Influence of pH and Etridiazole on *Pythium* Species

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SUMMARY. *Pythium* root rot (*Pythium* sp.) is ubiquitous in Michigan greenhouses that produce herbaceous ornamentals, an industry worth \$393 million in the state. Disease symptoms include stunting, flowering delay, root rot, and death. Fungicides that are highly effective against *pythium* root rot are limited, and pathogen resistance has been documented. The objectives of this study were to determine the sensitivity of *Pythium irregulare*, *Pythium ultimum*, and *Pythium aphanidermatum* isolates from symptomatic herbaceous greenhouse ornamentals to the fungicide etridiazole and to determine the influence of pH and etridiazole on *Pythium* mycelial growth and asexual reproduction. Isolates were tested in vitro for sensitivity to etridiazole by growing the pathogen on amended V8-agar plates sealed in plastic containers to minimize fungicide loss from the vapor phase. The majority of isolates of all three species were sensitive to the fungicide with EC₅₀ (effective concentration resulting in 50% inhibition of linear growth) values ranging from 0.10 to 5.03 µg·mL⁻¹. Two isolates of *P. irregulare* had an EC₉₀ (effective concentration resulting in 90% inhibition of linear growth) value >80 µg·mL⁻¹. The acidity of the medium influenced the ability of etridiazole to inhibit *Pythium* mycelial growth and asexual reproduction. Agar plates amended with 1 µg·mL⁻¹ etridiazole and adjusted to pH 4.5 limited the mycelial growth of two *P. aphanidermatum* isolates and two *P. irregulare* isolates by 90% and 56%, respectively, compared with amended agar at pH 6.5. Sporangial formation by *P. aphanidermatum* was less frequent on mycelial disks incubated in etridiazole-amended sterile distilled water (SDW) at pH 4.5 than pH 6.5 ($P < 0.05$). *P. aphanidermatum* zoospore cyst germination was less sensitive to etridiazole than sporangia or mycelial growth; however, the influence of pH and fungicide on cyst germination was significant ($P < 0.01$). At 250 µg·mL⁻¹ etridiazole and solution pH 4.5, zoospore cyst germination was inhibited 99.9% compared with 94.2% at pH 6.5. In a greenhouse experiment, disease symptoms were observed on ‘Pinto White’ geranium (*Pelargonium × hortorum*) in a potting medium infested with *P. aphanidermatum* and adjusted to pH 4.5 or 6.5; however, plant health and fresh weight were greater in low pH potting medium. Etridiazole, applied as a drench at transplant, did not improve control of root rot for plants grown at low pH ($P > 0.05$). Fresh weight of plants grown in infested potting medium adjusted to pH 4.5 and amended with a single drench of etridiazole (100 µg·mL⁻¹) was reduced 20%, statistically similar to the untreated control. Adjusting the acidity of irrigation water at the time of etridiazole application in ebb and flow and flood floor production systems could be beneficial in *pythium* root rot management of certain ornamental crops if plants have tolerance to low pH.

Pythium root rot causes significant losses in ornamental greenhouse production in Michigan, an industry worth an estimated \$393 million (U.S. Department of Agriculture, 2014). *Pythium* species infect roots and root hairs of ornamental

plants, causing wilting, stunting, delayed flowering, and plant death. Catastrophic losses can occur if plants develop symptoms near the

time of retail sale (Hausbeck et al., 1987; Moorman, 1986). Numerous *Pythium* species cause disease on ornamental crops; however, *P. ultimum*, *P. aphanidermatum*, and *P. irregulare* are isolated frequently from symptomatic plants in commercial production (Del Castillo Múnera and Hausbeck, 2016; Moorman et al., 2002). *Pythium* species are ubiquitous in natural environments, and maintaining production facilities free of the pathogen is difficult. Pathogenic *Pythium* species were found in irrigation water (Bush et al., 2003; Shokes and McCarter, 1979) and dust from greenhouse walk alleys (Stephens et al., 1983). Soilless potting medium can be conducive to *pythium* root rot because of limited microbial activity (Bolton, 1977; Stephens and Stebbins, 1985). However, reductions in seedling diseases and root rot after amending potting medium with biological controls have heightened the use of such management tools in ornamental crop production (Lewis and Lumsden, 2001; Thrane et al., 2000). Sanitation and preventive measures remain important to reduce inoculum levels in the greenhouse (Stephens et al., 1983). Additionally, greenhouses may inadvertently purchase plantlets or cuttings that are infected but asymptomatic from propagation greenhouses (Moorman and Kim, 2004; van der Gaag et al., 2001). Infected roots or root mucilage support the production of oospores that may become lodged in greenhouse fixtures and piping (Zheng et al., 2000) and are a source of primary inoculum. Survival of mycelium is also possible in the controlled greenhouse environment (Stanghellini, 1974), making *Pythium* an intractable problem. Greenhouse operations

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	µL	3.3814×10^{-5}
29,5735	fl oz	mL	0.0338
25.4	inch(es)	mm	0.0394
645.1600	inch ²	mm ²	0.0016
1	micron(s)	µm	1
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274×10^{-5}
0.001	ppm	g·L ⁻¹	1,000
1	ppm	mg·L ⁻¹	1
1	ppm	µg·mL ⁻¹	1
0.001	ppm	mL·L ⁻¹	1,000
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

that use flood floor or ebb and flow bench systems that recycle irrigation water are at increased risk of spreading *Pythium* species as multiple ranges may be irrigated in the same day and effectively disperse pathogen propagules (Hoitink, 1991).

