Characterization of *Phytophthora* spp. Isolated from Ornamental Plants in Florida

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Abstract

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This report investigates population structure and genetic variability of *Phytophthora* spp. isolated from botanically diverse plants in Florida. Internal transcribed spacer-based molecular phylogenetic analyses indicate that *Phytophthora* isolates recovered from ornamental plants in Florida represent a genetically diverse population and that a majority of the isolates belong to *Phytophthora nicotianae* (73.2%), *P. palmivora* (18.7%), *P. tropicalis* (4.9%), *P. katsurae* (2.4%), and *P. cinnamomi* (0.8%). Mating type analyses revealed that most isolates were heterothallic, consisting of both mating type A1 (25.2%) and mating type A2 (39.0%), and suggesting that they could outcross. Fungicide sensitivity assays

determined that several isolates were moderate to completely insensitive to mefenoxam. In addition, several isolates were also moderately insensitive to additional fungicides with different modes of action. However, correlation analyses did not reveal occurrence of fungicide cross-resistance. These studies suggest that a genetically diverse *Phytophthora* population infects ornamental crops and the occurrence of mefenoxam-insensitive *Phytophthora* populations raises concerns about disease management in ornamentals. Mitigating fungicide resistance will require prudent management strategies, including tank mixes and rotation of chemicals with different modes of actions.

Over 100 *Phytophthora* spp. have been described (Kroon et al. 2012), several of which infect economically important plants, including numerous vegetables, fruit, oil crops, ornamental plants, and landscape and forest trees (Erwin and Ribeiro 1996; Gevens et al. 2007; Grünwald et al. 2012; Martin et al. 2012). Worldwide, *Phytophthora* diseases are responsible for billions of dollars in crop losses (Kamoun et al. 2015; Wawra et al. 2012). *Phytophthora* spp. display substantial variation in host ranges, with some causing disease on a wider variety of plants whereas others infect only a single crop. They are regularly isolated from diverse ornamental plants in greenhouses and landscapes throughout the world (Bienapfl and Balci 2014; Leonberger et al. 2013; Parke et al. 2014; Prigigallo et al. 2015; Schlenzig et al. 2015). For example, *Phytophthora nicotianae* infects close to 255 genera in 90 families, including many herbaceous and woody plants, and is present in nurseries worldwide (Cline et al. 2008). *P. nicotianae* also causes root rot in citrus, resulting in substantial yield and quality losses where the disease occurs (Cacciola and Lio 2008). *P. ramorum*, which is an invasive and quarantined species, causes sudden oak death disease and infects over 60 ornamental trees and shrubs with devastating impacts on natural ecosystems (Grünwald et al. 2008, 2012). Another species, *P. capsici*, which was initially thought to infect pepper species only, was also found to infect cucurbits, tomato, eggplant, and bean (Martin et al. 2012; Wang et al. 2013). Common to the infection process of all *Phytophthora* spp. is the requirement of moist conditions, which

favors dispersal and germination of sporangia and zoospores. Long-distance dispersal of *Phytophthora* spp. is facilitated by run-off water from nurseries and by natural streams. The spread of *Phytophthora* spp. at a global level is believed to be through commercial trade of ornamentals (Bienapfl and Balci 2014; Goss et al. 2011b). For example, since the first detection of *P. ramorum* in the 1990s, it has spread to several U.S. states and Canada through shipments of infested ornamental plants (Goss et al. 2011b; Prospero et al. 2009; Rizzo et al. 2002).

Pesticides are the primary means of managing diseases caused by *Phytophthora* spp. (Foster and Hausbeck 2010), which add to the total cost of production. A number of fungicides with different modes of action such as phenyl amides, benzamides, phosphonate, dimethomorph, quinone outside inhibitors, and quinone inside inhibitors have been recommended to control diseases caused by *Phytophthora* spp. These fungicides are very effective because most of them inhibit single molecular sites in essential cellular processes such as mitochondrial function, ribosomal (r)RNA synthesis, cell wall biosynthesis, and cell division. Due to frequent use of single-site fungicides, *Phytophthora* spp. pose a high risk of developing resistance to fungicides and, therefore, potentially disrupting effective disease management programs (FRAC 2015; Hu et al. 2012). Fungicide-insensitive isolates of various *Phytophthora* spp. have been reported throughout the world (Dobrowolski et al. 2008; Gisi and Cohen 1996; Hu et al. 2005; Hu et al. 2012; Hu et al. 2008, 2010; Hwang and Benson 2005; Meng et al. 2011; Pérez-Sierra et al. 2011; Timmer et al. 1998). Therefore, regular monitoring of *Phytophthora* populations is essential for sustainable disease management (Brent 1992; Grünwald et al. 2006; Hu et al. 2012; Staub and Sozzi 1984).

