Sensitivity of Isolates of *Phytophthora capsici* from the Eastern United States to Fluopicolide

Anthony P. Keinath, Clemson University, Coastal Research and Education Center, and Chandrasekar S. Kousik, USDA, ARS, U.S. Vegetable Laboratory, Charleston, SC 29414-5329

Abstract

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Fluopicolide, a pyridinylmethyl-benzamide fungicide, was registered in the United States in 2008 to control diseases caused by Oomycete pathogens, such as *Phytophthora capsici*, on cucurbit and solanaceous vegetables. The main objective of this study was to determine baseline sensitivity to fluopicolide in isolates of *P. capsici* from the southeastern and midwestern United States. A total of 69 isolates from Florida (14 isolates), Georgia (14 isolates), Michigan (24 isolates), North Carolina (3 isolates), and South Carolina (17 isolates) that had not been previously exposed to fluopicolide were grown on fungicide-amended medium to determine sensitivity of mycelia, sporangia, and zoospores to the fungicide. All isolates of *P. capsici* tested (range of 54 to 69 isolates per assay) were sensitive to fluopicolide in all four assays. The median EC₅₀ fluopicolide concentration was 0.22, 2.08, 0.048, and

0.10 mg/liter in the mycelial growth, zoospore germination, sporangia production, and zoospore production assays, respectively. For mycelial growth and zoospore germination, isolates from Michigan had a higher mean EC₅₀ value than isolates from the four southeastern states. This is the first report of variation in baseline sensitivity to a fungicide by *P. capsici* isolates from different regions of the United States. In the sporangia production and zoospore production assays, isolates from different states did not differ in sensitivity. Single rates of fluopicolide were tested with additional isolates to validate discriminatory rates for monitoring sensitivity. A concentration of 0.3 or 1.0 mg/liter is recommended for mycelial growth, and 0.1 mg/liter is recommended for sporangia and zoospore production.

Phytophthora capsici Leon. is an Oomycete pathogen of vegetable crops that causes Phytophthora crown, root, and fruit rot on cucurbits and Phytophthora blight on pepper and eggplant. *P. capsici* is one of the *Phytophthora* species that causes buckeye fruit rot and root rot on tomato. In addition to cucurbit and solanaceous vegetables, the host range of *P. capsici* includes lima and snap beans, Fraser fir seedlings, and weeds in three botanical families (2,4,7,15,20). In the past 15 years, diseases caused by *P. capsici* have become widespread on vegetable crops throughout the eastern United States (3,4,9,11,13,14).

Oospores are the resistant structures produced by *P. capsici* and other *Phytophthora* species that allow for long-term survival in soil (9,16). Oospores may germinate directly and infect host plant parts in contact with soil, or they may produce sporangia, which also may germinate directly or produce motile zoospores (9). Zoospores and sporangia can be spread within and between fields by splashing rain and irrigation water. *P. capsici* has been recovered from bodies of surface water that are located adjacent to or downstream from infested fields (6). Because *P. capsici* produces three types of spores, fungicides that are active against multiple spore types, particularly sporangia and zoospores, are considered to be the most effective (19). Fluopicolide (Presidio 4SC, Valent U.S.A. Corporation, Walnut Creek, CA), along with mandipropamid (Revus, Syngenta Crop Protection, Greensboro, NC), is one of the main fungicides currently used to manage mefenoxam-insensitive *P. capsici* on cucurbits, peppers, and eggplant throughout the eastern half of