The fungicides, mefenoxam [active enantiomer of metalaxyl (Subdue Maxx; Syngenta Crop Protection, Greensborough, NC)] and etridiazole (Terrazole; OHP, Mainland, PA), have been used for ≈ 40 years to manage pythium root rot of ornamental crops (McCain and Byrne, 1966; Moorman and Kim, 2004; Raabe et al., 1981). Historically, these fungicides have provided effective control of pythiaceus organisms when applied as soil drenches (Benson, 1979; Hausbeck and Harlan, 2013; Raabe et al., 1981; Stephens and Stebbins, 1985) or mixed into the potting medium (McCain and Byrne, 1966). Resistance to mefenoxam has developed in greenhouse populations of *Pythium* and *Phytophthora* because of the site-specific mode of action of the fungicide and selection pressure from the repeated use of this active ingredient (Del Castillo Múnera and Hausbeck, 2016; Lamour et al., 2003; Moorman et al., 2002). In Michigan, greater than 35% of *Pythium* species isolates collected from greenhouses were resistant to mefenoxam (Del Castillo Múnera and Hausbeck, 2016). Control failures have been reported in greenhouses and nurseries (Ferrin and Rohde, 1992; Moorman and Kim, 2004) and may become more common if resistant isolates are accidentally moved among greenhouses with prefinished plants and propagative material (Moorman et al., 2002). Greenhouses that recycle irrigation water face additional challenges as resistant isolates may be selected when mefenoxam is applied repeatedly (Ferrin and Rohde, 1992) and sublethal fungicide residues that leach from pots into recirculating irrigation water can enhance disease (Garzón et al., 2011).

Etridiazole is a lipophilic fungicide that contains a heterocyclic nitrogen ring and has specificity toward oomycetes (Lyr, 1995; Vuik et al., 1990). Etridiazole disrupts the lipid structure of cell membranes (Radzuhan and Lyr, 1984) and inhibits respiration by binding to the

mitochondrial membrane between cytochromes b and c (Halos and Huisman, 1976b). The mode of action of etridiazole is considered multisite as cellular proteins may also be disrupted (Lyr, 1995). Resistance to etridiazole has not been observed in *Pythium* species (Hausbeck and Harlan, 2013; Jamart et al., 1988; Price and Fox, 1986; Raabe et al., 1981; Stephens and Stebbins, 1985), although mechanisms for tolerance to the fungicide are recognized (Halos and Huisman, 1976a). Rotation of fungicides with different modes of action has been recommended to delay the development of fungicide resistance in *Pythium* and *Phytophthora* populations (Ferrin and Rohde, 1992; Hausbeck and Harlan, 2013); however, there are limited numbers of additional fungicide options that effectively control pythium root rot. Recently, labeled fungicides with activity toward phytophthora root rot (*Phytophthora* sp.), such as fenamidone (Hausbeck and Harlan, 2013), have been determined to be ineffective against pythium root rot in greenhouse trials (Enzenbacher et al., 2011; Santamaria and Uribe, 2013).

Cultural practices that reduce growth and dissemination of *Pythium* species are important in disease-management programs (Hausbeck and Harlan, 2013; Price and Fox, 1986; Stephens and Stebbins, 1985). Limiting the duration of irrigation in ebb and flow systems reduced pythium root rot of poinsettia (*Euphorbia pulcherrima*), geranium, and chrysanthemum (*Chrysanthemum morifolium*) compared with standard irrigation practices (Elmer et al., 2012). Poinsettia grown in a *Pythium*-infested potting medium adjusted to pH 4.0–4.5 remained healthy compared with plants grown in an infested potting medium at pH levels >5.5 (Bateman, 1962; Bolton, 1980). Substrate pH can also influence the effectiveness of certain biocides. Chlorine, a disinfectant widely used in greenhouse production facilities, was the most effective in reducing *Streptococcus* species growth in an acidic solution (Shannon et al., 1965). The influence of solution pH on fungicides used to control *Pythium* species in ornamental production is not fully understood; however, pH is known to affect fungicide efficacy (Smith et al., 1946; Wolfe et al., 1976).

Pythium root rot continues to cause losses in commercial greenhouses despite routine fungicide use (Hausbeck and Harlan, 2013; Moorman and Kim, 2004), planting resistant cultivars (Chagnon and Bélanger, 1991), and preventive measures (Stephens and Stebbins, 1985), heightening the importance of integrated disease management. Our objectives were to a) determine the sensitivity of *P. ultimum*, *P. aphanidermatum*, and *P. irregulare* isolates from Michigan greenhouses to etridiazole; and b) determine the influence of pH and etridiazole on *P. aphanidermatum* and *P. irregulare* growth and asexual reproduction.

