Sensitivity of Populations of *Phytophthora capsici* from South Carolina to Mefenoxam, Dimethomorph, Zoxamide, and Cymoxanil

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ABSTRACT

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In summer and fall 2003, *Phytophthora* blight and crown rot, caused by *Phytophthora capsici*, was found in three fields each of summer squash and pepper on three farms in two counties in South Carolina. Although this disease had been confirmed previously in the state, five of these outbreaks were in fields thought to be free of *P. capsici*. The objectives of this study were to determine whether isolates of *P. capsici* in South Carolina were sensitive to mefenoxam and to determine baseline sensitivities to dimethomorph, zoxamide, and cymoxanil, fungicides recently registered to control *Phytophthora* blight. Of 120 isolates tested for sensitivity to mefenoxam at 100 mg/liter, 8 isolates were resistant (relative colony diameter [RCD] > 90% of nonamended control), 60 isolates were sensitive (RCD < 30%), and 52 isolates were intermediately sensitive. Only sensitive isolates were found in two fields in which no mefenoxam-containing fungicides had ever been used. Intermediately sensitive or resistant isolates were found in the four fields in which mefenoxam had been applied previously. In all, 15 to 61 isolates were tested for sensitivity to dimethomorph, zoxamide, and cymoxanil. The concentrations at which RCD, percent cyst germination, and relative zoospore production were reduced to 50% (EC₅₀ values) for mycelial growth were 0.19 ± 0.02 (± standard deviation) mg/liter for dimethomorph, 0.50 ± 0.50 mg/liter for zoxamide, and mostly >50 mg/liter for cymoxanil. EC₅₀ values for zoospore cyst germination were 0.07 ± 0.02 mg/liter for dimethomorph and >50 mg/liter for cymoxanil. EC₅₀ values for zoospore production were 0.63 ± 0.42 mg/liter for dimethomorph, 0.47 ± 0.51 mg/liter for zoxamide, and <50 mg/liter for cymoxanil. Sensitivity values obtained in this South Carolina study can be used as a comparative baseline to monitor shifts in sensitivity to the fungicides mefenoxam, dimethomorph, zoxamide, and cymoxanil in populations of *P. capsici*.

Additional keywords: *Capsicum annuum*, *C. frutescens*, *Cucurbita pepo*

Diseases caused by *Phytophthora capsici* on vegetable crops are known variously as *Phytophthora* blight on pepper; *Phytophthora* crown, root, and fruit rot on cucurbits; and buckeye fruit and root rot on tomato. Although this soilborne Oomycete was first described in New Mexico in 1922, in the past 15 years it has become widespread throughout the eastern United States (3,6,8,15,16). Losses caused by *P. capsici* can be as high as tens or hundreds of thousands of U.S. dollars per farm per year (6; unpublished data).

Phytophthora blight and other diseases caused by *Phytophthora* spp. often have been managed with applications of mefenoxam, a systemic phenylamide fungicide. In *P. capsici*, a single, incompletely dominant gene regulates insensitivity to mefenoxam (9). Isolates of *P. capsici* resistant to mefenoxam at 100 mg/liter have been reported from North Carolina (6,15),

New Jersey (15), Georgia (14), Michigan (9), Florida (3), Connecticut (6), New York (6), northern Italy (21), and Korea (7). In addition, isolates that were moderately insensitive have been found in Florida (3,16) southern Italy (1), and Spain (18). Resistance to mefenoxam, a very effective fungicide against sensitive isolates, has made management of *Phytophthora* diseases in vegetable crops more difficult.