the United States. Fluopicolide is a pyridinylmethyl-benzamide (acylpicolide) fungicide that disrupts cell division and mitosis by acting on spectrin-like proteins (1,5). It has strong translaminal activity and limited systemic movement in the xylem of potato and grape (17). Fluopicolide, which was registered in 2008, is effective against *P. capsici* when applied alone or with other fungicides. For example, fluopicolide applied alone was as effective as fluopicolide applied in alternation with potassium phosphite and dimethomorph in reducing mortality of bell pepper due to Phytophthora blight (3). Similarly, application of fluopicolide alone was effective in managing Phytophthora fruit rot of watermelon (14). However, the fungicide label mandates that fluopicolide be mixed with another fungicide that has an active ingredient from a different chemical class to prevent or slow development of resistance in target pathogens. For example, when mixed with copper hydroxide (10) and when mixed with copper hydroxide plus maneb or with cymoxanil plus famoxadone (12), fluopicolide reduced incidence of Phytophthora blight on bell pepper. The number of applications of fluopicolide is limited to four per season, and no more than two applications may be made in sequence.

To date, there have been no reports of insensitivity to fluopicolide in *P. capsici*. Because of widespread insensitivity to mefenoxam in *P. capsici* (6,7,9,11,13), it is important to establish the baseline sensitivity level of *P. capsici* to fluopicolide, so that any future shifts in populations toward insensitivity can be detected by comparison to baseline sensitivities in isolates not exposed to fluopicolide. Mycelial growth and zoospore germination of isolates of *P. capsici* from Georgia and Korea have been reported to be sensitive to ≤ 4.5 mg/liter fluopicolide (11,21). Sporangia production by isolates from Georgia was less inhibited by fluopicolide than mycelial growth or zoospore germination. EC₅₀ values were ≤ 9 mg/liter (11). The effect of fluopicolide on zoospore production by sporangia has not been reported. It also would be valuable to know if sensitivities varied among isolates from different states or regions, as most studies of fungicide sensitivity of *P. capsici* have examined isolates from only one state (6,7,11,13,16).

The objectives of this study were to (i) determine baseline sensitivity of mycelia, zoospores, and sporangia of isolates of *P. capsici*

Corresponding author: A. P. Keinath, E-mail: tknth@clemson.edu

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from South Carolina, Georgia, Florida, North Carolina, and Michigan to fluopicolide; (ii) compare sensitivities of isolates from different states within the eastern United States; (iii) compare sensitivity values (EC_{50} values) among four assays assessing sensitivity of different life stages of *P. capsici*; and (iv) determine and verify threshold discriminatory single concentrations for each assay that can be used for future monitoring.

Materials and Methods

All isolates of *P. capsici* used in this study were derived from a single zoospore and had been stored in screw-top glass vials on cornmeal agar at 10°C (16). Isolates (104) were selected from collections of *P. capsici* maintained at the Clemson Coastal Research and Education Center and the USDA, ARS, U.S. Vegetable Laboratory in Charleston, SC by the authors, or were obtained from researchers in Florida, Michigan, and North Carolina (Table 1). Isolates were selected to represent geographic (state) and host diversity and for the ability to readily produce sporangia in culture. The 42 isolates from pepper (*Capsicum annuum* and *C. frutescens*) and summer squash (*Cucurbita pepo*) grown in South Carolina were collected on one farm in Bamberg County and two farms in Lexington County in 2003 (13). The 14 isolates from Georgia were recovered from two watermelon (*Citrullus lanatus*) fields in 2007. The 25 isolates from Michigan were collected between 2000 and 2006 (7). The 21 isolates from Florida were collected prior to 2004 (18) through spring 2008. Three isolates came from North Carolina. By host of origin, 10 isolates originated from snap or wax bean (*Phaseolus vulgaris*), 6 from cucumber (*Cucumis sativus*), 27 from pepper, 20 from summer squash, and 26 from watermelon (Table 1). For 15 isolates, the host was unknown.

In October 2009, a separate collection of 144 isolates was made from watermelon in six fields in Beaufort County, SC that were managed by the same grower. Three fields were sampled on each of two farms, which were approximately 35 km apart. Samples were collected by walking along one or two diagonal lines and excising small pieces of infected rind from individual fruit. Between 10 and 40 isolates were obtained per field depending on the incidence of fruit rot.