Materials and methods

P. aphanidermatum ($n = 9$), *P. irregulare* ($n = 14$), and *P. ultimum* ($n = 14$) isolates originally recovered from symptomatic floriculture crops in Michigan were selected from the culture collection of M.K. Hausbeck at Michigan State University (Table 1). The isolates were maintained on cornmeal agar [CMA (17 g·L⁻¹ cornmeal)]. Molten V8 agar [163 mL·L⁻¹ V8 juice, 3 g·L⁻¹ calcium carbonate (CaCO₃), 16 g·L⁻¹ agar] was cooled to ≈ 50 °C and amended with etridiazole (Terrazole 35 WP dissolved in SDW) at concentrations of 0, 0.1, 1.0, 2.5, and 6.2 $\mu\text{g}\cdot\text{mL}^{-1}$ a.i. For six isolates of *P. irregulare*, preliminary EC₅₀ values were outside of this range of concentrations. These isolates were additionally tested at 62 $\mu\text{g}\cdot\text{mL}^{-1}$ etridiazole. A 7-mm diameter colonized agar plug from the margin of a 2- to 4-d-old CMA culture was placed in the center of an amended plate, and the plates were incubated in the dark at ambient temperature (21 ± 1 °C) in a plastic chamber that was sealed to minimize the loss of etridiazole to the vapor phase (Ioannou and Grogan, 1984). Radial growth on two axes was measured 3 d post inoculation. There was one plate per etridiazole concentration for each isolate, and the experiment was conducted three times. Growth inhibition (percent) was calculated by comparing radial growth on amended agar plates with radial growth on non-amended control plates. Amended plates were used 1 to 2 d after preparing the medium.

P. aphanidermatum isolates 106 and 319 and *P. irregulare* isolates 125

Table 1. Isolates of *P. aphanidermatum*, *P. irregulare*, and *P. ultimum* obtained from infected ornamental plants in Michigan, and evaluated in vitro for sensitivity to etridiazole.

Isolate no. ^z	<i>Pythium</i> species	Host ^y	Location ^x
106	<i>P. aphanidermatum</i>	Geranium (<i>P. ×hortorum</i>)	B
670	<i>P. aphanidermatum</i>	Geranium	G
672	<i>P. aphanidermatum</i>	Geranium	G
283	<i>P. aphanidermatum</i>	Poinsettia (<i>E. pulcherrima</i>)	D
288	<i>P. aphanidermatum</i>	Poinsettia	D
290	<i>P. aphanidermatum</i>	Poinsettia	D
292	<i>P. aphanidermatum</i>	Poinsettia	D
302	<i>P. aphanidermatum</i>	Poinsettia	D
319	<i>P. aphanidermatum</i>	Poinsettia	D
108	<i>P. irregulare</i>	Geranium	H
115	<i>P. irregulare</i>	Geranium	D
125	<i>P. irregulare</i>	Geranium	D
464	<i>P. irregulare</i>	Geranium	C
468	<i>P. irregulare</i>	Geranium	C
667	<i>P. irregulare</i>	Geranium	G
458	<i>P. irregulare</i>	Geranium	A
49	<i>P. irregulare</i>	Poinsettia	E
94	<i>P. irregulare</i>	Poinsettia	B
98	<i>P. irregulare</i>	Poinsettia	B
306	<i>P. irregulare</i>	Poinsettia	D
308	<i>P. irregulare</i>	Poinsettia	D
147	<i>P. irregulare</i>	Snapdragon (<i>Antirrhinum majus</i>)	D
149	<i>P. irregulare</i>	Snapdragon	D
19	<i>P. ultimum</i>	Poinsettia	E
26	<i>P. ultimum</i>	Poinsettia	E
46	<i>P. ultimum</i>	Poinsettia	E
50	<i>P. ultimum</i>	Poinsettia	E
52	<i>P. ultimum</i>	Poinsettia	F
56	<i>P. ultimum</i>	Poinsettia	F
65	<i>P. ultimum</i>	Poinsettia	F
69	<i>P. ultimum</i>	Poinsettia	F
76	<i>P. ultimum</i>	Poinsettia	F
80	<i>P. ultimum</i>	Poinsettia	F
422	<i>P. ultimum</i>	Poinsettia	F
424	<i>P. ultimum</i>	Poinsettia	F
433	<i>P. ultimum</i>	Poinsettia	F
439	<i>P. ultimum</i>	Poinsettia	F
Total	37		

^zIsolates selected from the culture collection of M.K. Hausbeck at Michigan State University.

^yHost plant of original isolation.

^xIsolates with the same letter designation were collected from the same greenhouse.

and 147 were used to determine the influence of pH and etridiazole on *Pythium* growth inhibition. To determine radial growth inhibition, the pH of the agar medium was adjusted with sterile 1 N sodium hydroxide (NaOH) and 1 N hydrochloric acid (HCl) before autoclaving to obtain CMA at pH 4.5 and 6.5. Adjusting the pH of CMA to 7 before autoclaving was necessary to obtain a pH of 6.5 (C.S. Krasnow, unpublished data). Etridiazole was incorporated into molten CMA to achieve final concentrations of 0, 0.5, 1.0, 2.0, 4.0, and 8.0 $\mu\text{g}\cdot\text{mL}^{-1}$. A 5-mm

diameter agar plug of *P. aphanidermatum* or *P. irregulare* from an actively growing CMA culture was transferred to the center of each plate, and the plates were incubated at ambient temperature ($21 \pm 1^\circ\text{C}$) in a sealed chamber under constant fluorescent light for 3 d. Colony diameter was measured on two axes, and the percentage of growth inhibition was calculated as described. There were two replicate plates per fungicide concentration and pH, and the experiment was conducted three times.