To manage *Phytophthora* diseases on cucurbit and solanaceous crops, new fungicide active ingredients have been registered. The fungicide dimethomorph was registered on cucurbits in 2002 as Acrobat (BASF Corp., Research Triangle Park, NC). In vitro, *P. capsici* is sensitive to low concentrations of dimethomorph. This fungicide reduces mycelial growth, sporangium formation, and zoospore cyst germination, but its effect on zoospore production is unknown (17,21,24). Zoxamide, marketed in a mixture with mancozeb as Gavel (Dow AgroSciences, Indianapolis, IN), was registered in 2002 to control *Phytophthora* diseases on tomato, cucumber, watermelon, muskmelon, and summer squash. *P. capsici* also is sensitive to low concentrations of zoxamide in vitro. Zoxamide reduces mycelial growth and sporangium and zoospore production but not

zoospore cyst germination (23,25). However, only one isolate of *P. capsici* was used in three previous screenings of dimethomorph and zoxamide (13,24,25). Shishkoff et al. (17) reported that dimethomorph at 1 mg/liter greatly reduced mycelial growth and zoospore germination of 16 isolates from Massachusetts, New York, and Georgia. Tamietti and Valetino (21) reported a mean concentration at which relative colony diameter (RCD) was reduced to 50% (EC₅₀) for dimethomorph of 0.48 mg/liter for mycelial growth of 26 Italian isolates. In order to establish more reliable baseline sensitivities for dimethomorph and zoxamide, a greater number of isolates should be tested. In fall 2003, cymoxanil plus famoxadone (Tanos; DuPont Agricultural Products, Wilmington, DE) was registered on pepper to suppress the foliar phase of *Phytophthora* blight. In 2005, Tanos registration was expanded to include cucurbits and tomato. Baseline sensitivities of *P. capsici* to cymoxanil and famoxadone have not been reported.

In summer and fall 2003, severe *Phytophthora* blight and crown rot was observed in six fields on three farms in two counties in South Carolina; Lexington County in the center of the state and Bamberg County in the southwestern part of the state. Although this disease had been confirmed previously in several counties in South Carolina, five of the 2003 outbreaks were in fields in which *Phytophthora* blight had not been observed previously. On farm 1 in Lexington County, symptoms of *Phytophthora* crown rot on squash and *Phytophthora* foliar blight and fruit rot on pepper were limited to one area in each field. On farm 2 in Lexington County, symptomatic wilted bell pepper plants were observed in 0.5 ha at one end of a 16-ha field; however, later, the pepper crop in this field was lost entirely to *Phytophthora* blight. *Phytophthora* crown rot and blight had been observed previously in this field on summer squash in 2000, on bell pepper in fall 2002, and on summer squash in spring 2003. In a jalapeno pepper field on farm 2 in Lexington County, fewer than 25 plants exhibited slight wilting. In a 6-ha field of yellow summer squash and in an adjacent field of zucchini in Bamberg County, all plants were killed by *P. capsici*. Symptoms of *Phytophthora* crown rot, blight, and fruit rot in all six fields were typical of those previously described and illustrated on summer squash and pepper (6).

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The objectives of this study were to determine whether isolates of *P. capsici* in South Carolina were sensitive to mefenoxam and to determine baseline sensitivities to dimethomorph, zoxamide, and cymoxanil in isolates never exposed to these fungicides. Mycelial growth, germination of encysted zoospores, and zoospore production were evaluated for sensitivity to dimethomorph, zoxamide, and cymoxanil. Technical-grade famoxadone was not available and so was not tested.

MATERIALS AND METHODS

***P. capsici* collection.** In August and September 2003, fruit and crowns of yellow crookneck and zucchini summer squash (*Cucurbita pepo*) with symptoms of Phytophthora fruit or crown rot were collected from three fields, one in Lexington County on farm 1 and two adjacent fields on a farm in Bamberg County (Table 1). Fruit of bell pepper (*Capsicum annuum*) with symptoms of Phytophthora fruit rot also were collected on farm 1 in Lexington County. Stems and roots of bell pepper and jalapeno pepper (*C. frutescens*) with symptoms of Phytophthora blight were collected on farm 2 in Lexington County. In all fields, only symptomatic plants and fruit were sampled. Both pepper fields on farm 2 in Lexington County had been treated with one application of mefenoxam (Ridomil Gold 2E) approximately 1 week prior to sampling. Mefenoxam had not been applied to the other four crops prior to sampling, and no mefenoxam-containing fungicides had been applied to the fields in Bamberg County. None of the fields had been treated with dimethomorph, zoxamide, cymoxanil, or famoxadone.

