005, 0.01, 0.05, 0.1, 0.5, 1, and 5 μg ml^{-1}. It is estimated that the concentrations of fluopicolide in soil are in the range of 1.5–490 μg ml^{-1} based on the recommended rate of Presidio at 0.29 l per ha (i.e. 115.4 g of fluopicolide per ha) and amount of water applied through drip irrigation and foliar sprays. Hence the concentrations of fluopicolide in the range 0–5 μg ml^{-1} are good choices to determine the baseline sensitivity of *P. capsici* to this compound. Technical grade of fluopicolide dissolved in acetone was used to amend the agar medium. Duplicate plates were used for each concentration and the plates were incubated at 25 °C in the dark. Two perpendicular colony diameters were measured per plate 4 days after incubation and diameter of colonies was calculated for each concentration using the mean of the two perpendicular colony diameters as described above. The experiments were conducted twice under similar conditions and EC_{50} values of fluopicolide in suppressing *P. capsici* were calculated for each isolate. Data from two experiments were pooled together and EC_{50} value was calculated by fitting linear regression lines of probit-transformed inhibition data against the $\log_{10}$-transformed fungicide concentration according to the method described by Stein and Kirk (2003).

Additionally, a total of 150 *P. capsici* isolates, including 89 from vegetable crops and 61 from irrigation ponds, was assessed for sensitivity to 10 μg ml^{-1} of fluopicolide. The isolates were grown on V8 agar plates and 5 agar plugs, each from a *P. capsici* culture, were placed on a V8 agar plate amended with fluopicolide at a final concentration of 10 μg ml^{-1}. The plates were incubated at 25 °C in the dark and growth of the isolates was observed 4 and 6 days after incubation.

2.4. Effect of fluopicolide on zoospore germination

Evaluation of the effect on zoospore germination was as previously described with minor modifications (Koné et al., 2009). The 25 *P. capsici* cultures were grown on V8 juice agar plates at 25 °C in the dark for 4 days. The plates were then incubated at room temperature under constant fluorescent light for 2 days. A volume of 10 ml of SDW was added to each plate, and the plates were chilled at 4 °C for 20 min and moved to room temperature (23–25 °C) for 20 min to allow zoospore release. Zoospore suspensions were collected in sterile 50-ml centrifuge tubes and vortexed for 1 min. A volume of 50 μl of zoospore suspension was spread plated on clarified V8 agar plates amended with fluopicolide at different concentrations ranging from 0 to 10 μg ml^{-1}. The experiment was conducted twice with duplicate plates used for each concentration in each experiment. The plates were incubated at room temperature (23–25 °C) for 4 h and germinated and non-germinated zoospores were counted for 100 zoospores on each plate under an SZ × 16 Olympus stereo microscope