To determine the influence of pH and etridiazole on sporangial

formation and zoospore cyst germination, SDW was buffered with 20 mL citric acid and dibasic phosphate, adjusted to pH 4.5 or 6.5 with NaOH or HCl, and amended with etridiazole at 0, 0.1, 0.5, and 2.5 $\mu\text{g}\cdot\text{mL}^{-1}$. An agar slice (5 mm diameter, 0.5–1.0 mm thickness) was removed from the surface of a 5-mm diameter plug of a 2-d-old CMA culture of *P. aphanidermatum* (106 and 319), placed into a 60-mm petri dish containing 6 mL of etridiazole-amended solution, and incubated at 30°C in the dark for 12 h. The agar slice was removed with forceps to a glass microscope slide and rated for the density of lobate and inflated sporangia at three random 1-mm² focal planes per slice with a compound microscope (100 $\times$) using a 0–2 scale, where 0 = no sporulation, 1 = light sporulation (1–5 sporangia/mm²), and 2 = heavy sporulation (6–20 sporangia/mm²). Two agar slices were evaluated per plate with two plates per pH $\times$ concentration; the experiment was conducted three times.

Zoospores of *P. aphanidermatum* (319) were produced according to the method of Rahimian and Banihashemi (1979). A 5-d-old V8-agar culture was divided into six strips and separated into two sterile 100-mm diameter petri dishes. The dishes were flooded with SDW, incubated at 30°C for 24 h, drained, rinsed, and flooded with SDW. After incubation for 10 h, zoospores were enumerated with a hemocytometer. Motile zoospores (1×10^4 in 1 mL) were added to 1 mL of solution adjusted to a pH of 4.5 or 6.5 containing etridiazole at 0, 50, 100, and 250 $\mu\text{g}\cdot\text{mL}^{-1}$ in a 2-mL screwcap vial and incubated for 10 min. The vial was vortexed briefly, and a 100- μL aliquot was removed and added to a flask containing 100 mL SDW. The suspension was immediately filtered through 2.5- μm pore-size quantitative filter paper (GE Healthcare, Pittsburgh, PA), and the filter paper was plated onto BARPR (50 ppm benomyl, 100 ppm ampicillin, 30 ppm rifampicin, 200 ppm pentachloronitrobenzene, and 10 ppm rose bengal)-amended CMA. After 1 d, the filter paper was removed, and the colonies were enumerated. Two vials per pH $\times$ concentration were used with three replicates per vial, and the experiment was conducted twice.

A greenhouse experiment to assess the influence of potting medium pH and etridiazole on pythium root rot of geranium was conducted. ‘Pinto White’ geranium was seeded into 288-cell plug flats and grown for 4 weeks in a research greenhouse at Michigan State University. Soilless-peat potting mixture (Suremix Michigan Grower Products, Galesburg, MI) was adjusted to pH 4.0 to 4.5 and 6.5 to 7.0 by periodically adding sulfuric acid (1% H₂SO₄) or potassium hydroxide (1% KOH) for 3 weeks before the experiment to permit pH equilibration. Potting mixture samples were taken weekly, and the acidity was measured (1 potting medium:2 SDW) using a glass electrode pH meter (Mettler-Toledo, Columbus, OH). The potting mixture was autoclaved for 45 min at 121 °C immediately before the experiment. Millet (*Pennisetum glaucum*) inoculum (Quesada-Ocampo and Hausbeck, 2010) was prepared by autoclaving millet seed (100 g), distilled water (72 mL), and L-asparagine (0.08 mg) in mushroom bags (RJG Sales, Port Richey, FL) twice consecutively, and adding seven 7-mm agar plugs colonized by *P. aphanidermatum*. Infested millet seed was grown under constant fluorescent light for 3 to 4 weeks and mixed weekly before use. Isolates 106 and 319 were used to infest millet singly and were mixed 1:1 (v/v) immediately before the experiment. The geranium seedlings were transplanted into 3-inch diameter pots containing potting mixture adjusted to pH 4.0 to 4.5 or 6.5 to 7.0 and 3 g·L⁻¹ *Pythium*-infested millet, an inoculum density selected based on previous research on root rot symptom development in geranium (Hausbeck et al., 1989). Control pots received 3 g·L⁻¹ sterilized millet seeded with sterile V8-agar plugs. Etridiazole was applied at transplanting as an 80 mL/plant drench at 0, 10, 50, and 100 µg·mL⁻¹, followed by an 80-mL drench of water to improve etridiazole movement into the potting medium. The greenhouse day/night temperatures were 27/26 °C; supplemental lighting was provided by sodium lamps for 16 h per day. Plants were rated for disease severity 4 weeks post inoculation using a 1–5 scale; 1 = healthy; 2 = minor chlorosis and stunting; 3 = chlorotic or necrotic lower leaves