Small pieces of tissue were cut from the margins of lesions on stems, roots, or fruits. Tissue pieces were surface disinfested in 70% ethanol and placed on unclarified V8 juice agar amended with benomyl at 25 mg/liter, ampicillin at 100 mg/liter, rifampicin at 30 mg/liter, and pentachloronitrobenzene at 100 mg/liter (9). One single-zoospore isolate of *P. capsici* was obtained from each field isolate as described by Lamour and Hausbeck (9) and stored in screw-top glass vials on corn-

meal agar at 10°C. *P. capsici* from pepper roots was isolated from plants that also yielded the pathogen from stem tissue (Table 1).

Sensitivity to mefenoxam. For sensitivity tests with all four fungicides, isolates of *P. capsici* were grown on V8 agar for 3 days at 23 to 25°C in the dark. Agar plugs (7 mm in diameter) cut from these cultures were transferred to V8 agar amended with mefenoxam (Ridomil Gold 2E) (9,12) at 0 or 100 mg/liter. All 120 isolates were tested twice with two replicate plates in each test, and two perpendicular colony diameters were measured per plate. Isolate reaction to mefenoxam was based on RCD after 3 days of growth at 23 to 25°C in the dark. The diameter of the agar plug was subtracted from the colony diameter before calculating RCD. Isolates with an RCD <30% were classified as sensitive to mefenoxam, isolates with an RCD ≥30 and ≤90% were classified as intermediately sensitive, and isolates with an RCD >90% were classified as resistant to mefenoxam (9).

Mycelial sensitivity to dimethomorph, zoxamide, and cymoxanil. In all, 61 isolates were selected so that 10 isolates from each sampled field were included; 11 isolates from jalapeno pepper were included. Isolates were cultured on V8 agar as described above, and one 7-mm-diameter plug was transferred to V8 agar amended with dimethomorph (technical grade, 98%; Chem Service, West Chester, PA) at 0, 0.03, 0.10, 0.31, or 1.0 mg/liter and to V8 agar amended with zoxamide (technical grade, 98.1%; Dow Agro-Sciences) at 0, 0.10, 0.31, 1.0, or 3.1 mg/liter. Fifteen isolates, including one to five isolates from each field, were transferred to V8 agar amended with cymoxanil (technical grade, 99%; Chem Service) at 0, 0.1, 0.5, 1, 5, 10, or 50 mg/liter. Stock solutions of each fungicide were prepared in dimethyl sulfoxide (DMSO) (20). Non-amended agar received the equivalent volume of DMSO, 0.5 ml/liter, as amended agar. After 3 days of growth at 23 to 25°C in the dark, two perpendicular colony diameters were measured per plate. All isolates were tested twice with two replicate plates in each test. RCDs were calculated for each concentration of each fungicide.

Zoospore germination on fungicide-amended medium. Five isolates from each field were grown on V8 agar plates that were not wrapped with parafilm (to promote formation of sporangia) and held at 23 to 25°C for 4 days with a 16-h photoperiod (9). Plates were flooded with 10 ml of 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 (9). Four drops of zoospore suspension were streaked on V8 agar amended with dimethomorph at 0, 0.03, 0.10, 0.31, or 1.0 mg/liter. Plates were held at 23 to 25°C at ambient lighting for 2 h and 100 zoospore cysts were examined at ×200 magnification. Germinated, nongerminated, and lysed cysts were counted. All isolates were tested twice with two replicate plates in each test. As with RCD, the relative percentage of germinated zoospore cysts was calculated by dividing the number of germinated cysts per 100 cysts examined on fungicide-amended medium by the number of germinated cysts per 100 cysts examined on nonamended medium. The same procedure was used with zoospore suspensions of 15 isolates streaked on V8 agar amended with cymoxanil at 0 or 50 mg/liter. In preliminary tests, no reduction in germination of cysts was observed at cymoxanil concentrations of <50 mg/liter (*data not shown*). Therefore, no additional tests were done with low concentrations.