Determination of EC_{50} values. Stock solutions of fluopicolide (V-10161 technical grade, 97.9% purity) were prepared at 10 mg/liter and 100 mg/liter in dimethyl sulfoxide (DMSO). Nonamended agar received the equivalent volume of DMSO, 1.0 ml/liter, as amended agar. For each sensitivity assay, isolates were first grown on V8 medium for 3 days at 23 to 25°C with a 16-h photoperiod (13). For each isolate, each fungicide concentration was replicated twice, and each assay was repeated (11,13). Isolates from all five states were used in each assay.

Mycelial growth. For the mycelial growth assay, fluopicolide concentrations were 0, 0.032, 0.10, 0.32, and 1.00 mg/liter; two isolates also were tested at 3.2 mg/liter. Agar plugs (7 mm diameter) were cut from source cultures of 69 isolates and placed onto V8 medium amended with fluopicolide and incubated 5 days at 23 to 25°C with a 16-h photoperiod. Two perpendicular colony diameters per plate were measured and averaged. The diameter of the agar plug was subtracted from the colony diameter before calculating the mean. Relative colony diameter was calculated by dividing the mean colony diameter on each fungicide-amended plate by the mean colony diameter across the two nonamended plates for each isolate (13).

Zoospore germination. V8 agar source plates with 67 isolates were not wrapped with Parafilm to promote formation of sporangia (13). After 3 days growth, source plates were flooded with 5 ml of cold, sterile distilled water, chilled at 10°C for 30 min, and then held at 23 to 25°C for another 30 min to allow for zoospore release (16). Four 10- μ l drops were streaked on clarified V8 medium amended with fluopicolide at concentrations of 0, 0.10, 1.00, and 10.0 mg/liter. For 10 isolates, preliminary EC_{50} values lay outside this range of concentrations. Germination with these isolates also was assessed at 0.032 (3 isolates) or 3.16 and 31.6 mg/liter (7 isolates). Plates were held at 23 to 25°C at ambient lighting for 2 h,

and 100 zoospore cysts were examined at $\times 200$ magnification. Germinated and nongerminated (including lysed) cysts were counted. The relative zoospore germination was calculated by dividing the number of germinated cysts per 100 cysts examined on each plate of fungicide-amended medium by the mean number of germinated cysts per 100 cysts examined on the two nonamended plates.

Sporangia production. Agar plugs (7 mm diameter) cut from source cultures were placed on V8 medium amended with 0, 0.032, 0.10, and 0.32 mg/liter fluopicolide. One plate per isolate was used as the source culture for plugs for all concentrations. Cultures were incubated for 5 days at 23 to 25°C with a 16-h photoperiod. After 5 days of growth, plugs were removed and the plates were flooded with 5 ml of sterilized water and vortexed to release the caducous sporangia. Three drops, 1 μ l each, from each plate were placed on a glass microscope slide with glass coverslip and examined at $\times 100$ magnification. All sporangia were counted in each drop and recorded as full (normal), empty of cytoplasm, or lysed. Sixty-three isolates were tested, seven of which were tested with the additional concentration of 0.01 mg/liter fluopicolide. Relative sporangia production was calculated by dividing the mean count of normal sporangia produced on each fungicide-amended plate by the mean normal sporangia counts from the two nonamended plates.