and stunting; 4 = necrotic lower leaves, stunting, and necrotic stem tissue (black leg) at the soil line; and 5 = plant death. Plant height and width were measured at planting and at the conclusion of the experiment, and plant volume was determined using the shape of a cylinder to approximate plant size (Enzenbacher et al., 2015). Plants were excised at the soil line, and the aboveground plant fresh weight was recorded. About 10% of inoculated plants were randomly selected, the roots rinsed, and three water-soaked and symptomatic roots per plant isolated onto BARPR-amended CMA and confirmed as *P. aphanidermatum* based on sporangial morphology (van der Plaats-Niterink, 1981). The trial was arranged as a completely randomized design with 10 plants per treatment and was conducted once.

SAS (version 9.4; SAS Institute, Cary, NC) was used to analyze data from the study. Mycelial growth-inhibition data were analyzed with linear regression in PROC REG. The EC₅₀ and EC₉₀ were interpolated from growth inhibition data. Growth inhibition data were transformed to probits, and etridiazole concentration was log-transformed before analysis to straighten the dosage–response curve. The data for the influence of pH and etridiazole on mycelial growth inhibition, sporangial production, and inhibition of zoospore cyst germination are presented as inhibition relative to the nonamended control plates, and differences were separated with Fisher’s protected least significant difference test (LSD; *P* = 0.05) using PROC MIXED. Normality of residual data was assessed using PROC UNIVARIATE. Data from each experiment were pooled before analysis as assumptions for homogeneity of variance were met. Data on plant fresh weight, volume, and disease severity rating were analyzed using PROC MIXED with etridiazole

concentration and pH as fixed effects. There was a significant pH × fungicide concentration interaction (*P* < 0.05) so a slice statement was used to test simple main effects. Residual data were analyzed to ensure heteroscedasticity.

Results

Based on mycelial growth, *P. irregulare* was not as sensitive to etridiazole as *P. aphanidermatum* and *P. ultimum* (Table 2). Mean EC₅₀ values were 2.64, 0.97, and 0.58 µg·mL⁻¹ etridiazole for *P. irregulare*, *P. aphanidermatum*, and *P. ultimum*, respectively. Two *P. irregulare* isolates exhibited a notably reduced sensitivity to etridiazole compared with the other isolates, with EC₉₀ values of 134.0 and 84.8 µg·mL⁻¹ (data not shown). The mean slope values for mycelial growth inhibition of *P. irregulare*, *P. aphanidermatum*, and *P. ultimum* were 2.90, 2.36, and 2.34, respectively (data not shown).

Linear mycelial growth of pythium was influenced by the pH of etridiazole-amended CMA. At CMA pH 4.5 and 1 µg·mL⁻¹ etridiazole, there was a >96% reduction of mycelial growth of *P. aphanidermatum* isolates compared with the non-amended controls (Table 3); growth inhibition was <70% of the controls at CMA pH 6.5. Radial growth after 3 d on pH 4.5 CMA at 1 µg·mL⁻¹ etridiazole was <5 mm for *P. aphanidermatum* isolates and <25 mm for *P. irregulare* isolates, compared with >60 mm for the controls at both pH levels (Table 3). The slope of the dosage–response curve for mycelial growth on the log-probit scale was steeper at pH 4.5 than pH 6.5 for *P. aphanidermatum* and *P. irregulare* isolates (Fig. 1), indicating greater inhibition at low pH.

Etridiazole inhibited *P. aphanidermatum* sporangial production more effectively at low pH (*P* < 0.01). Sporangia were absent on mycelial disks immersed in a solution at

Table 2. Mean concentration resulting in 50% and 90% inhibition of linear growth (EC₅₀ and EC₉₀, respectively) for *Pythium* species grown on etridiazole-amended V8 agar.

<i>Pythium</i> species	EC ₅₀ ^z		EC ₉₀	
	Mean ± SD	Range	Mean ± SD	Range
<i>P. aphanidermatum</i>	0.97 ± 0.276	0.55–1.36	6.89 ± 3.81	2.68–14.67
<i>P. irregulare</i>	2.64 ± 1.16	0.36–5.03	25.20 ± 37.33	6.29–134.02
<i>P. ultimum</i>	0.58 ± 0.86	0.10–3.29	7.03 ± 4.70	3.59–20.50

^zEC₅₀ and EC₉₀ values interpolated from regression equations describing the dosage–response relationship.

Table 3. Influence of solution pH and etridiazole on radial growth of *P. aphanidermatum* and *P. irregulare* isolates on corn meal agar.