Zoospore production on fungicide-amended medium. The “sandwich” plate technique devised by Young and Vjugina (25) was used to test inhibition of zoospore production. In all, 15 of the 61 isolates used for mycelial sensitivity tests, including 1 to 5 isolates per field, were grown on V8 agar plates that were not wrapped with parafilm and held at 23 to 25°C with a 16-h photoperiod for 4 days to promote formation of sporangia. A 6-cm-diameter disk was cut from the culture with the bottom half of a 6-cm petri plate. Each culture disk was placed with the mycelium side up on a separate water agar plate amended with the test fungicide. After 2 days at 23 to 25°C with a 16-h photoperiod, the culture disk was transferred to an empty, sterile petri plate, flooded with approximately

Table 1. Number of isolates of *Phytophthora capsici* collected from six fields on three farms in South Carolina in 2003 and sensitivity to mefenoxam in vitro^a

County (farm) ^b	Crop	Plant part sampled				Sensitive	Intermed	Resistant
		Fruit	Crown	Stem	Root			
Lexington (1)	Bell pepper	6	0	4	0	0	10	0
Lexington (1)	Summer squash	12	0	0	0	0	7	5
Bamberg	Summer squash	16	3	0	0	19	0	0
Bamberg	Zucchini	17	0	0	0	17	0	0
Lexington (2)	Bell pepper	0	0	46	4	19	29	2
Lexington (2)	Jalapeno pepper	0	0	10	2	5	6	1
Total	...	51	3	60	6	60	52	8

^a Isolates with a relative colony diameter (RCD) <30% were classified as sensitive, isolates with an RCD equal to or between 30 and 90% were classified as intermediately sensitive (Intermed), and isolates with an RCD >90% were classified as resistant to mefenoxam at 100 mg/liter.

^b The fields on farm 1 in Lexington County were approximately 2 km apart; fields on the other two farms were adjacent.

10 ml of sterile deionized water to cover the disk, chilled at 10°C in the dark for 30 min, and then held at 23 to 25°C for 30 min at ambient lighting (9). The culture disk was scraped gently with a sterile spatula to release zoospores from sporangia. Aliquots of the zoospore suspension were placed on a hemacytometer. Young and Vjugin's procedure was modified by passing the hemacytometer quickly through an alcohol flame to stop zoospore motility. The number of zoospores was counted, with four subsamples (counts) per replication (petri plate). Fungicide concentrations tested were 0, 0.03, 0.31, and 3.1 mg/liter for zoxamide; 0, 0.01, 0.10, and 1.0 mg/liter for dimethomorph; and 0 and 50 mg/liter for cymoxanil. The relative proportion of zoospores produced was calculated by dividing the number of zoospores counted from culture disks placed on fungicide-amended medium by the number of zoospores produced on nonamended medium. There were two replicate plates in each test, and all isolates were tested twice with dimethomorph and cymoxanil and three times with zoxamide.

Calculation of EC₅₀ and statistical analysis. RCD, relative percentage of zoospore cyst germination, and relative zoospore production were calculated for each replication in each repeat and then regressed against the base-10 logarithm of three or four fungicide concentrations (22). Except for relative zoospore production on dimethomorph, the plots of observed and predicted values were linear with respect to the base-10 logarithm of fungicide concentration; therefore, the probit transformation was not needed. Quadratic equations were used for relative zoospore production in the presence of dimethomorph when plots of relative zoospore production against log₁₀ (fungicide concentration) were nonlinear. The regression equation obtained for each isolate with PROC GLM of SAS (version 8e) was solved to obtain EC₅₀. Using PROC GLM, isolates were compared by farm and field within farm based on EC₅₀ values for mycelial growth and zoospore cyst germination in the presence of dimethomorph or zoxamide and zoospore production in the presence of cymoxanil. Pearson correlation coefficients (*r*) were calculated with SAS PROC CORR for EC₅₀ values with isolates that were used in both mycelial growth and zoospore tests with dimethomorph and zoxamide.