Zoospore production. Large disks (6 cm diameter) with sporangia of 54 isolates of *P. capsici* cut from V8 agar source cultures that had not been sealed with Parafilm were transferred to petri dishes of water agar amended with 100 mg/liter streptomycin sulfate and 0, 0.032, 0.10, or 0.32 mg/liter fluopicolide (13,22). This arrangement was necessary because fluopicolide reduced or inhibited sporangia formation at ≥ 0.10 mg/liter. After 2 days at 23 to 25°C with a 16-h photoperiod, the disk was transferred to an empty petri dish. Cold, sterile distilled water (5 ml) was added, and the dish was chilled at 10°C for 30 min, and then held at 23 to 25°C for another 30 min. The sporangia on the disk were rubbed gently with a sterile glass rod to release zoospores. The zoospore suspension was transferred to a 50-ml test tube and vortexed 30 to 50 s to encyst zoospores. The number of zoospores in three aliquots of each suspension was counted with a hemacytometer at $\times 200$ magnification. Relative zoospore production was calculated by dividing the mean zoospore count for each fungicide-amended plate per replication by the mean zoospore count of the nonamended plates across two replicates.

Threshold concentrations. Threshold concentrations were determined from EC_{50} assays. Additional isolates from the collection of 104 isolates were selected from Michigan (1 isolate), Florida (10 isolates), and South Carolina (24 isolates). For mycelial growth, an additional 31 isolates were tested at 0 and 1.0 mg/liter fluopicolide. In addition, 144 isolates collected in 2009 from Beau-

Table 1. Isolates of *Phytophthora capsici* used to determine or verify fluopicolide EC_{50} values in this study

Geographic origin	Host	No. of isolates
Florida	Bell pepper	1
	Summer squash	2
	Watermelon	12
	Unknown	5
Georgia	Watermelon	14
Michigan	Snap bean	5
	Wax bean	5
	Cucumber	6
	Bell pepper	1
	Zucchini squash	1
	Unknown	7
North Carolina	Unknown	3
South Carolina	Bell pepper	19
	Jalapeno pepper	6
	Yellow summer squash	13
	Zucchini squash	4
Total		104

fort County, SC were tested at 0 and 0.3 mg/liter to assess variation within a population of isolates collected at the same time. For zoospore germination, 32 additional isolates were tested at 0 and 10.0 mg/liter fluopicolide. Numbers of sporangia were counted for 33 additional isolates grown on V8 amended with 0 and 0.1 mg/liter fluopicolide. No additional isolates were tested in the zoospore production assay. For each isolate, each fungicide concentration was replicated twice, and each assay was done once.

Statistical analysis. Data were analyzed with SAS v. 9.1 (SAS, Inc., Cary, NC). PROC UNIVARIATE was used to calculate the Shapiro-Wilk W statistic to determine if EC₅₀ values were normally distributed. Relative colony diameter, relative zoospore germination, relative sporangia production, and relative zoospore production were plotted against the base-ten logarithm of fluopicolide concentration. The plots for the first three data sets were linear with respect to the logarithm of fungicide concentration, and the plots for relative zoospore production were not linear for most isolates. Relative zoospore production was probit-transformed before regression to linearize the response. Relative colony diameter, relative zoospore germination, relative sporangia production, and relative zoospore production were regressed against the base-ten logarithm of fluopicolide concentration with PROC GLM. The resulting linear regression equations were solved to calculate the concentration of fluopicolide at which the relative responses were reduced to 50% of the value of the nonamended control (EC₅₀).

EC₅₀ values were compared across the five states from which isolates originated with PROC MIXED with state as a fixed effect and isolate as a random effect in the model. EC₅₀ values were compared between pairs of assays with PROC CORR by calculating Pearson correlation coefficients (*r*) between EC₅₀ values of isolates

used in both assays in each pair. Forty-one isolates were used in all four assays and 62 isolates were used in the mycelial growth, zoospore germination, and sporangia production assays.

Results

All isolates of *P. capsici* tested were sensitive to fluopicolide in all four assays. The EC₅₀ values for mycelial growth, zoospore germination, and sporangia production were non-normally distributed, and EC₅₀ values for zoospore production were normally distributed.