Isolate ^z	<i>Pythium</i> species	Etridiazole concn ($\mu\text{g}\cdot\text{mL}^{-1}$) ^y	Radial growth (mm) ^x	
			pH 4.5 ^w	pH 6.5
106	<i>P. aphanidermatum</i>	0.0	64.0	82.0*
		0.5	23.8	—
		1.0	2.2	25.7*
		2.0	0.0	8.4*
		4.0	0.0	6.7*
		8.0	—	0.0
319	<i>P. aphanidermatum</i>	0.0	77.7	82.0
		0.5	31.4	—
		1.0	2.5	26.5*
		2.0	0.0	9.8*
		4.0	0.0	6.2*
		8.0	—	0.0
125	<i>P. irregulare</i>	0.0	81.1	82.0
		0.5	53.5	—
		1.0	8.5	19.5*
		2.0	0.2	9.8*
		4.0	0.0	6.1*
		8.0	—	0.8
147	<i>P. irregulare</i>	0.0	82.0	82.0
		0.5	70.3	—
		1.0	24.7	56.7*
		2.0	0.0	30.8*
		4.0	0.0	7.9*
		8.0	—	0.3

^zIsolates selected from the culture collection of M.K. Hausbeck at Michigan State University.

^y1 $\mu\text{g}\cdot\text{mL}^{-1}$ = 1 ppm.

^xRadial diameter on pH-adjusted corn meal agar (CMA) amended with etridiazole measured on two axes per plate after 3 d growth. Two plates/pH/concentration were used, and the experiment was conducted 3 $\times$; 1 mm = 0.0394 inch.

^wCMA pH adjusted with 1 N hydrochloric acid or sodium hydroxide before autoclaving and amending with etridiazole.

*Indicates significant difference between pH treatments at $P < 0.05$, according to Fisher's protected LSD test.

— = not tested.

pH 4.5 containing 2.5 $\mu\text{g}\cdot\text{mL}^{-1}$ etridiazole while sporangia developed in a solution at pH 6.5 (Table 4). Zoospores of *P. aphanidermatum* were less sensitive to etridiazole than sporangia and mycelial growth. At 100 $\mu\text{g}\cdot\text{mL}^{-1}$ etridiazole, there was 37.7% and 2.9% inhibition of zoospore cyst germination at pH 4.5 and pH 6.5, respectively (Table 5). Cyst germination was inhibited 99.9% in a pH 4.5 solution containing 250 $\mu\text{g}\cdot\text{mL}^{-1}$ etridiazole, but inhibition was less (about 94%) in a solution containing the same concentration etridiazole adjusted to pH 6.5 ($P < 0.05$).

The potting medium pH had a significant effect on geranium health in the greenhouse experiment [$P < 0.001$ (Table 6)]. Plants grown in infested potting medium at pH 4.5 remained healthy (average disease severity rating 1.7) but were stunted with chlorotic foliage when grown at pH 6.5 (average disease severity rating 3.5,

data not shown). Plants grown in potting medium adjusted to pH 4.5 or 6.5 and treated with etridiazole did not differ in root rot symptoms or fresh weight at any fungicide concentration [$P > 0.05$ (Table 6)]. However, plants grown in infested potting medium at pH 4.5 and treated with etridiazole had significantly greater plant volume than treated plants at pH 6.5 [$P < 0.05$ (data not shown)]. There was no effect of potting medium pH on plant health for the noninoculated control plants (data not shown).

Discussion

Etridiazole has provided effective control of pythium root rot in greenhouse production. Most of the *Pythium* isolates tested in this study were sensitive to etridiazole in vitro and displayed reduced mycelial growth at concentrations $< 1.0 \mu\text{g}\cdot\text{mL}^{-1}$. The inhibition levels observed were similar to results with other *Pythium* and

Phytophthora species (Benson, 1979; Ioannou and Grogan, 1984; Jamart et al., 1988). *P. parasitica* and *P. cinnamomi* mycelial growth and sporangial formation had EC₅₀ values $< 1.0 \mu\text{g}\cdot\text{mL}^{-1}$ etridiazole (Benson, 1979; Ioannou and Grogan, 1984). Price and Fox (1986) reported similar etridiazole sensitivity for a *P. irregulare* isolate from Australian soil with an EC₅₀ value $< 1.0 \mu\text{g}\cdot\text{mL}^{-1}$ and 86% mycelial growth inhibition at 10 $\mu\text{g}\cdot\text{mL}^{-1}$. An isolate of *P. irregulare* from ivy (*Hedera* sp.) had EC₅₀ values of 3.44 and 0.28 $\mu\text{g}\cdot\text{mL}^{-1}$ for mycelial growth and oospore formation, respectively, on etridiazole-amended CMA (Jamart et al., 1988). The growth of some *P. irregulare* isolates in the current study at $> 50 \mu\text{g}\cdot\text{mL}^{-1}$ etridiazole and detection of EC₉₀ values of 84.8 and 134.0 $\mu\text{g}\cdot\text{mL}^{-1}$ for two isolates of this species suggest that there may be diversity in etridiazole sensitivity of *P. irregulare* from Michigan greenhouses. A limited number of *P. irregulare* isolates were tested in previous studies, and baseline threshold values for etridiazole sensitivity were not established (Jamart et al., 1988; Price and Fox, 1986). Including *Pythium* isolates collected from ornamental crops in multiple production regions and years in future in vitro fungicide screening would provide meaningful comparisons for Michigan isolates.