RESULTS

Sensitivity to mefenoxam. Of 120 isolates of *P. capsici* collected in 2003, 8 isolates (7%) were resistant (RCD > 90%), 60 (50%) were sensitive (RCD < 30%), and 52 (43%) were intermediately sensitive to mefenoxam at 100 mg/liter in vitro (Table 1). Only sensitive isolates were obtained from the adjacent fields of yellow crookneck and zucchini summer squash in

Bamberg County, whereas each of the four fields sampled in Lexington County had intermediately sensitive isolates. Resistant isolates were found in three of the four

fields in Lexington County; the two fields on farm 1 yielded no sensitive isolates.

In the two pepper fields on farm 2 in Lexington County that had been treated

Fig. 1. Concentrations at which relative colony diameter, percent cyst germination, and relative zoospore production were reduced to 50% (EC₅₀) for *Phytophthora capsici* isolates on dimethomorph-amended medium. **A**, Mycelial growth for 61 isolates after 3 days. **B**, Germination of encysted zoospores of 30 isolates after ≥2 h. **C**, Production of zoospores by 15 isolates.

with mefenoxam prior to sampling, 24 isolates (39%) were sensitive, 35 (56%) were intermediately sensitive, and 3 isolates (5%) were resistant. Among the six isolates from pepper roots in the treated fields, two isolates were sensitive and four isolates were intermediately sensitive. Four of five pairs of isolates recovered from stems and roots of the same pepper plant had identical sensitivities (i.e., both isolates were sensitive or intermediate). In the fifth pair, the isolate from a jalapeno pepper root was intermediately sensitive, but the isolate from the stem was sensitive.

Sensitivity to dimethomorph. Mean colony diameters of 61 isolates of *P. capsici* grown on nonamended V8 agar differed for the three farms from which they were collected ($P < 0.0001$). Differences were consistent across the control plates for the mycelial growth assays for dimethomorph and zoxamide. Mean colony diameters were 58.7 ± 0.7 mm for isolates

from Bamberg County, 36.2 ± 0.7 mm for isolates from Lexington farm 1, and 44.2 ± 0.8 mm for isolates from Lexington farm 2. There were no significant differences in colony diameters between fields on a single farm.

Mean RCDs for all isolates were $<50\%$ for dimethomorph at 0.31 mg/liter (range 12 to 48%). Mean RCDs across isolates at 0.31 mg/liter were identical in the two tests, 0.28 ± 0.8 (standard deviation). Four isolates, LJ1, LJ2, RCZ6, and RCS1, two isolates each from Lexington farm 2 and Bamberg, respectively, had a mean RCD for dimethomorph at 1 mg/liter that was greater than 0 (t test, $P \leq 0.04$). EC_{50} values for all isolates were approximately normally distributed (Shapiro-Wilk W statistic = 0.96, $P = 0.07$) around the mean of dimethomorph at 0.19 ± 0.02 mg/liter (Fig. 1A). The difference between the minimum and maximum EC_{50} values for individual isolates (dimethomorph at 0.14

and 0.26 mg/liter) was only 1.9-fold. Nevertheless, the mean EC_{50} for isolates from the farm in Bamberg County (0.20 ± 0.02) was significantly higher ($P < 0.0001$) than for the two farms in Lexington County (mean of 0.18 ± 0.02).

