Identification, Mefenoxam Sensitivity, and Compatibility Type of *Phytophthora* spp. Attacking Floriculture Crops in North Carolina

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ABSTRACT

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Phytophthora isolates were collected from floriculture crops grown in commercial greenhouses in North Carolina for species identification, compatibility type determination, and mefenoxam sensitivity tests. Isolation from 41 symptomatic plant species at 29 production locations resulted in 483 isolates from eight crops at seven locations. *Phytophthora cryptogea* (184 isolates) was recovered from dusty miller and gerbera daisy. All isolates of *P. cryptogea* were insensitive or intermediate in sensitivity to mefenoxam at 1 µg a.i./ml and were A1 compatibility type. *P. nicotianae* (273 isolates) was isolated from African violet, lavender, pansy, petunia, and vinca. Of these isolates, 21% were insensitive to mefenoxam at either 1 or 100 µg a.i./ml. Isolates of *P. nicotianae* from five locations were A2 compatibility type, whereas isolates on pansy at one location were A1 compatibility type. English ivy grown at two locations was infected with *P. palmivora*. All 26 isolates of *P. palmivora* were sensitive to mefenoxam and were A1 compatibility type. Mating type and mefenoxam sensitivity were uniform among isolates of *P. nicotianae* and *P. palmivora* from a given crop at a given location, suggesting that epidemics within a location may have originated from a single source of inoculum.

Additional keywords: EC₅₀, phenotypic diversity, survey

The wholesale value of floriculture crops in the United States was estimated at \$4.88 billion in 2002 (29). Floriculture crops are relatively high-value crops with intensive labor needs and little tolerance for plant damage; therefore, economic loss can be severe if plant diseases develop. Several *Phytophthora* spp. are known to cause root rot, crown rot, and foliar blight on a variety of floriculture crops grown in greenhouses (5,7). Managing *Phytophthora* diseases on floriculture crops can be difficult, considering favorable environmental conditions for the pathogen inside greenhouses and the biology of the pathogen. Disease development may be favored by warm and humid conditions, with pathogen inoculum and ample host plants in year-round production. Dispersal of motile zoospores of *Phytophthora* spp. in recycling irrigation water may further compound the problem (27,30). In addition, regional, national, and international move-

ment of large volumes of plant material is common in the floriculture industry. It is highly possible that propagules of the pathogen can be dispersed long distances in a short period of time along with infected plant materials, and cause an outbreak where they have been introduced.

Fungicide application is a major component in the management of *Phytophthora* diseases on floriculture crops (5). Metaxyl and a similar isomer, mefenoxam, are systemic phenylamide fungicides selective for Oomycete pathogens (23). Growers continue to rely on these compounds due to the effectiveness of the products, but fungicide resistance has now developed (8,15).

Monitoring the proportion of a pathogen population resistant to a fungicide can be

essential to develop management strategies that can delay or prevent development of resistance to fungicides and fungicide failure (25,26). Monitoring a pathogen population also can detect the movement of indigenous pathogen propagules as well as the introduction of exotic pathogens. In a previous study (14), the movement of isolates of *Phytophthora drechsleri* and *P. nicotianae* within a production site and among production facilities was investigated by comparison of mefenoxam sensitivity, compatibility type, and amplified fragment length polymorphism (AFLP) profile. Because the pathogen population was clonal, asexual reproduction was responsible for epidemics within a facility. Spread of the pathogen among facilities occurred via movement of infected plant materials (14).

An inclusive survey for a pathogen over an extended period of time can provide valuable information on the occurrence and distribution of the pathogen. Characterization of a pathogen population allows monitoring of fungicide resistance development as well as dispersal of the pathogen, information that is critical for successful disease control. However, little is known concerning the current status of *Phytophthora* diseases on floriculture crops in North Carolina because an extensive, statewide survey has not been done. The objectives of this study were to identify *Phytophthora* spp. attacking floriculture crops grown in North Carolina and to characterize these isolates based on their mefenoxam sensitivity and compatibility type.