Mycelial growth. In the mycelial growth assay, the median EC₅₀ fluopicolide concentration across 69 isolates was 0.22 (range of 0.085 to 1.16) mg/liter, and 97% of the isolates had an EC₅₀ value <1.0 mg/liter (Table 2). Two isolates, one from bean in Michigan and one from yellow summer squash in South Carolina, that had relative colony diameters >50% at 1.0 mg/liter fluopicolide were grown on V8 amended with 3.16 mg/liter fluopicolide. EC₅₀ values for these two isolates then were calculated at 1.05 and 1.16 mg/liter, respectively (Fig. 1). The ratio between the least sensitive and the most sensitive isolate of the 69 isolates tested was 13.6. Mean EC₅₀ values differed among states (*F* value significant at *P* = 0.004). Isolates from Michigan had a higher mean EC₅₀ value, 0.38 mg/liter, than isolates from other states (*t* tests of least-squares means, *P* ≤ 0.02) (Table 3).

Zoospore germination. Calculated across all tests and isolates, a mean of 92.6% of encysted zoospores germinated on nonamended V8 medium. The median EC₅₀ concentration was 2.08 (range of 0.14 to 13.74) mg/liter across 66 isolates (Table 2). EC₅₀ values were <10.0 mg/liter for 96% of the isolates tested (Fig. 2). The EC₅₀ value calculated for one isolate from South Carolina was

Table 2. EC₅₀ values for isolates of *Phytophthora capsici* exposed to three or more concentrations of fluopicolide in four assays

Assay	No. isolates	Median EC ₅₀ (mg/liter)	Range (mg/liter)	Mean ± SE EC ₅₀ (mg/liter)	Ratio ^a	Threshold (mg/liter)	No. add'l isolates	Isolates with any growth at threshold (%)	No. above threshold ^b
Mycelial growth	69	0.22	0.085 to 1.16	0.27 ± 0.022	14.5	1.0	31	100	0
Zoospore germination	66	2.08	0.14 to 13.74	3.2 ± 0.38	97.4	10.0	34	29	0
Sporangia production	50	0.048	0.010 to 0.14	0.058 ± 0.0041	13.6	0.1	33	100	8
Zoospore production	53	0.10	0.034 to 0.19	0.10 ± 0.0046	5.7	0.1	0	ND ^c	ND

^a Ratio between the most sensitive and least sensitive isolate.

^b Number of additional isolates tested that had a response greater than 50% relative to the nonamended control.

^c ND = not done.

Fig. 1. Range of EC₅₀ values for growth of mycelia of 69 isolates of *Phytophthora capsici* from Florida (11 isolates), Georgia (14 isolates), Michigan (24 isolates), North Carolina (3 isolates), and South Carolina (17 isolates) on fluopicolide-amended V8 medium for 5 days.

very low, 0.015 mg/liter, and less than the lowest concentration (0.032 mg/liter) tested with this isolate.

Mean EC_{50} values for zoospore germination differed among states (F value significant at $P = 0.02$). Isolates from Michigan had a higher mean EC_{50} value, 4.91 mg/liter, than isolates from other states (t tests of least-squares means, $P \leq 0.03$) (Fig. 2, Table 3). The ratio between the least sensitive isolate, which originated from cucumber in Michigan, and the most sensitive isolate, which was obtained from bell pepper in South Carolina, was 97.4 across all isolates. The ratios between the least and most sensitive isolates within states ranged from 3.7 for 3 isolates from North Carolina to 44.6 for 17 isolates from South Carolina (data not shown).

Sporangia production. In the sporangia production assay, a mean of 65.6 sporangia per microliter were produced on nonamended V8 medium. Seventeen of the 63 isolates from Florida, Georgia, Michigan, and South Carolina had an EC_{50} value <0.03 mg/liter fluopicolide after preliminary calculations. Eight of these isolates were retested at 0, 0.010, 0.032, and 0.100 mg/liter. Four isolates then had EC_{50} values >0.010 , and four isolates had EC_{50} values <0.010 . For the 50 isolates that had EC_{50} values within the ranges of concentration tested, the median was 0.048 (range 0.010 to 0.14) mg/liter (Fig. 3). The ratio between the least and most sensitive isolates was low, 13.6. Mean EC_{50} values did not differ significantly among the five states (Table 3). Among the 50 isolates used to determine the EC_{50} values, 88% had an EC_{50} value <0.1 mg/liter.