Historically, *Phytophthora* spp. have been characterized through morphological features, which may not be practical for in-depth analyses of larger populations. More recently, several groups have used DNA-based molecular phylogenetic analyses for characterizing *Phytophthora* communities in agricultural lands, nurseries, and natural ecosystems (Blair et al. 2008; Grünwald et al. 2011; Park et al. 2008). One of the first DNA regions to be used in phylogenetic analysis of *Phytophthora* spp. was the 5.8S rRNA gene and the flanking

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internal transcribed spacers (ITS) 1 and 2 (Lee and Taylor 1992). These studies have revealed important information about the genetic makeup, lineages, and spread of previously known and some newly discovered *Phytophthora* spp. throughout the world.

Several *Phytophthora* spp. are heterothallic, with two known mating types, type A1 and A2 (Gisi and Cohen 1996). Hybridization between opposite mating types increases genetic variability and promotes survival of the pathogen (Lamour and Hausbeck 2000). Sexual reproduction may also spread fungicide resistance through genetic recombination and, therefore, it is important to monitor mating types as well as fungicide sensitivity of *Phytophthora* spp.

Florida is home to thousands of commercial nurseries that produce a wide variety of ornamental plants ranging from annual herbaceous plants to tropical foliage and woody trees and shrubs (DPI 1998). Florida is the major port of entry for ornamental plants arriving in the United States (Merritt et al. 2012) and, subsequently, has a high number of pest and disease introductions. *Phytophthora* spp. are no exception and are frequently isolated from various ornamental plants in Florida. Based on morphological characteristics and previous pathological history, these isolates are tentatively assigned to specific species. However, molecular species identification is not routine. Accurate characterization of the genetic composition of the *Phytophthora* spp. infecting ornamentals is necessary for investigating the evolution, spread, and adaptation of *Phytophthora* spp. to new hosts and environments. Many commercial nurseries where these isolates were collected cocultivate many host plants of different *Phytophthora* spp., potentially increasing risk of development of hybrid species. Because the ornamental crop industry in Florida relies on frequent fungicide sprays for controlling diseases, the risks of selecting fungicide resistant *Phytophthora* isolates are high. The objectives of this study were to (i) conduct molecular phylogenetic analyses of a spatiotemporal population of *Phytophthora* recovered from ornamental crops in Florida, (ii) determine mating types of this population, and (iii) investigate the sensitivity of these *Phytophthora* spp. to commonly used fungicides with different modes of actions. Findings in this report will help in formulating effective strategies and policies for preventing the spread of *Phytophthora* spp. to new niches and mitigate risks of fungicide resistance development.

Materials and Methods

***Phytophthora* isolates.** Between 1993 and 2014, in total, 156 putative *Phytophthora* isolates were recovered from the leading edges of lesions of different diseased plants with characteristic symptoms of Phytophthora diseases at the University of Florida Plant Disease Clinics at the Mid-Florida Research and Education Center, Apopka, FL and the Tropical Research and Education Center, Homestead, FL. These isolates were initially recovered on water agar and then stored in sterile water or 10% V8 juice agar slants in glass bottles at room temperature. For this study, we attempted to recover all these isolates on 10% V8, potato dextrose agar (PDA), and water agar. All cultures that grew were checked for any bacterial or fungal contamination, and contaminated cultures were excluded from further analysis, resulting in a population of 123 isolates (Table 1). A single hyphal tip from pure isolate cultures was transferred to and maintained on selective medium V8-PARP agar (20% clarified V8 juice, 0.4% CaCO₃, 1.5% Bacto-agar, pimaricin at 25 µg ml⁻¹, ampicillin at 100 µg ml⁻¹, rifampicin at 25 µg ml⁻¹, and pentachloronitrobenzene at 25 µg ml⁻¹). The first two digits in an isolate designation indicate year of collection. For this study, all isolates were grown on V8-PARP at 22°C for 6 to 8 days.