MATERIALS AND METHODS

Isolate collection. From 2001 to 2002, a total of 41 plant species with symptoms of

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Fig. 1. Production locations of greenhouses in North Carolina surveyed for *Phytophthora* spp. in 2001 and 2002. *Phytophthora* spp. were isolated (+) from greenhouse locations in 6 of 17 counties surveyed. No *Phytophthora* sp. was recovered from greenhouse locations in the other 11 counties surveyed (-).

leaf blight, crown rot, or root rot were sampled in greenhouses at 29 production locations from 17 counties in North Carolina (Fig. 1). Production facilities were selected randomly and were diverse in size and production scale. Some of these sites were family-operated greenhouses with less than 2,000 m² of production area, whereas others were large-scale operations with over 100 employees on site. Up to 120 symptomatic plant samples were collected throughout the greenhouse ranges for a given plant species at each location. Plant samples were kept in an insulated chest with ice to minimize tissue deterioration during the transportation of the samples from the survey site to the laboratory, where the samples were stored at 4°C until processed. Plant samples were washed under running tap water and leaf, stem, or root tissues with margins of developing lesions were selected for isolation. *Phytophthora* spp. were isolated from plant samples by plating infected tissues on cornmeal agar (CMA) amended with pimarcin, ampicillin, rifampicin, and pentachloronitrobenzene (PARP) with or without hymexazol (12). The plates were kept at ambient temperature (23 to 25°C) in the dark for up to 14 days. Mycelial growth on the media was examined under a microscope for the presence of hyphae of *Phytophthora* spp. Developing *Phytophthora* colonies were transferred to new PARP-CMA media. After 48 h, a sequential transfer of actively growing hyphal tips to a fresh CMA plate was done to establish an axenic culture. For long-term storage, a 5-mm-diameter agar plug from the edge of a colony was placed into a 25-ml tube with 9 ml of CMA. After incubation at ambient temperature in the dark for 7 days, the isolates were stored at 18°C. An isolate was defined as a culture of a *Phytophthora*

sp. recovered from an individual plant grown at a given location on a given survey date. A group was defined as several isolates of *Phytophthora* spp. collected from the same plant species grown at a given production location on a given date of survey.

Species identification. Isolates were identified to species based on morphological and physiological characteristics (7,24). Morphology of sporangia was examined after incubation of the cultures on either CMA or V8 broth (200 ml of V8 clarified juice plus 800 ml of sterile deionized water) under continuous fluorescent light for 3 to 7 days. Clarified V8 juice was prepared by filtering V8 juice through a 3-cm-thick layer of Celite 545 (Fisher Scientific, Fair Lawn, NJ) after adding 2 g of CaCO₃. Twice-autoclaved vinca (*Catharanthus roseus*) leaves colonized with a *Phytophthora* isolate were placed in a petri plate, then flooded with sterile tap water. After the formation of sporangia, flooded vinca leaves were shaken on a rotary shaker at 200 rpm for 1 min for determination of sporangial caducity and length of pedicels.

Species identifications were confirmed with a polymerase chain reaction (PCR) technique with internal transcribed spacer (ITS) primers ITS 5 (5' GGAAGTAAAGTAACAAGG) and ITS 4 (5' TCC-TCCGCTTATTGATATGC) as described by Ristaino et al (22). Three to four isolates representing each group were grown in pea broth for 7 days at ambient temperature. Pea broth was prepared by autoclaving 120 g of frozen peas in 500 ml of deionized water. After filtration through several layers of cheesecloth, the filtrate was brought to 1 liter with deionized water and autoclaved for 25 min. Mycelia of isolates were filtered and collected into

1.5-ml microtubes. DNA was extracted by a hexadecyltrimethylammonium bromide (CTAB) procedure (11). The PCR with the ITS 5 and ITS 4 primers was conducted in 50-μl reaction volumes. Each reaction tube contained 1 μl of a 1-ng/μl DNA template, 36.6 μl of sterile distilled water, 5 μl of 10× PCR buffer (Boehringer Mannheim, Indianapolis, IN), 2 μl of 2 mM deoxynucleoside triphosphates (Invitrogen, Carlsbad, CA), 1 μl of 10 mM MgCl₂ (Sigma-Aldrich, St. Louis), 2 μl of each primer, and 0.4 μl of *Taq* (5 U/μl, Boehringer Mannheim). The PCR cycle consisted of an initial denaturation at 96°C for 2 min followed by 35 cycles of 96°C for 1 min, 55°C for 1 min, and 72°C for 2 min, and a final extension at 72°C for 10 min. Amplified fragments were digested with a series of restriction enzymes including *AluI*, *HaeIII*, *MspI*, and *RsaI*. Each digestion tube consisted of 15 μl of amplified PCR product and 2 μl of enzyme mixture (1 μl of REact buffer [Gibco BRL, Gaithersburg, MD], 1 μl of restriction enzyme, and 8 μl of sterile distilled water). Amplified PCR product was digested at 37°C for 3 h and the reaction was stopped by incubating the product at 65°C for 10 min. Digested DNA was resolved and visualized on a 1.5% agarose gel containing ethidium bromide (0.5 μg/ml) in Tris-borate-EDTA buffer at 55 mA for 2 h. Fragment sizes in base pairs were calculated with the shareware software SEQAID II (20). Reference isolates for corresponding species were obtained from cooperators (Table 1).