On nonamended V8 agar, a mean of $90.0 \pm 1.3\%$ of *P. capsici* zoospore cysts germinated after 2 h of incubation at ambient temperature. On V8 agar amended with dimethomorph at 0.31 and 1.0 mg/liter, $\geq 75\%$ of the zoospore cysts lysed. Mean germination of zoospore cysts exposed to dimethomorph at 1.0 mg/liter ranged from 0.0 to 2.5% for 30 isolates of *P. capsici*. Because 12 of 30 isolates did not germinate at this concentration, dimethomorph at 1.0 mg/liter was excluded from analyses to ensure linearity of the regression equations used to calculate EC_{50} values. EC_{50} values for all isolates were normally distributed (Shapiro-Wilk W statistic = 0.96, $P = 0.24$) around the mean of dimethomorph at 0.07 ± 0.02 mg/liter (Fig. 1B). The mean EC_{50} for isolates from the squash (0.08 ± 0.01) and bell pepper (0.04 ± 0.01) fields from Lexington County farm 1 differed significantly from each other ($P = 0.002$). EC_{50} values for zoospore germination of 29 isolates were not correlated with EC_{50} values for mycelial growth on dimethomorph-amended medium ($r = 0.13$, $P = 0.49$).

Dimethomorph inhibited zoospore production. A mean of $9.1 \pm 3.5 \times 10^5$ zoospores/ml were produced by *P. capsici* isolates on V8 agar disks placed on non-amended water agar. Mean relative zoospore production with dimethomorph at 0.1 mg/liter was not significantly different from mean relative zoospore production at 0.01 mg/liter (t test, $P = 0.34$). Based on this test, analysis of variance with and without a quadratic term for $\log_{10}$ (concentration), and visual examination of plots of relative zoospore production and $\log_{10}$ (concentration), a quadratic equation was used to calculate EC_{50} for 13 of the 15 isolates, and a linear equation was used for the remaining 2 isolates. One isolate, RCS4 from the yellow summer squash field in Bamberg County, had higher relative zoospore production with dimethomorph at 1.0 mg/liter than the other 14 isolates tested (Waller-Duncan k -ratio t test, $k = 500$). Therefore, relative zoospore production with dimethomorph at 3.1 mg/liter was determined in two additional tests with this isolate. EC_{50} values for 15 isolates were nonnormally distributed (Shapiro-Wilk W statistic = 0.63, $P < 0.0001$) with a median of dimethomorph at 0.53 mg/liter (Fig. 1C). The mean EC_{50} was 0.63 ± 0.42 mg/liter. EC_{50} values for zoospore production by seven isolates were not correlated with EC_{50} values for mycelial growth or zoospore germination on dimethomorph-amended medium ($P \geq 0.63$).

Sensitivity to zoxamide. Margins of fungal colonies grown on zoxamide-

Fig. 2. Concentrations at which relative colony diameter and relative zoospore production were reduced to 50% (EC_{50}) for *Phytophthora capsici* isolates on zoxamide-amended medium. **A**, Mycelial growth for 61 isolates after 3 days. **B**, Production of zoospores by 15 isolates.

amended medium were somewhat irregular. Mean RCDs across isolates were 3 to 5% higher in trial 2 than in trial 1 at each zoxamide concentration but did not differ significantly between trials (t tests, $P > 0.10$). Mean RCDs for all isolates were <50% with zoxamide at 3.1 mg/liter (range 9 to 49%). EC_{50} values for mycelial growth of individual isolates ranged from 0.07 to 2.36 mg/liter, with a mean of 0.50 ± 0.50 mg/liter across isolates (Fig. 2A). Values were nonnormally distributed (Shapiro-Wilk W statistic = 0.65, $P < 0.0001$) with median of zoxamide at 0.33 mg/liter.

Zoxamide also reduced zoospore production by *P. capsici*. A mean of $5.4 \pm 2.7 \times 10^5$ zoospores/ml was produced by *P. capsici* isolates on nonamended agar. EC_{50} values for 15 isolates were nonnormally distributed (Shapiro-Wilk W statistic = 0.76, $P = 0.001$) with a median of zoxamide at 0.29 mg/liter (Fig. 2B). The mean EC_{50} was 0.47 ± 0.51 mg/liter for zoxamide. EC_{50} values for zoospore production of 13 isolates were not correlated with EC_{50} values for mycelial growth on zoxamide-amended medium ($r = -0.21$, $P = 0.49$).