Polymerase chain reaction and sequencing of ITS. Approximately 100 mg of mycelia of each isolate, grown on clarified 20% V8 PARP agar plates, was harvested by scraping them off with a sterile spatula into sterile 1.5-ml Eppendorf tubes. The mycelia were flash frozen with liquid nitrogen and ground using a pestle and mortar. DNA was isolated from the ground mycelia according to a previously described method (Aljanabi and Martinez 1997), and DNA was quantified using NanoDrop 2000C Spectrophotometer (Thermo Fisher Scientific). To accurately identify the isolates to species level, ITS regions were sequenced using the ITS5 forward primer and

ITS4 reverse primers to amplify the ITS1, 5.8s rRNA, and ITS2 regions (White et al. 1990) (Fig. 1). Polymerase chain reaction (PCR) consisted of 1× Ex Taq reaction buffer, 0.8 mM all dNTPs, 0.3 µM each primer, TaKaRa Ex Taq polymerase (TaKaRa) at 0.1 U/µl, and genomic DNA at 0.8 ng/µl in a final volume of 25 µl. PCR conditions consisted of an initial denaturation step at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). Both strands of the amplified DNA products were directly sequenced using the ITS4 reverse and ITS5 forward primers. ITS sequences were searched against both the nonredundant nucleotide sequence database at the National Center for Biotechnology Information (NCBI; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the *Phytophthora* database (Kang et al. 2007).

Phylogenetic analysis. Genetic diversity of all isolates was determined using phylogenetic analyses. To infer genetic relationships of the *Phytophthora* isolates reported in this study, phylogenetic analyses were conducted using the ITS sequences. Representative ITS sequences of *Phytophthora* spp. were also included in constructing the phylogenetic tree for comparison. Sequences were aligned using ClustalW; alignment with the Muscle algorithm returned similar results. The ClustalW alignments were examined manually and ambiguous overhanging sequences from both ends were trimmed, resulting in an alignment block consisting of 772 sites, which spanned the ITS1, 5.8S rRNA, and ITS2 regions. For multiple sequence alignment, a FASTA file containing ITS sequences determined in this study and representative ITS sequences of *Phytophthora* spp. were imported into Mega 6.0 software (Larkin et al. 2007; Tamura et al. 2011) and aligned using the ClustalW algorithm (Larkin et al. 2007). Alignments were also performed using the Muscle algorithm (Edgar 2004) implemented in Mega 6.0. A phylogenetic tree was constructed using the maximum-likelihood (ML) method based on the Tamura-Nei model, with 1,000-bootstrap replication (Tamura and Nei 1993; Tamura et al. 2011); the neighbor-joining method was also employed, which yielded results similar to the ML method. Evolutionary diversity estimates were calculated for the *P. nicotianae* group. Estimates of average evolutionary divergence over all isolate pairs were calculated as number of base substitutions per nucleotide site. Analyses were conducted using the maximum composite likelihood model (Tamura et al. 2004). All positions containing gaps and missing data were not included in analyses. There were 772 base positions in the final dataset.

Mating type analysis. Mating type of isolates was determined using known *P. nicotianae* mating type A1 (number 13-724) and type A2 (number 13-723) tester strains in 24-well culture plates (Nunc; Thermo Fisher). Each well of the culture plate contained 0.5 ml of V8 juice agar (20% clarified V8 juice, 0.4% CaCO₃, and 1.5% Bacto-agar). A mycelial plug (2 mm in diameter) from a test isolate was placed at the margin of the well of a culture plate opposite a mycelial plug (2 mm in diameter) of either mating type A1 or A2. Each isolate was also tested for homothallism using two agar plugs from the same isolate. Plates were wrapped with Parafilm and incubated in a growth chamber at 22 to 25°C for up to 6 weeks under dark. Plates were checked for the presence of oospores every week for at least 6 weeks.