Even though etridiazole is one of the primary fungicides used in nursery and ornamental crop production to manage root rot caused by *Pythium* and *Phytophthora* species (Benson, 1979; Hausbeck and Harlan, 2013; McCain and Byrne, 1966; Raabe et al., 1981), the level of control can be variable. A single drench of etridiazole at 222 $\mu\text{g}\cdot\text{mL}^{-1}$ did not provide control of phytophthora root rot (*P. cinnamomi*) of azalea (*Rhododendron obtusum*), although four drenches at that rate or a single drench at 444 $\mu\text{g}\cdot\text{mL}^{-1}$ effectively limited the disease (Benson, 1979). Etridiazole prevented phytophthora root rot (*P. nicotianae*) of tomato (*Solanum lycopersicum*) when incorporated into potting soil at 250 $\mu\text{g}\cdot\text{mL}^{-1}$ in a greenhouse study (Ioannou and Grogan, 1984), and a single drench of etridiazole at 100 $\mu\text{g}\cdot\text{mL}^{-1}$ reduced root rot of larkspur (*Delphinium* sp.) to $< 5\%$ (Bloch et al., 1976). Etridiazole limited symptoms

of geranium root rot at 50 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ in this Michigan study; however, applying etridiazole at a greater rate (labeled rate = 92–262 $\mu\text{g}\cdot\text{mL}^{-1}$) may improve disease control.

In greenhouse experiments using ebb and flow systems, control of pythium root rot by etridiazole has been variable (Jamart et al., 1988; Sanogo and Moorman, 1993), and *Pythium* species were recovered from

Fig. 1. Dosage–response curves for (A) *Pythium irregularis* isolates 125 (○) and 147 (Δ) and (B) *P. aphanidermatum* isolates 106 (○) and 319 (Δ) on etridiazole-amended corn meal agar adjusted to pH 4.5 (shaded) or 6.5 (open). Radial growth was determined on two axes of each petri plate after 3 d. Each data point is the mean of six replicate plates; 1 $\mu\text{g}\cdot\text{mL}^{-1}$ = 1 ppm.

diseased root tissue when fungicide and zoospore or mycelial inoculum were added consecutively to the recirculating irrigation water (Sanogo and Moorman, 1993; S. Jeon and C.S. Krasnow, unpublished data). The relatively low sensitivity of *P. aphanidermatum* zoospores to etridiazole observed in the current study suggests that these propagules may not be killed when infested recirculating irrigation water is treated with the fungicide. Etridiazole is not mobile in the potting medium and soil (Helling et al., 1974; King and Zentmyer, 1979), and fungicide effectiveness in flood floor and ebb and flow production systems may be affected only if the roots in the lower region of pots are protected when etridiazole is applied in the irrigation water. Conceivably, zoospores or cysts present in fungicide-treated irrigation water could enter pots from the base during irrigation and move with capillary force to roots and potting medium with an ineffective concentration of fungicide. Additionally, etridiazole concentration in recirculating irrigation water is likely diluted by the periodic addition of untreated water to holding tanks (Themann et al., 2002) or by volatilization (Ioannou and Grogan, 1984) and may affect disease control during the 1-month labeled application interval.

Inhibition of radial growth and asexual reproduction of *Pythium* species by etridiazole was influenced by pH in this study; however, the mechanisms responsible for inhibition at low pH were not determined in the study. Solution pH may directly affect the stability of a fungicide (Wolfe et al., 1976) or the degree of

ionization of the toxophore (Smith et al., 1946). Captan, a fungicide used to control pythium root rot on some crops in greenhouses, hydrolyzes to nonfungitoxic compounds at alkaline pH (Wolfe et al., 1976), and Spergon (tetra-chloro-p-benzoquinone) seed treatment was least inhibitory to *Rhizoctonia solani* in alkaline soil as a result of chemical conversion of the fungicide (Kelman, 1947). Uptake of 2,5-dimercapto-1,3,4-thiadiazole homologs by *Monolinia fructicola* conidia was 2× greater at pH 1.5 than at pH 6.0 (Somers, 1958). The fungicide homologs were almost completely unionized (99.9%) at low pH, which might have improved cellular penetration and pathogen inhibition (Lukens, 1971; Somers, 1958). Low pH may indirectly affect inhibition by affecting fungicide solubility (Buchenauer and Erwin, 1972). Benomyl and benzimidazole were more effective at reducing verticillium wilt (*Verticillium dahliae*) of cotton (*Gossypium hirsutum*) when applied as a drench in acidic solution than at alkaline pH levels (Buchenauer and Erwin, 1972). Greater water solubility of these fungicides at low pH increased uptake by cotton plants, improving disease control. However, the effect of benomyl drenches on cylindrocladium root and petiole rot of lily (*Spathiphyllum* sp.) was not influenced by the potting medium pH [pH 3.8–7.0 (Chase and Poole, 1987)]. Additionally, the pH of V8 agar medium (pH 4.5–8.0) did not influence mefenoxam, dimethomorph, or fluopicolide inhibition of *Phytophthora capsici* mycelial growth (C.S. Krasnow, unpublished data). Additional testing is necessary to determine the effect of pH on other fungicides and biological control agents labeled for pythium root rot control in greenhouses.