Compatibility type determination. Fresh cultures of each isolate were obtained by transferring agar plugs from stock cultures onto CMA plates. A 6-mm-diameter agar plug from the margin of a growing colony was transferred to either V8-juice agar (200 ml of clarified V8 juice, 800 ml of deionized water, and 17 g of agar per liter) or amended V8-juice agar (100 ml of V8 juice, 2.5 g of calcium carbonate, 30 mg of β-sitosterol, 20 mg of tryptophan, 100 mg of CaCl₂ · H₂O, 1 mg of thiamine, and 15 g of agar per liter) as described by Chee et al. (3). Each isolate initially was paired with a known compatibility type isolate of *P. capsici* by placing agar plugs of tester isolates 2 cm apart from each unknown isolate. Tester isolates of *P. cryptogea* and *P. nicotianae* were used for the isolates which failed to form oospores in pairing with *P. capsici*. The tester isolates were obtained from cooperators and our own collection (Table 1). After incubating the plates at ambient temperature in the dark for 3 to 14 days, the compatibility type of each isolate was determined microscopically based on the presence or absence of oospores.

Mefenoxam sensitivity and 50% growth inhibition assay. All isolates were screened for fungicide sensitivity based on hyphal growth on CMA amended with mefenoxam. An agar plug from each stock

Table 1. Species, host plant, and location where isolates were recovered, and designation of isolates of *Phytophthora* spp. used as a reference in polymerase chain reaction confirmation of species identification and as testers in compatibility type tests

Species, host, and location	Isolate designation	Compatibility type ^a
<i>Phytophthora capsici</i>		
Cucurbits, Michigan	OP97 ^b	A1
Cucurbits, Michigan	SP98 ^b	A2
<i>P. cryptogea</i>		
Aster, California	ATCC15402 ^c	A1
Dusty miller, North Carolina	FDM	A1
<i>Godetia</i> sp., South Africa	ATCC15403 ^c	A2
<i>P. nicotianae</i>		
African violet, North Carolina	VAV	A2
Pansy, Maryland	223 ^d	A2
Pansy, North Carolina	FBPS	A1
Verbena, New York	221 ^d	A2
<i>P. palmivora</i>		
Citrus, Florida	P8 ^e	A1
Citrus, Florida	P29 ^e	A1

^a All *Phytophthora* isolates from the survey initially were paired with *P. capsici* OP97 and SP98. *P. cryptogea* ATCC15402 and ATCC15403, *P. nicotianae* 221 and 223, and *P. palmivora* P8 and P29 were used as reference species.

^b Isolates provided by K. H. Lamour.

^c Isolates provided by S. H. Kim.

^d Isolates provided by M. L. Daughtrey.

^e Isolates provided by J. B. Ristaino (22).

culture was transferred to CMA, then incubated at ambient temperature for 3 days to obtain a fresh culture. A 6-mm-diameter agar disk was taken from the margin of each resulting culture and placed at the center of each of three replicate CMA plates containing mefenoxam (Subdue MAXX; Syngenta, Greensboro, NC) at either 0, 1, or 100 µg a.i./ml. The plates were incubated at ambient temperature for 4 to 10 days. The diameter of hyphal growth in all plates was measured when colonies in the absence of mefenoxam had reached the edge of the plate. Inhibition by mefenoxam was determined as the percentage of growth in diameter at a given mefenoxam concentration compared with the growth on the nonamended plate. At a given concentration, isolates were defined as (i) sensitive if hyphal growth was none, (ii) intermediate if hyphal growth was less than 50% compared with the growth on the nonamended plate, or (iii) insensitive if hyphal growth was

greater than 50% of growth of that on the nonamended plate.