Fungicide sensitivity tests. Most isolates were tested for sensitivity to mefenoxam (Fungicide Resistance Action Committee [FRAC] group 4, Subdue Maxx; Syngenta Crop Protection), fluopicolide (FRAC group 43, Adorn; Valent USA Corporation), aluminum Tris (FRAC group 33, Aliette WDG; Bayer Crop Science), azoxystrobin (FRAC group 11, Heritage; Syngenta Crop Protection), cyazofamid (FRAC group 21, Segway; FMC Corporation), and dimethomorph (FRAC group 40, Stature SC; BASF). Fungicides formulated as commercial products were dissolved in autoclaved clarified V8 medium at the following active ingredient rates: mefenoxam, 0.1 mg ml⁻¹; fluopicolide, 0.1 mg ml⁻¹; aluminum Tris, 1 mg ml⁻¹; azoxystrobin, 1 mg ml⁻¹; cyzofamid, 1 mg ml⁻¹; and dimethomorph, 1 mg ml⁻¹. Fungicide concentrations chosen in this study were based on previous studies (Jackson et al. 2010; Kousik and Keinath 2008; Kuhajek et al. 2003; Stein and Kirk 2004; Ziogas et al. 2006). A single 5-mm

Table 1. Isolates of *Phytophthora* spp. collected from various ornamental hosts in Florida

Species ^a	Isolates	ITS identity (%) ^b	Host	Mating types ^c		Mefenoxam sensitivity ^d
<i>cinnamomi</i>	07-385	99.9	<i>Rhododendron</i> sp.	...	A2	S
<i>katsurae</i>	14-374B	99.0	<i>Podocarpus</i> sp.	A1A2	A1A2	S
<i>katsurae</i>	14-374C	99.0	<i>Podocarpus</i> sp.	A1A2	A1A2	S
<i>katsurae</i>	14-761	99.0	<i>Podocarpus</i> sp.	A1A2	A1A2	S
<i>palmivora</i>	01-118	100.0	<i>Hedera</i> sp.	...	A2	S
<i>palmivora</i>	03-550	99.4	<i>Chamaedorea elegans</i>	A1	...	S
<i>palmivora</i>	11-65	99.9	<i>Spathiphyllum</i> sp.	...	A2	S
<i>palmivora</i>	10-1733	99.0	<i>Liriope muscari</i>	–	–	S
<i>palmivora</i>	13-1483A	100.0	<i>Chrysanthemum</i> sp.	–	–	S
<i>palmivora</i>	13-1483B	100.0	<i>Chrysanthemum</i> sp.	–	–	S
<i>palmivora</i>	13-1726	100.0	<i>Mandevilla</i> sp.	–	–	S
<i>palmivora</i>	13-1734	98.0	<i>Phoenix dactylifera</i>	–	–	...
<i>palmivora</i>	13-1735	97.0	<i>Catharanthus</i> sp.	–	–	...
<i>palmivora</i>	14-130	100.0	<i>Cordyline</i> sp.	...	A2	S
<i>palmivora</i>	14-32	100.0	<i>Latania</i> sp.	–	–	S
<i>palmivora</i>	14-33	100.0	<i>Latania</i> sp.	–	–	S
<i>palmivora</i>	14-34	99.0	<i>Latania</i> sp.	–	–	S
<i>palmivora</i>	14-35	100.0	<i>Latania</i> sp.	–	–	S
<i>palmivora</i>	14-37	99.0	<i>Latania</i> sp.	–	–	S
<i>palmivora</i>	14-684A	100.0	<i>Podocarpus</i> sp.	–	–	S