Growing plants in potting medium maintained at low pH has been recommended to control pythium root rot of poinsettia (Bateman, 1962; Bolton, 1980) and in nursery production to control *Phytophthora* species (Blaker and Macdonald, 1983). In a hydroponic growing system, greater control of pythium root rot of tomatoes was realized when the nutrient solution was maintained at pH 4.5–5.0 compared with pH 6.0–6.5 as a result of reduced zoospore motility and attachment to roots;

Table 4. Influence of solution pH and etridiazole on *P. aphanidermatum* sporangial formation for each of the two isolates.

Etridiazole concn ($\mu\text{g}\cdot\text{mL}^{-1}$) ^z	Sporangial formation (0–2 scale) ^y			
	Isolate 106 ^x		Isolate 319	
	pH 4.5	pH 6.5	pH 4.5	pH 6.5
0	1.8	2.0*	1.5	2.0*
0.1	1.6	1.2	1.6	2.0*
0.5	0.3	1.0	0.8	1.9*
2.5	0.0	0.1	0.0	0.1

^z1 $\mu\text{g}\cdot\text{mL}^{-1}$ = 1 ppm.

^yDensity of sporangia at three random 1-mm² (0.0016 inch²) focal planes of a 5-mm (0.2 inch) mycelial disk soaked in pH-adjusted solution amended with etridiazole at each concentration estimated using a 0–2 scale, where 0 = no sporulation, 1 = light sporulation (1–5 sporangia/mm²), and 2 = heavy sporulation (6–20 sporangia/mm²). Values represent the mean of six mycelial disks; 1 sporangium/mm² = 645.1600 sporangia/inch².

^xIsolate 106 and 319 refer to isolate designation from the culture collection of M.K. Hausbeck at Michigan State University.

*Indicates significant difference between pH treatments at $P < 0.05$, according to Fisher's protected LSD test.

Table 5. Influence of solution pH and etridiazole on *P. aphanidermatum* zoospore cyst germination inhibition.

Etridiazole concn ($\mu\text{g}\cdot\text{mL}^{-1}$) ^z	Zoospore cyst germination inhibition (%) ^y	
	pH 4.5	pH 6.5
50	7.2	0.0
100	37.7	2.9*
250	99.9	94.2*

^z1 $\mu\text{g}\cdot\text{mL}^{-1}$ = 1 ppm.

^yZoospore cyst germination inhibition (percent) of *P. aphanidermatum* (319) zoospores after the addition to fungicide-amended solution adjusted to pH 4.5 or pH 6.5, filtering the zoospores on 2.5 μm (micron) filter paper, plating on benomyl, pentachloronitrobenzene, ampicillin, rifampicin, and rose bengal-amended corn meal agar and determining the reduction in cfu per milliliter compared with the nonamended control plates.

*Indicates significant difference between pH treatments at $P < 0.05$, according to Fisher's protected LSD test.

Table 6. Influence of potting medium pH and etridiazole on pythium root rot of geranium.

Etridiazole concn ($\mu\text{g}\cdot\text{mL}^{-1}$) ^z	Reduction in plant fresh wt (%) ^y	
	pH 4.5 ^x	pH 6.5
0	22.0	70.8*
10	30.0	43.8
50	26.0	22.9
100	20.0	25.0

^z1 $\mu\text{g}\cdot\text{mL}^{-1}$ = 1 ppm.

^yReduction (percent) in fresh weight compared with the untreated noninoculated control plants. Etridiazole applied as a transplant drench [80 mL (2.71 fl oz) per plant] followed by an 80-mL drench of water to improve fungicide movement into the potting medium. Means represent a single greenhouse experiment with 10 plants/treatment.

^xPotting medium pH adjusted with sulfuric acid or potassium hydroxide for 3 weeks before the experiment.

*Indicates significant difference between pH treatments at $P < 0.05$, according to Fisher's protected LSD test.

plant health and yield were unaffected in uninfested nutrient solution maintained at a low pH (Huang and Tu, 1998). Geranium plant health did not appear to be affected by low pH potting medium in the present study, similar to poinsettia that remained healthy when grown in potting medium adjusted to pH 4.0–4.5 (Bateman 1962; Bolton 1980). However, Biernbaum et al. (1988) reported iron and manganese toxicity in herbaceous ornamentals grown at low pH, and soilless potting medium is typically maintained at pH 5.5–6.0 in commercial greenhouses to optimize nutrient uptake (Biernbaum et al., 1988; Warncke and Krauskopf, 1983).

In this study, the growth of all three *Pythium* species was reduced by etridiazole at an acidic pH. The influence of low pH and etridiazole on *Pythium* species highlights the importance of monitoring water acidity when etridiazole is applied with irrigation water in flood floor or ebb and flow production systems. Etridiazole may provide insufficient control of root rot when applied in irrigation water maintained near a neutral pH. Improvements in integrated *Pythium* management may be realized if irrigation water acidity can be augmented

without adversely affecting plant health. Tolerance of greenhouse ornamentals to extended periods of low pH should be tested further.

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