Isolates that grew at 1 µg a.i. mefenoxam/ml were further studied to determine the effective concentration of fungicide giving 50% growth inhibition (EC₅₀). Based on the results from the mefenoxam sensitivity test, three or four mefenoxam concentrations were selected for each group of isolates for appropriate regression analysis. A subset of three isolates within a group was transferred to three replicate plates of CMA amended with mefenoxam at either 0.1, 0.2, 0.5, 10, 20, 50, 200, 500, or 1,000 µg a.i./ml. The growth of each isolate relative to the growth on the nonamended plate was determined. The mean across isolates within a subset was subjected to a regression analysis with percent growth expressed on a probability scale and fungicide concentration on a logarithmic scale. The resulting slope value was used to interpolate EC₅₀ (2). Both the mefenoxam sensitivity test and EC₅₀ assay

experiments were repeated twice with a completely randomized design. Data were analyzed with mefenoxam concentration as a fixed effect in one-way analysis of variance. The data were pooled across experiments based on homogeneity of variances.

RESULTS

Phytophthora isolation and species identification. A total of 483 *Phytophthora* isolates were recovered from 3,831 symptomatic plants (41 species) sampled from 29 production locations (Table 2). Among 41 plant species sampled, *Phytophthora* spp. were isolated from African violet, dusty miller, English ivy, gerbera daisy, lavender, pansy, petunia, and vinca at one or more of seven locations in six counties in North Carolina (Fig. 1).

These isolates were identified as *P. cryptogea*, *P. nicotianae*, and *P. palmivora* based on morphological and physiological characteristics and PCR (Fig. 2). Isolates of *P. cryptogea* produced nonpapillate, nondeciduous sporangia with internal proliferation on V8 broth. Amphigynous antheridia were produced in the presence of the opposite compatibility type on amended V8-juice agar. Oospores were typically 25 µm in diameter with a range of 20 to 29 µm. Isolates of *P. nicotianae* produced abundant papillate sporangia and chlamydospores on CMA. Sporangia were not caducous. Oospores with amphigynous antheridia were formed in paired cultures on V8-juice agar and ranged in diameter from 22 to 29 µm. Sporangia of *P. palmivora* were prominently papillate on V8-juice agar. Deciduous sporangia had a rounded base and short pedicels when they were detached from sporangiophores. On amended V8-juice agar, oospores were

Table 2. Plant species, number of plants sampled and locations surveyed, and number of *Phytophthora* isolates recovered from a survey of floriculture crops in North Carolina in 2001 and 2002

Plants	Number of		
	Samples	Locations ^a	Isolates
African violet, <i>Saintpaulia ionantha</i>	150	1	96
Artemisia, <i>Artemisia</i> spp.	21	1	0
Begonia, <i>Begonia</i> spp.	134	3	0
Campanula, <i>Campanula</i> spp.	46	1	0
Carnation, <i>Dianthus caryophyllus</i>	19	2	0
Chrysanthemum, <i>Chrysanthemum</i> spp.	5	1	0
Cladium, <i>Cladium</i> spp.	2	1	0
Cyclamen, <i>Cyclamen persicum</i>	5	2	0
Dahlia, <i>Dahlia pinnata</i>	34	1	0
Dusty Miller, <i>Cineraria maritima</i>	161	3	101
English ivy, <i>Hedera helix</i>	178	5	26
Fuchsia, <i>Fuchsia</i> spp.	46	1	0
Garden sage, <i>Salvia officinalis</i>	20	1	0
Geranium, <i>Pelargonium</i> × <i>hortorum</i>	21	3	0
Gerbera daisy, <i>Gerbera jamesonii</i>	339	9	83
Gloxinia, <i>Gloxinia perennis</i>	88	2	0
Impatiens, <i>Impatiens</i> spp.	134	5	0
Lamb's ears, <i>Stachys byzantina</i>	18	1	0
Lantana, <i>Lantana</i> spp.	76	1	0
Lavender, <i>Lavandula angustifolia</i>	65	2	60
Lisianthus, <i>Eustoma grandiflorum</i>	30	1	0
Marigold, <i>Calendula officinalis</i>	37	1	0
Miniature rose, <i>Rosa</i> spp.	25	1	0
New guinea impatiens, <i>Impatiens</i> × <i>hybrida</i>	12	1	0
Oregano, <i>Origanum</i> spp.	86	2	0
Pansy, <i>Viola</i> × <i>wittrockiana</i>	253	4	8
Pentas, <i>Pentas</i> spp.	150	4	0
Petunia, <i>Petunia</i> × <i>hybrida</i>	233	4	40
Plectranthus, <i>Plectranthus</i> spp.	26	1	0
Poinsettia, <i>Euphorbia pulcherrima</i>	93	3	0
Portulaca, <i>Portulaca grandiflora</i>	87	1	0
Pothos, <i>Pothos</i> spp.	24	1	0
Rhodochiton, <i>Rhodochiton</i> spp.	76	1	0
Rosemary, <i>Rosmarinus officinalis</i>	40	1	0
Salvia, <i>Salvia</i> spp.	120	2	0
Scotch moss, <i>Sagina subulata</i>	18	1	0
Snapdragon, <i>Antirrhinum majus</i>	128	3	0
Syngonium, <i>Syngonium</i> spp.	25	1	0
Verbena, <i>Verbena</i> × <i>hybrida</i>	83	3	0
Vinca, <i>Catharanthus roseus</i>	603	11	69
Viola, <i>Viola cornuta</i>	120	1	0