<i>palmivora</i>	14-684B	99.0	<i>Sabal palmetto</i>	–	–	S
<i>palmivora</i>	14-704	97.0	<i>Pothos</i> sp.	–	–	S
<i>palmivora</i>	14-705	97.0	<i>Pothos</i> sp.	–	–	S
<i>palmivora</i>	14-708	99.0	Palm	...	A2	S
<i>palmivora</i>	14-709	99.0	Palm	...	A2	S
<i>palmivora</i>	14-710	98.0	<i>Pachira</i> sp.	–	–	S
<i>palmivora</i>	14-711	98.0	<i>Pachira</i> sp.	–	–	S
<i>nicotianae</i>	93-653	99.9	<i>Vinca</i> sp.	...	A2	S
<i>nicotianae</i>	99-191	99.9	<i>Spathiphyllum</i> sp.	...	A2	S
<i>nicotianae</i>	00-334	100.0	<i>Vinca</i> sp.	A1	...	S
<i>nicotianae</i>	01-138	98.4	<i>Spathiphyllum</i> sp.	...	A2	S
<i>nicotianae</i>	01-280	99.7	<i>Anthurium</i> sp.	...	A2	S
<i>nicotianae</i>	01-302	99.3	<i>Viola</i> sp.	–	–	S
<i>nicotianae</i>	02-29	99.1	<i>Calceolaria</i> sp.	...	A2	S
<i>nicotianae</i>	03-131	99.9	<i>Spathiphyllum</i> sp.	...	A2	S
<i>nicotianae</i>	03-132	100.0	<i>Spathiphyllum</i> sp.	–	–	S
<i>nicotianae</i>	03-361	100.0	<i>Dieffenbachia</i> sp.	A1	–	S
<i>nicotianae</i>	03-407	99.3	<i>Pothos</i> sp.	A1	–	S
<i>nicotianae</i>	04-130	99.9	<i>Spathiphyllum</i> sp.	A1	...	S
<i>nicotianae</i>	04-325	100.0	<i>Spathiphyllum</i> sp.	...	A2	S
<i>nicotianae</i>	04-410	99.8	<i>Spathiphyllum</i> sp.	...	A2	S
<i>nicotianae</i>	05-215	99.6	<i>Alocasia</i> sp.	...	A2	S
<i>nicotianae</i>	05-217	100.0	<i>Coleus</i> sp.	–	–	S
<i>nicotianae</i>	05-222	99.6	<i>Gerbera</i> sp.	...	A2	S
<i>nicotianae</i>	05-287	97.2	<i>Pothos</i> sp.	...	A2	S
<i>nicotianae</i>	05-297	98.5	Poinsettia	...	A2	R
<i>nicotianae</i>	06-141	99.9	<i>Spathiphyllum</i> sp.	...	A2	S
<i>nicotianae</i>	06-174	100.0	<i>Cattleya</i> sp.	...	A2	S
<i>nicotianae</i>	07-57	99.8	<i>Alocasia</i> sp.	A1	...	S
<i>nicotianae</i>	08-168	99.7	<i>Spathiphyllum</i> sp.	–	–	S
<i>nicotianae</i>	10-43	99.9	<i>Spathiphyllum</i> sp.	A1	...	S
<i>nicotianae</i>	11-1	99.9	<i>Spathiphyllum</i> sp.	...	A2	S
<i>nicotianae</i>	11-3	100.0	<i>Spathiphyllum</i> sp.	–	–	S
<i>nicotianae</i>	11-21	99.0	<i>Rhoeo discolor</i>	...	A2	S
<i>nicotianae</i>	11-38	100.0	<i>Impatiens</i> sp.	...	A2	S
<i>nicotianae</i>	12-1	98.6	<i>Spathiphyllum</i> sp.	...	A2	S
<i>nicotianae</i>	12-5	98.2	<i>Hibiscus</i> sp.	...	A2	S
<i>nicotianae</i>	10-524A	99.0	<i>Iris</i> sp.	...	A2	S
<i>nicotianae</i>	10-524B	99.0	<i>Iris</i> sp.	...	A2	S
<i>nicotianae</i>	10-534B	100.0	<i>Spathiphyllum</i> sp.	–	–	S