Zoospore production. Across 53 isolates, the mean number of zoospores produced on nonamended medium was 97.5×10^4 /ml. The median fluopicolide concentration for a 50% reduction in zoospore production was 0.10 mg/liter (range of 0.034 to 0.19

mg/liter) (Table 2). One isolate from bell pepper from South Carolina was very sensitive and had an $EC_{50} < 0.03$ mg/liter. The ratio between the least and most sensitive isolates was very low, 5.7. The EC_{50} values were normally distributed, and the mean, 0.10 ± 0.034 , was identical to the median (Fig. 4). Mean EC_{50} values for zoospore production did not differ significantly among the five states (Table 3).

Threshold concentrations. Threshold concentrations were selected based on the response of the isolates tested in the four assays described previously. All of the 31 additional isolates tested grew on V8 medium amended with 1.0 mg/liter fluopicolide. The mean relative colony diameter was 0.18 ± 0.016 ; only one isolate had a colony diameter that equaled 50% of the control. For 34 additional isolates tested at 10 mg/liter, some zoospores of 10 isolates (29% of the isolates tested) germinated, but germination was less than 50% for all isolates. All of the 33 additional isolates produced some sporangia at the threshold concentration of 0.1 mg/liter. Mean relative sporangia production was $36.5 \pm 3.2\%$ (range of 1.95 to 76.8%) of the number of sporangia produced on nonamended medium (Table 2).

For the additional isolates, which were tested at only one concentration, the EC_{50} can be estimated from the threshold concentration. The number of isolates with a relative response $<50\%$ is the number of isolates that have an EC_{50} less than the threshold concentration (Tables 2 and 4). For mycelia growth, 97% of the additional isolates (30 of 31) had an $EC_{50} < 1.0$ mg/liter, and one isolate had an EC_{50} that equaled 1.0 mg/liter. Of the 144 isolates from watermelon in Beaufort County, SC tested at 0.3 mg/liter, 141 (98%) had an $EC_{50} < 0.3$ mg/liter (Table 4). The mean relative mycelial growth differed slightly between the isolates recovered

Table 3. Mean EC_{50} values (with standard errors) by state of origin for isolates of *Phytophthora capsici* exposed to three or more fluopicolide concentrations in four assays

Assay	Mean EC_{50} (mg/liter)				
	Florida	Georgia	Michigan	North Carolina	South Carolina
Mycelial growth	0.20 ± 0.051	0.19 ± 0.046	0.38 ± 0.035^a	0.14 ± 0.099	0.24 ± 0.041
Zoospore germination	2.49 ± 0.93	1.80 ± 0.78	4.91 ± 0.61^a	1.89 ± 1.69	2.63 ± 0.73
Sporangia production	0.047 ± 0.011	0.065 ± 0.0082	0.057 ± 0.0070	0.056 ± 0.021	0.059 ± 0.0094
Zoospore production	0.098 ± 0.0095	0.10 ± 0.0095	0.11 ± 0.010	0.12 ± 0.020	0.093 ± 0.0095

^a Value for isolates from Michigan was significantly greater than the values for isolates from the other four states in the same row, t tests of least-squares means, $P < 0.03$.

Fig. 2. Range of EC_{50} values for germination of zoospores of 65 isolates of *Phytophthora capsici* from Florida (10 isolates), Georgia (14 isolates), Michigan (23 isolates), North Carolina (3 isolates), and South Carolina (15 isolates) that were incubated on fluopicolide-amended V8 medium for 2 h.

from the two farms (F significant at $P = 0.038$). For zoospore germination, 100% of the 34 additional isolates had an $EC_{50} < 10.0$ mg/liter; and for sporangia production, 76% (29 of 37) of the isolates had an $EC_{50} < 0.1$ mg/liter.