^a In all, 29 production locations in 17 North Carolina counties were surveyed during 2001 and 2002. For some plants, more than one production location was surveyed. For some locations, the survey was repeated over 2 years.

Fig. 2. Restriction analysis of DNA of *Phytophthora cryptogea* ATCC 15402 (lane 2), *P. cryptogea* ATCC 15403 (lane 3), *P. cryptogea* isolated from gerbera daisy (lane 4), *P. nicotianae* 221 (lane 5), *P. nicotianae* 223 (lane 6), *P. nicotianae* isolated from petunia (lane 7), *P. palmivora* P8 (lane 8), *P. palmivora* P29 (lane 9), and *P. palmivora* isolated from English ivy (lane 10) amplified with internal transcribed spacer (ITS) primers ITS 5 and ITS 4. Lanes 4, 7, and 10 are representative isolates of each *Phytophthora* sp. collected from floriculture crops grown in North Carolina in 2001 and 2002. Amplified DNA of *P. cryptogea* was digested with *HaeIII*. DNA of *P. nicotianae* and *P. palmivora* was digested with *RsaI* after amplification. Lanes 1 and 11 contain a 100-bp DNA ladder.

formed with amphigynous antheridia in paired cultures and typically were 23 µm in diameter.

Digestion of amplified DNA of representative isolates of each species identified by restriction enzymes resulted in the same fragment pattern and base pair size previously reported for these species by Ristaino et al (22). In comparison with corresponding reference species (Table 1), isolates of *P. nicotianae* and *P. palmivora* collected in this study showed the same restriction fragment pattern when DNA amplified with ITS 5 and ITS 4 was digested with *Rsa*I. After digestion of amplified DNA of *P. cryptogea* isolates with *Hae*III, the restriction pattern of the isolates was consistent with the pattern of reference species (Fig. 2).

Among all the isolates, 38% (184 isolates) were *P. cryptogea* isolated from dusty miller and gerbera daisy at two locations and 57% (273 isolates) were *P. nicotianae* isolated from African violet, lavender, pansy, petunia, and vinca at five locations. A total of 26 isolates (5%) of *P. palmivora* were recovered from English ivy at two locations. Even though they were isolated from different plants, *P. cryptogea* and *P. nicotianae* coexisted at one location (Table 3).

Compatibility type of isolates. Regardless of host, all isolates of *P. cryptogea* were identified as compatibility type A1 when the isolates were paired with a tester isolate of a known A2 compatibility type (ATCC 15403). Isolates of *P. nicotianae* were cultured with tester isolates of A1 compatibility type (OP97 and FBPS) and A2 compatibility type (SP98). Seven groups (groups 5, 6, 7, 9, 10, 11, and 12) of isolates collected from African violet, lavender, petunia, and vinca at four locations were A2 compatibility type, while

only one group (group 8) of isolates recovered from pansy at one location was A1 compatibility type (Table 3). All isolates of *P. palmivora* collected from English ivy at two locations were A1 compatibility type when they were tested with an A2 compatibility type isolate (VAV).

Mefenoxam sensitivity screening. Of the 483 *Phytophthora* isolates tested, 248 isolates (51%) were sensitive to mefenoxam at either 1 or 100 µg a.i./ml (Table 3). All isolates of *P. cryptogea* were either intermediate in sensitivity (3%) or insensitive to mefenoxam (97%) at 1 µg a.i./ml. Only five isolates (3%) of *P. cryptogea* were sensitive to mefenoxam at 100 µg a.i./ml. In all, 57 isolates of *P. cryptogea* (31%) were intermediate whereas 122 isolates (66%) showed insensitivity to mefenoxam at 100 µg a.i./ml. For *P. nicotianae* isolates, 79% of the isolates (217 isolates) were sensitive to mefenoxam at either 1 or 100 µg a.i./ml and the rest (56 isolates) were insensitive at 1 and 100 µg a.i./ml. All isolates of *P. palmivora* were sensitive to mefenoxam at either 1 or 100 µg a.i./ml (Table 3). The isolates that showed hyphal growth at 1 µg a.i./ml were evaluated over a range of mefenoxam concentrations for EC₅₀. A wide range of the EC₅₀ values (0.1 to 549.5 µg a.i./ml) was obtained for *P. cryptogea* isolates, depending on the production location and host plant. One group of *P. nicotianae* isolates (group 9) had an EC₅₀ value of 269.2 µg a.i./ml and the other group (group 10) had a value of 346.7 µg a.i./ml (Table 3; Fig. 3).