(continued on next page)

^a *Phytophthora* spp. were identified based on BLAST match in the National Center for Biotechnology Information database and *Phytophthora* database.^b Percent identity to ITS sequences in the *Phytophthora* database.^c A1 and A2 mating types were determined using known tester mating type A1 (*P. nicotianae* #13-724) and A2 (*P. nicotianae* #13-723); each isolate was also tested with itself for homothallism; – indicates sterile, defined as those that did not display any evidence of oospores, antheridia, or oogonia; and A1A2 represents homothallic isolates.^d S and R indicate sensitive and resistant isolates, respectively, which were defined as those that displayed $\geq 80\%$ (S) or $\leq 50\%$ (R) growth inhibition on V8 agar plates containing mefenoxam at 100 $\mu\text{g ml}^{-1}$.

Table 1. (continued from preceding page)

Species ^a	Isolates	ITS identity (%) ^b	Host	Mating types ^c		Mefenoxam sensitivity ^d
<i>nicotianae</i>	10-616A	100.0	<i>Spathiphyllum</i> sp.	–	–	S
<i>nicotianae</i>	10-616B	99.0	<i>Spathiphyllum</i> sp.	–	–	S
<i>nicotianae</i>	10-616C	100.0	Unknown	–	–	S
<i>nicotianae</i>	13-1133A	100.0	<i>Mandevilla</i> sp.	–	–	S
<i>nicotianae</i>	13-1134B	100.0	<i>Rhododendron</i> sp.	A1	...	S
<i>nicotianae</i>	13-1667	100.0	<i>Mandevilla</i> sp.	–	–	S
<i>nicotianae</i>	13-1727	100.0	<i>Mandevilla</i> sp.	–	–	S
<i>nicotianae</i>	13-1728	99.0	<i>Mandevilla</i> sp.	–	–	S
<i>nicotianae</i>	13-1729	100.0	<i>Allamanda</i> sp.	–	–	S
<i>nicotianae</i>	13-1730	100.0	<i>Spathiphyllum</i> sp.	–	–	S
<i>nicotianae</i>	13-1731	100.0	<i>Spathiphyllum</i> sp.	–	–	S
<i>nicotianae</i>	13-1732	100.0	<i>Spathiphyllum</i> sp.	A1	...	S
<i>nicotianae</i>	13-1815	100.0	<i>Kalanchoe</i> sp.	...	A2	S
<i>nicotianae</i>	13-1816	100.0	Not determined	...	A2	S
<i>nicotianae</i>	13-1817	100.0	Not determined	A1	...	S
<i>nicotianae</i>	13-1818	99.0	<i>Swietenia</i> sp.	–	–	S
<i>nicotianae</i>	13-1819	100.0	<i>Strelitzia nicolai</i>	...	A2	S
<i>nicotianae</i>	13-1820	100.0	<i>Bursera</i> sp.	A1	...	S
<i>nicotianae</i>	13-1821	99.0	<i>Syagrus romanzoffiana</i>	...	A2	S
<i>nicotianae</i>	13-1822	100.0	<i>Casia</i> sp.	A1	...	S
<i>nicotianae</i>	13-1823	100.0	<i>Casia</i> sp.	A1	...	S
<i>nicotianae</i>	13-1824	99.0	<i>Impatiens</i> sp. <i>walleriana</i>	...	A2	S
<i>nicotianae</i>	13-1825	100.0	<i>Impatiens</i> sp. <i>walleriana</i>	A1	...	S
<i>nicotianae</i>	13-1826	99.0	<i>Croton</i> sp.	...	A2	S
<i>nicotianae</i>	13-1827	100.0	<i>Oncidium</i> sp.	A1	...	S
<i>nicotianae</i>	13-1828	100.0	<i>Catharanthus</i> sp.	A1	...	S
<i>nicotianae</i>	13-1829	100.0	<i>Catharanthus</i> sp.	A1	...	S
<i>nicotianae</i>	13-1830	100.0	<i>Catharanthus</i> sp.	A1	...	S
<i>nicotianae</i>	13-1831	100.0	<i>Tilandsia</i> sp.	A1	...	S
<i>nicotianae</i>	13-1832	100.0	<i>Dracaena</i> sp.	A1	...	S
<i>nicotianae</i>	13-1833	100.0	<i>Dracaena</i> sp.	A1	...	S
<i>nicotianae</i>	13-1834	100.0	<i>Schefflera</i> sp.	A1	...	S
<i>nicotianae</i>	13-1835	99.0	<i>Schefflera</i> sp.	A1	...	S
<i>nicotianae</i>	13-1836	100.0	<i>Dracaena</i> sp.	A1	...	S
<i>nicotianae</i>	13-1837	100.0	<i>Anthurium</i> sp.	–	–	S
<i>nicotianae</i>	13-1838	99.0	<i>Sansevieria</i> sp.	A1	...	S
<i>nicotianae</i>	13-1839	100.0	<i>Epipremnum</i> sp.	A1	...	S
<i>nicotianae</i>	13-1840	99.0	<i>Mandevilla</i> sp.	...	A2	S
<i>nicotianae</i>	13-1841	100.0	<i>Philodendron</i>	...	A2	S
<i>nicotianae</i>	13-1842	99.0	<i>Epipremnum</i> sp.	...	A2	S
<i>nicotianae</i>	13-1843	99.0	<i>Adenium</i> sp.	...	A2	S
<i>nicotianae</i>	13-1844	100.0	<i>Mandevilla</i> sp.	...	A2	S
<i>nicotianae</i>	13-1845	100.0	<i>Geranium</i> sp.	...	A2	S
<i>nicotianae</i>	14-1030	99.0	<i>Anthurium</i> sp.	–	–	...
<i>nicotianae</i>	14-1585B	99.0	<i>Gardenia</i> sp.	–	–	S
<i>nicotianae</i>	14-1585C	99.0	<i>Gardenia</i> sp.	–	–	S
<i>nicotianae</i>	14-214A	100.0	<i>Phoenix</i> sp.	...	A2	S
<i>nicotianae</i>	14-214B	100.0	<i>Gardenia</i> sp.	...	A2	S
<i>nicotianae</i>	14-215	100.0	<i>Gardenia</i> sp.	...	A2	S
<i>nicotianae</i>	14-271A	98.0	<i>Podocarpus</i> sp.	...	A2	S
<i>nicotianae</i>	14-707	99.0	<i>Pothos</i> sp.	...	A2	S
<i>nicotianae</i>	14-712	99.0	<i>Spathiphyllum</i> sp.	A1	...	S
<i>nicotianae</i>	14-754A	99.0	<i>Spathiphyllum</i> sp.	A1	...	S
<i>nicotianae</i>	14-754B	99.0	<i>Spathiphyllum</i> sp.	–	–	S
<i>nicotianae</i>	14-754C	99.0	<i>Spathiphyllum</i> sp.	A1	...	S
<i>nicotianae</i>	14-773A	99.0	<i>Strelitzia reginae</i>	...	A2	S
<i>nicotianae</i>	14-820	99.0	<i>Petunia</i> sp.	...	A2	S
<i>tropicalis</i>	96-83	100.0	<i>Hedera</i> sp.	A1	...	S
<i>tropicalis</i>	03-387	100.0	<i>Hedera</i> sp.	...	A2	S
<i>tropicalis</i>	03-452	100.0	<i>Hedera</i> sp.	A1	–	S
<i>tropicalis</i>	08-228	99.8	<i>Hedera</i> sp.	...	A2	S
<i>tropicalis</i>	14-138	99.0	Pepper	–	–	R
<i>tropicalis</i>	14-822A	99.0	<i>Rosmarinus officinalis</i>	–	–	S