Sensitivity to cymoxanil. Mean RCDs on V8 medium amended with cymoxanil at 50 mg/liter ranged from 0.25 to 0.80 for 15 isolates of *P. capsici*, with an overall mean of 0.56 ± 0.13 . EC_{50} values for mycelial growth on cymoxanil-amended medium were >50 mg/liter for 13 isolates and between 10 and 50 mg/liter for two isolates tested. Relative proportions of zoospore cysts germinating with cymoxanil at 50 mg/liter ranged from 0.76 to 1.07 with a mean of 0.95 ± 0.08 . For two isolates (CRP12A from Lexington farm 1 and RCZ17 from Bamberg County), percent germination was lower on cymoxanil-amended medium than on nonamended medium (least squares means t tests, $P < 0.05$).

Cymoxanil greatly inhibited zoospore production. The mean proportion of zoospores formed with cymoxanil at 50 mg/liter relative to nonamended medium was 0.11 ± 0.15 , with a median value of 0.004 and a range of 0.000 to 0.42 (Fig. 3). Thus, EC_{50} values for zoospore production were <50 mg/liter for cymoxanil for all 15 isolates. Of 15 isolates tested, 6 did not produce any zoospores when exposed to cymoxanil at 50 mg/liter. Mean relative proportion of zoospores produced by isolates from Bamberg County (0.29 ± 0.11) was significantly greater ($P = 0.0003$) than the mean proportion for isolates from both farms in Lexington County (0.02 ± 0.05 and 0.03 ± 0.07 for farms 1 and 2, respectively).

DISCUSSION

Although isolates of *P. capsici* recovered from diseased squash and pepper in South Carolina showed a range of sensitivities to mefenoxam, one-half of the isolates were

intermediately sensitive or resistant. Prior application of mefenoxam-containing fungicides was a good predictor of the level of sensitivity to mefenoxam in field isolates. All isolates collected from the two Bamberg County fields in which no mefenoxam-containing fungicides had ever been used were sensitive to mefenoxam. However, intermediately sensitive and resistant isolates were identified in three and four of the Lexington County fields, respectively. It is likely that applications of fungicides containing mefenoxam in years prior to 2003 affected the sensitivity of the population. On both farms in Lexington County, Ridomil Gold 2E, mefenoxam-chlorothalonil (Ridomil Gold Bravo), and mefenoxam-mancozeb (Ridomil Gold MZ) had been applied infrequently to control downy mildew on summer squash. Deposition of foliar-applied mefenoxam onto soil in open spaces between young plants during spraying or runoff from the plant canopy may have exposed *P. capsici* in soil to mefenoxam. Use of mefenoxam-containing foliar fungicides could be an additional risk factor in selecting for mefenoxam resistance in *P. capsici*.

Parra and Ristaino (15) reported a higher recovery (mean of 75%) of resistant isolates from fields in which metalaxyl or mefenoxam had been applied alone to manage Phytophthora blight than when applied in combination with other fungicides (mean of 34%). This association could not be tested in South Carolina, because no fields were sampled in which mefenoxam alone had been applied during the past several years.

In all fields that have been sampled for *P. capsici* in Florida, Michigan, and several

other states, both the A1 and A2 mating types were found (6,9,16). In Illinois and Spain, however, both mating types were present in only a few of the fields sampled, although the number of isolates collected per field was much lower than in other studies (8,18). The mating type of isolates was not determined in the present study; however, it is likely that both mating types, and oospores, were present in the fields sampled. The reappearance of Phytophthora blight in the bell pepper field on farm 2 in Lexington County in 2002, after the initial outbreak in 2000, supports the assumption that oospores were present in this field. In a previous study in Michigan, the frequency of mefenoxam-resistant isolates did not change during three consecutive years of sampling (10). Likewise, 59 and 63% of *P. capsici* isolates collected in North Carolina were resistant to mefenoxam in 1997 and 2001, respectively (2). Therefore, mefenoxam-resistant isolates likely will persist in the fields sampled in South Carolina.