Phenotypic diversity. Within a given group, 12 of 14 groups of *Phytophthora* isolates were phenotypically homogeneous based on sensitivity to mefenoxam and compatibility type (Table 3). Among these 12 groups, all isolates within a group showed the same level of sensitivity to

mefenoxam at a given concentration. All isolates within a group consisted of the same compatibility type. Isolates in group 2 of *P. cryptogea* from dusty miller were of the same compatibility type but heterogeneous for mefenoxam sensitivity. Among 49 isolates within the group, five isolates (group 2-a) had an EC₅₀ value of 0.1 µg a.i./ml and were identified as intermediate and sensitive at 1 and 100 µg a.i./ml, respectively. Another 44 isolates (group 2-b) were insensitive and intermediate at 1 and 100 µg a.i./ml, respectively, and their EC₅₀ value was 27.5 µg a.i./ml. Isolates in group 3 of *P. cryptogea* from gerbera daisy also showed differences in mefenoxam sensitivity. Among 30 isolates within the group, the EC₅₀ value for 13 isolates in group 3-a was 5.4 µg a.i./ml and the value for 17 isolates in group 3-b was 549.5 µg a.i./ml (Table 3; Fig. 3).

Over 2 years, isolates of *P. nicotianae* were collected from African violet at the same location. Both groups collected in 2001 and 2002 (groups 5 and 6) showed the same level of sensitivity to mefenoxam at 1 and 100 µg a.i./ml and were A2 compatibility type (Table 3). In contrast, two groups of *P. cryptogea* isolates collected from dusty miller at the same location in 2001 (group 1) and 2002 (group 2) showed differences in mefenoxam sensitivity (Table 3). Isolates of *P. cryptogea* collected in 2001 (group 1) had EC₅₀ value of 407.4 µg a.i./ml, whereas the isolates collected in 2002 (group 2) had EC₅₀ values of 0.1 or 27.5 µg a.i./ml (group 2-a or 2-b, respectively). Both groups of isolates were A1 compatibility type.

DISCUSSION

P. cryptogea, *P. nicotianae*, and *P. palmivora* were isolated from eight floriculture crops grown at seven production

Table 3. Compatibility type and sensitivity to mefenoxam of *Phytophthora* isolates collected from floriculture crops in North Carolina

Group ^b	Location ^c	Year isolated	Host	Species	No. of isolates	Compatibility type	Mefenoxam sensitivity ^a		
							1 µg a.i./ml	100 µg a.i./ml	EC ₅₀ ^d
1	A	2001	Dusty miller	<i>P. cryptogea</i>	52	A1	Insensitive	Insensitive	407.4
2-a	A	2002	Dusty miller	<i>P. cryptogea</i>	5	A1	Intermediate	Sensitive	0.1
2-b	A	2002	Dusty miller	<i>P. cryptogea</i>	44	A1	Insensitive	Intermediate	27.5
3-a	A	2002	Gerbera daisy	<i>P. cryptogea</i>	13	A1	Insensitive	Intermediate	5.4
3-b	A	2002	Gerbera daisy	<i>P. cryptogea</i>	17	A1	Insensitive	Insensitive	549.5
4	B	2002	Gerbera daisy	<i>P. cryptogea</i>	53	A1	Insensitive	Insensitive	416.9
5	C	2001	African violet	<i>P. nicotianae</i>	77	A2	Sensitive	Sensitive	NA
6	C	2002	African violet	<i>P. nicotianae</i>	19	A2	Sensitive	Sensitive	NA
7	A	2001	Lavender	<i>P. nicotianae</i>	60	A2	Sensitive	Sensitive	NA
8	D	2002	Pansy	<i>P. nicotianae</i>	8	A1	Sensitive	Sensitive	NA
9	E	2002	Petunia	<i>P. nicotianae</i>	40	A2	Insensitive	Insensitive	269.2
10	F	2001	Vinca	<i>P. nicotianae</i>	16	A2	Insensitive	Insensitive	346.7
11	D	2001	Vinca	<i>P. nicotianae</i>	9	A2	Sensitive	Sensitive	NA
12	A	2002	Vinca	<i>P. nicotianae</i>	44	A2	Sensitive	Sensitive	NA
13	C	2001	English ivy	<i>P. palmivora</i>	17	A1	Sensitive	Sensitive	NA
14	G	2002	English ivy	<i>P. palmivora</i>	9	A1	Sensitive	Sensitive	NA