Correlations of EC_{50} values between assays. When Pearson correlation coefficients (r) were calculated between the EC_{50} values of isolates used in pairs of assays, there were no correlations between EC_{50} values for mycelial growth or zoospore germination and EC_{50} values from the other assays. Of the six possible correlations, the only one with a significant correlation coefficient was the correlation between sporangia production and zoospore production ($r = 0.44$, $P = 0.003$).

Discussion

Mycelial growth, sporangia production, zoospore production, and zoospore germination of 104 isolates of *P. capsici* were sensitive to ≤ 10 mg/liter fluopicolide, except for six isolates whose zoospores germinated at concentrations up to 31.6 mg/liter. Zoospore germination was approximately nine times less sensitive to fluopicolide than mycelial growth was. In another study, the EC_{90} for zoospore cyst germination of *P. infestans* was >10 mg/liter (17). Sporangia and zoospore production were approximately four and two times more sensitive to fluopicolide than mycelial growth was. Mycelial growth of *P. capsici* was approximately equally sensitive to fluopicolide, di-

methomorph, and zoxamide (EC_{50} values ≤ 0.50 mg/liter) and was less sensitive to cymoxanil (>50 mg/liter) than to fluopicolide (13,19). Germination of zoospores of isolates from South Carolina was more sensitive to dimethomorph and less sensitive to cymoxanil than to fluopicolide (13). However, the single isolate of *P. capsici* that Matheron and Porchas (19) used was less sensitive to dimethomorph than isolates were to fluopicolide. Zoospore production was slightly more sensitive to fluopicolide than to dimethomorph or zoxamide (13).

Variation in baseline fungicide sensitivity in *P. capsici* isolates from different regions of the United States is reported here for the first time. The current study is one of the few studies on fungicide sensitivity of *P. capsici* to include isolates from more than one state (6,7,11,13,16). Isolates from Michigan were less sensitive to fluopicolide than isolates from four southeastern states in the mycelial growth and zoospore germination assays. Why sensitivity values differed in these two assays and did not differ in the sporangia and zoospore production assays is unknown. The different responses, however, indicate that fungicide sensitivity differs among life stages of this oomycete. EC_{50} values for fluopicolide were not correlated with mating type, mefenoxam sensitivity, or year of isolation (data not shown). Genetic diversity of isolates of *P. capsici* was high in each of six eastern states, including North Carolina and Michigan (9). This genetic diversity may include variation in sensitivity to fungicides.

Fig. 3. Frequency distribution of EC_{50} values for production of sporangia by 63 isolates of *Phytophthora capsici* from five states in the eastern United States grown on fluopicolide-amended V8 medium for 5 days.

Fig. 4. Frequency distribution of EC_{50} values for production of zoospores by 54 isolates of *Phytophthora capsici* from five states in the eastern United States that were incubated on fluopicolide-amended medium for 2 days.

Table 4. Evaluation of isolates of *Phytophthora capsici* from watermelon fields in Beaufort County, SC, for mycelial growth at 0.3 mg/liter fluopicolide

Farm, field	No. of isolates	Mean relative growth ^a	No. of isolates with $>50\%$ relative growth	Percentage of isolates with $EC_{50} < 0.3$ mg/liter
Farm 1				
Field 1	16	0.343 ± 0.0082	0	100
Field 2	16	0.355 ± 0.013	0	100
Field 3	38	0.341 ± 0.015	0	100
Subtotal	70	0.345 ± 0.0084	0	100
Range		0.170 to 0.485		
Farm 2				
Field 1	10	0.373 ± 0.010	0	100
Field 2	24	0.401 ± 0.012	0	100
Field 3	40	0.353 ± 0.015	3	93
Subtotal	74	0.371 ± 0.0092	3	96
Range		0.172 to 0.58		
Grand total	144	0.358 ± 0.0063	3	98

^a Growth on V8 medium amended with 0.3 mg/liter fluopicolide divided by growth on nonamended medium.