mycelial plug from the leading edge of a 1-week-old *Phytophthora* culture, grown in the dark at 22°C, was placed on fungicide-amended and nonamended clarified V8 agar plates. Plates were incubated in the dark at 22 to 25°C for 7 days. Colony diameters were measured and percent growth inhibition was calculated using the following formula:

$$\text{Percent growth inhibition} = \frac{C - F}{C} \times 100$$

where *C* and *F* are average colony diameters on nonamended control and fungicide-amended V8 agar plates, respectively. To determine the effect of mefenoxam dosage on resistance, isolate 05-297 (resistant, with less than 10% growth inhibition) and isolate 05-217 (sensitive, with >90% growth inhibition) were assayed over a range of mefenoxam concentrations. All experiments were arranged in a completely randomized design with four replications for each fungicide. The experiment was repeated once. The emergence of fungicide-resistant populations has been reported in several *Phytophthora* spp. (Dobrowolski et al. 2008; Gisi and Cohen 1996; Hu et al. 2005; Hu et al. 2012; Hu et al. 2008, 2010; Hwang and Benson 2005; Meng et al. 2011; Pérez-Sierra et al. 2011; Timmer et al. 1998) and poses serious threat to effective management of *Phytophthora* diseases. Therefore, *Phytophthora* isolates collected in this study were screened against commonly used fungicides. Based on the percent growth inhibition, isolates were categorized into sensitive ($\geq 80\%$ growth inhibition), moderately sensitive (51 to 79% growth inhibition),

and resistant ($\leq 50\%$ growth inhibition). Cross-resistance of isolates to different fungicides was analyzed using the Spearman's rank correlation coefficients (ρ) on ranked percent growth inhibition data. Spearman's ρ values and their associated *P* values were calculated using the open source statistical software R (<http://www.r-project.org/>).

Results

Molecular identification of *Phytophthora* isolates. Consensus ITS contigs for each isolate were constructed from the alignment of forward and reverse sequences obtained with ITS5 and ITS4 primers. The resulting contigs were searched using BLASTn at NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and at the manually curated *Phytophthora* database (Park et al. 2008). BLAST search results from NCBI and the *Phytophthora* database were identical for each isolate (Table 1). Among the 123 isolates, 90 isolates were identified as *P. nicotianae*, 23 as *P. palmivora*, 6 as *P. tropicalis*, 3 as *P. katsurae*, and 1 as *P. cinnamomi* (Table 1). Because double peaks were not observed in the DNA sequence chromatograms of any of the isolates, this suggested that these isolates are homozygous for ITS.