^a Isolates were (i) sensitive if hyphal growth was little or none, (ii) intermediate if hyphal growth was less than 50% compared with the growth on control plate, or (iii) insensitive if hyphal growth was greater than 50% of growth of that on control plate.

^b Group defined as isolates of *Phytophthora* collected from one plant species grown at a given location at a given time of survey. Groups 2 and 3 were subdivided based on the differences in sensitivity to mefenoxam among isolates within each group.

^c *Phytophthora* isolates were recovered from seven production locations. Locations with the same letter represent a single production location.

^d Concentration of fungicide giving 50% growth inhibition (EC₅₀) for isolates that grew at 1 µg a.i./ml concentration of mefenoxam. NA = not applicable.

locations in North Carolina. Occurrence of these three species of *Phytophthora* on each corresponding host has been reported previously in other parts of the United States. In this study, *P. cryptogea* was isolated from dusty miller and gerbera daisy. Since the first report of crown and root rot on gerbera daisy in New Jersey, occurrence of this disease caused by *P. cryptogea* has been reported in other areas of the United States (1,17). *P. nicotianae* was the most prevalent species found in the survey, with isolations from African violet, lavender, pansy, petunia, and vinca. This pathogen is known to have a wide host range of more than 58 different plant families, including various floriculture crops (5), throughout the United States (1,9,10,14,18,19). Ploetz and Engelhard (18) described *P. nicotianae* (=parasitica) as a causal agent of crown and root rot of African violet. *P. nicotianae* also is known to cause root rot on lavender (19). Isolates of *P. palmivora* were found from English ivy grown in greenhouses in this survey. Although distribution of *P. palmivora* typically is limited to semitropical and tropical areas, *P. palmivora* caused an outbreak of stem and root rot on English ivy grown in greenhouses in California (13). The pathogen also was isolated from the same host in landscape and nursery settings in South Carolina (6). Species of *Phytophthora* were isolated from eight of 41 plant species collected. *Pythium*, *Rhizoctonia*, and *Thielaviopsis* spp. were isolated from many plant samples from which *Phytophthora* spp. were not recovered (data not shown). These fungi have host ranges that overlap with *Phytophthora* and they can cause symptoms that are similar to symptoms induced by *Phytophthora* spp. on floriculture crops; therefore, it is unlikely that a surveyor can selectively collect plants infected only with *Phytophthora* spp.

One half of the isolates (243 of 483) of *P. cryptogea*, *P. nicotianae*, and *P. palmivora* collected in this study were sensitive to mefenoxam at 1 μg a.i./ml. A high level of resistance to mefenoxam was observed for isolates of *P. cryptogea* and *P. nicotianae*. Three groups of isolates of *P. cryptogea* from dusty miller and gerbera daisy (groups 1, 3-a, and 4) had EC_{50} values greater than 100 μg a.i./ml; hence, disease control by use of mefenoxam alone may be impractical. The EC_{50} values for two groups of isolates of *P. nicotianae* from pansy and vinca (groups 8 and 10) also were greater than 100 μg a.i./ml. All of the production locations where the isolates highly resistant to mefenoxam were recovered had epidemics of *Phytophthora* blight and crown and root rot in spite of regular use of mefenoxam. Insensitivity of *Phytophthora* spp. to mefenoxam and metalaxyl has been reported for several floriculture crops (8,15). An isolate of *P. nicotianae* (=parasitica) from *Catharanthus roseus* in California had an EC_{50} value

of 717.4 μg a.i./ml, which was 19 times greater than the highest rate of metalaxyl use labeled for *C. roseus* (9).