The ranges of sensitivity to dimethomorph in three different populations of *P. capsici* were relatively narrow. Although Italian isolates of *P. capsici* had a slightly higher mean EC_{50} for mycelial growth on dimethomorph-amended medium (0.48 mg/liter) than isolates from South Carolina (0.19 mg/liter), both values are of the same order of magnitude (21). In a study by Shishkoff et al. (17), only 1 of 16 isolates grew with dimethomorph at 1.0 mg/liter. Similarly, only 4 of 61 isolates grew with dimethomorph at 1.0 mg/liter in the present study. Maximum germination of zoospore cysts with dimethomorph at 1.0 mg/liter, 2.3 and 2.5%, also was similar in

Fig. 3. Proportion of zoospores produced by 15 isolates of *Phytophthora capsici* exposed to cymoxanil at 50 mg/liter in vitro relative to the nonamended control. Six isolates from Lexington County did not produce any zoospores. Pooled standard error = 0.092.

the study by Shishkoff et al. (17) and in the present study, respectively. EC₅₀ values for mycelial growth of *P. capsici* on dimethomorph-amended medium were similar to values reported for *P. infestans*, but *P. capsici* was more sensitive to dimethomorph in production and germination of zoospores than *P. infestans* was (19).

Using the values reported by Young and Vjugina (25), an EC₅₀ value of 0.09 mg/liter was calculated for zoospore production in the presence of zoxamide. Young and Vjugina (25) counted only motile zoospores when examining the effects of zoxamide on *P. capsici* ATCC 15399. In the current study, the elevated EC₅₀ value of 0.47 mg/liter reflects a count of all zoospores, because they were immobilized by heat and readily visible on the hemacytometer. Differences in methodology and differences in the isolates used may account for the different EC₅₀ values.

Mycelial growth of *P. capsici* was much less sensitive to cymoxanil than mycelial growth of *P. infestans*. In tests of a worldwide collection of 266 isolates of *P. infestans*, mean EC₅₀ values were <1.0 mg/liter for cymoxanil (5,20). For *P. capsici*, EC₅₀ values were >10 mg/liter for all 15 isolates tested.

Differences in EC₅₀ values between the most and least sensitive isolates were <10 for all three assays with dimethomorph, 34 and 79 for mycelial growth and zoospore production in the presence of zoxamide, and 141 for zoospore production in the presence of cymoxanil. The sensitivity range to dimethomorph reported for *P. infestans* was similar in magnitude (value ≤ 11) to the value for *P. capsici*, but the range for *P. infestans* was smaller for cymoxanil (approximately 100) (19,20). According to Gisi et al. (4), baseline sensitivities of the most and least sensitive isolates in a population may differ by a factor of up to 100. Thus, all six South Carolina populations of *P. capsici* examined consisted of isolates that were sensitive to dimethomorph and zoxamide with no biologically significant variations within or among populations. However, the differences observed between South Carolina populations in sensitivity to cymoxanil may warrant further sampling.

Because isolates of *P. capsici* have become resistant to mefenoxam in many parts of the eastern United States, growers must rely on cultural practices and other fungicides to manage *Phytophthora* blight and fruit rot. *Phytophthora* crown and fruit

rot on summer squash and *Phytophthora* blight and fruit rot on pepper have continued to appear in other fields in South Carolina since the outbreaks in 2003. Growers who have had crop losses to *P. capsici* in the recent past currently use various combinations of mefenoxam, dimethomorph, zoxamide plus mancozeb, or cymoxanil plus famoxadone to attempt to manage this pathogen. The sensitivity values determined in this study can be used as a comparative baseline to monitor South Carolina populations of *P. capsici* for shifts in sensitivity to cymoxanil, dimethomorph, and zoxamide as these fungicides are used more widely.

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