Mean fluopicolide EC₅₀ values of 0.2 mg/liter for mycelial growth and 2.7 mg/liter for zoospore germination of 25 isolates of *P. capsici* from cucurbits and peppers in Georgia were similar to mean values for 66 to 69 isolates from the eastern United States examined in the current study (11). In addition, the maximum EC₅₀ values for an individual isolate reported by Jackson et al. (11) were similar to the maximum EC₅₀ values for isolates from Georgia in the current study, 0.35 and 0.33 mg/liter for mycelia growth and 4.5 and 5.2 mg/liter for zoospore germination, respectively. The mean EC₅₀ for sporangia production of the 25 Georgia isolates tested by Jackson et al. (11), however, was much higher (1.7 mg/liter) than the mean across the 50 isolates tested by us (0.058 mg/liter). The different methods used to measure sensitivity of sporangia formation to fluopicolide in the two studies may have affected the results. Jackson et al. (11) placed agar plugs from cultures grown on unamended medium into aqueous suspensions of fluopicolide, whereas we amended agar with fluopicolide. In the only other laboratory study with zoospores of *P. capsici* and fluopicolide, the EC₅₀ values of two Korean isolates were 0.2 and 3.8 mg/liter, which fell within the range of the 66 isolates tested in the current study (21).

It was not surprising that mycelial growth and zoospore germination were not correlated with either sporangia production or zoospore production. Growth and germination are different physiological processes than production of reproductive propagules. Based on previous results with dimethomorph and zoxamide, it was not unexpected that mycelial growth would not be correlated with zoospore germination (13). Although both processes involve extension of hyphae, the walls of encysted zoospores might be less sensitive or less permeable to fluopicolide than the cell walls in mycelia. The correlation between sporangia production and zoospore production may reflect the fact that sporangia are involved in both assays, as zoospores are produced inside sporangia at low temperatures (16). Although sporangia were formed before exposure to fluopicolide in the zoospore production assay, they absorbed enough fungicide after exposure to fluopicolide to greatly reduce zoospore production.

Among the four assays, zoospore germination had the highest ratio between the most and least sensitive isolates tested in this study. According to Gisi et al. (8), baseline sensitivities of the most and least sensitive isolates in a population of isolates of *P. infestans* differed by a factor of up to 100. Thus, the ratio of 97.4 is not an indication of an unusual lack of sensitivity among isolates of *P. capsici*. Zoospores are considered to be the main infective propagule in saturated soils (6,19). Preventing infection by isolates whose zoospores are less sensitive to fluopicolide may require use of the higher rate of Presidio, 0.29 liter/ha rather than 0.22 liter/ha.

The close agreement between EC₅₀ values for mycelial growth determined with the original group of isolates and estimated with two other populations of isolates, the additional isolates from the collection and the Beaufort County isolates, lends confidence to the use of the threshold concentrations. For future testing, 0.3 or 1.0 mg/liter is recommended for mycelial growth assays, and 0.1 mg/liter is recommended for sporangia production and zoospore production. The threshold concentration for zoospore germination should be <10 mg/liter, since zoospores of only 29% of the additional isolates germinated at this concentration. A concentration of 5.0 mg/liter is suggested, based on the EC₅₀ value for isolates from Michigan. Zoospore germination is the assay most likely to detect isolates with reduced sensitivity to fluopicolide, since the EC₅₀ values and the ratio between the most and the least sensitive isolate were highest for this assay among the four assays. Because isolates in Michigan had higher EC₅₀ values for mycelial growth and zoospore germination than isolates from the southeastern United

States, future monitoring should concentrate on isolates from Michigan.

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