The individual species of *Phytophthora* recovered were all heterothallic. At a given location, isolates were all the same compatibility type. The inability to form oospores during an epidemic can be a disadvantage to *Phytophthora* spp. Sexual reproduction enhances genetic variation and promotes survival (15,21). However, oospore survival may not be important for maintaining epidemics because year-round production of floriculture crops under controlled environmental conditions is favorable for asexual reproduction and dispersal inside greenhouses. For 12 of 14 groups, all isolates within a group had the same level of sensitivity to mefenoxam and were the same compatibility type. Similar results were reported for *P. drechsleri* and *P. nicotianae* isolates from several floriculture crops (14). All isolates of *P. drechsleri* and *P. nicotianae* recovered from each location had the same sensitivity to mefenoxam, compatibility type, and AFLP profile. In our study, asexual reproduction of three *Phytophthora* spp. may play a major role in epidemics within each production location because lack of phenotypic diversity and sexual reproduction by oospore formation were observed. Two groups of isolates (groups 2 and 3) showed a different level of mefenoxam sensitivity even though they were from the same host grown at a particular location. The isolates within each group were the same compatibility type; thus, segregation of mefenoxam sensitivity through sexual recom-

bination was impossible. Genetic analysis of these groups is needed to confirm that isolates with different mefenoxam sensitivity within two groups originated from different sources.

Over the 2-year survey, we were able, in some instances, to collect the same *Phytophthora* spp. from the same host grown at the same location. Isolates of *P. nicotianae* collected from African violet at one location in 2001 and 2002 (groups 5 and 6) had the same compatibility type and sensitivity to mefenoxam. It is probable that the same isolates survived the period between crops without forming oospores to cause epidemics again the following year. Lamour et al. (14) also reported that phenotypic and genetic characteristics were identical for isolates of *P. nicotianae* collected from snapdragons over 2 years. However, isolates of *P. cryptogea* we collected from dusty miller at one location in 2001 and 2002 (groups 1 and 2) showed a difference in mefenoxam sensitivity. The EC_{50} value for the isolate group from 2001 was 407.4 μg a.i./ml, whereas the values for the isolate groups from 2002 were 0.1 and 27.5 μg a.i./ml. It is unlikely that a shift toward mefenoxam sensitivity of the isolates was in progress over 2 years. The selection pressure for mefenoxam resistance was similar because fungicide usage was based on the same application schedule for both years. It is possible that the isolates more sensitive to mefenoxam were introduced to the production location during that period and became predominant. In a study of comparison of fitness ability of metalaxyl-resistant and -sensitive iso-

Fig. 3. Dosage-response curve for hyphal growth inhibition of *Phytophthora cryptogea* and *P. nicotianae* with mefenoxam collected from floriculture crops grown in North Carolina in 2001 and 2002. Groups 1, 2-a, and 2-b were isolates of *P. cryptogea* collected from dusty miller. Groups 3-a, 3-b, and 4 were *P. cryptogea* isolates recovered from gerbera daisy. Isolates of *P. nicotianae* collected from petunia and vinca were group 9 and 10, respectively. Percent inhibition of growth by mefenoxam amendment was transformed to probability unit based on a probit table.

lates of *P. nicotianae* on orange seedlings (28), resistant isolates were less competitive than sensitive isolates in some pairings. However, some resistant isolates were able to compete with sensitive isolates. For other *Phytophthora* spp., metalaxyl-resistant isolates were more fit than sensitive isolates in some studies, whereas others showed that there was no relationship between fitness and metalaxyl resistance (4,16). Future testing of competitiveness of our sensitive and insensitive isolates of *P. cryptogea* may help answer these questions.

Although in vitro insensitivity to mefenoxam may not always result in fungicide failure in control of *Phytophthora* diseases on floriculture crops grown in greenhouses, production locations with isolates highly resistant to mefenoxam should use mefenoxam only as a rotational product. It is believed that intensive use of a single fungicide that has a single mode of action can cause rapid development of fungicide resistance (25,26). Rotation of fungicides with different modes of action can reduce selection pressure for fungicide resistance.

Monitoring and characterization of the pathogen population, including fungicide resistance and sexual recombination, may play a significant role in developing an effective disease management strategy. Symptoms and lesions caused by all three species were not limited to root and crown tissues of the plants. Most plants infected by these species showed aboveground symptoms such as leaf blight and petiole rot as well as root rot and crown rot. Intensive production conditions for floriculture crops grown in greenhouses, including dense leaf canopy, tight plant spacing, and overhead irrigation, may increase aboveground dispersal of *Phytophthora* propagules within a production facility. Sanitation within a production facility and improving the quality of recirculating irrigation water should be included as an integrated approach to prevent introduction of the pathogen and to reduce the inoculum level of the pathogen once it is introduced (30,31). Growers will continue to be challenged in their attempts to manage *Phytophthora* diseases in floriculture crops due to the diversity of *Phytophthora* spp., development of fungicide resistance, and movement of plant stocks within the industry.

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