Diversity and genetic composition of *Phytophthora* isolates. ClustalW alignment showed that approximately 33% (252/772) of the sites were polymorphic. An ML tree was constructed from these alignments, which separated all isolates into five major groups, consistent with BLAST results, such that each group closely clustered with a representative published ITS sequence of *P. parasitica*

Fig. 1. Molecular phylogenetic analysis of *Phytophthora* isolates. Upper panel shows schematics of the internal transcribed spacer (ITS) region of *Phytophthora* spp. Locations of ITS4 and ITS5 primers are indicated. Lower panel shows the phylogenetic tree, which was constructed from the ITS sequences using the maximum-likelihood method. Analysis consisted of 128 sequences including five reported representative ITS sequence of *P. nicotianae* (GU983635), *P. palmivora* (KF263691), *P. tropicalis* (KC479199), *P. katsurae* (JQ901391), and *P. cinnamomi* (JX996044).

(GU983635), *P. palmivora* (KF263691), *P. tropicalis* (KC479199), *P. katsurae* (JQ901391), and *P. cinnamomi* (JX996044) (Fig. 1). Using the maximum composite likelihood model (Tamura et al. 2004), evolutionary diversity estimate, calculated as mean base substitution per site over all sequence pairs, was 0.0286 (2.86%).

The *P. nicotianae* group, which consisted of 90 isolates, displayed substantial variation and was further analyzed in detail for genetic diversity. These analyses classified the *P. nicotianae* isolates roughly into five subgroups (Fig. 1). Overall, mean divergence between *P. nicotianae* isolates was 0.64% (range, 0 to 7.5%). In pairwise comparisons, a majority of the *P. nicotianae* isolates displayed mean divergence below 2%. These isolates infected plants from diverse families. Groups *PpIV* and *PpV* displayed high within-group and between-group divergences (Tables 2 and 3). Similarly, *P. palmivora* isolates also displayed considerable variation in ITS sequence, with a 0.44% (range, 0 to 2.3%) overall mean divergence.

Phytophthora isolates are a mixture of A1 and A2 mating types. Mating types of all isolates were identified using known A1 and A2 mating type tester isolates. A summary of this analysis is presented in Table 4. Overall, 25.2% of all *Phytophthora* isolates belonged to mating type A1 and 39.0% belonged to mating type A2. The three isolates identified as *P. katsurae* were all homothallic and produced oospores. The remaining 33.3% did not develop any oospores, antheridia, or oogonia during up to 6 weeks of culture, suggesting that they are sterile.

Several Phytophthora isolates displayed mefenoxam-insensitivity. A majority of isolates were either sensitive (53%) or moderately sensitive (37%) to mefenoxam, suggesting that they can be controlled using mefenoxam. There was substantial variation in mefenoxam sensitivities of these isolates (Supplementary Fig. S1). One *P. tropicalis* and three *P. nicotianae* isolates were found to be resistant to mefenoxam. Among these, isolate 05-297 (*P. nicotianae*) was highly resistant to mefenoxam, with only 9.4% growth inhibition (Fig. 2). The other three isolates, 05-215 (*P. nicotianae*), 11-38 (*P. nicotianae*), and 14-138 (*P. tropicalis*) had 43.7, 47.7, and 23.9% growth reduction, respectively. Isolate 05-297 displayed a high level of resistance over all tested concentrations. Interestingly, at sublethal concentrations (0.1 to 2 µg ml⁻¹), the growth of this isolate was enhanced by approximately 20%, which was observed consistently in several independent experiments (Fig. 2A). Similar results were observed with the other three resistant isolates (05-215, 11-38, and 14-138; data not shown). Because mefenoxam is used extensively in nurseries, the resistant strains will likely be selected, thus increasing the probability of mefenoxam-resistance build-up in future *Phytophthora* populations.

Phytophthora isolates did not display any resistance to different fungicides. A majority of the tested isolates were sensitive to fluopicolide (Adorn), aluminum Tris (Aliette), azoxystrobin (Heritage), cyzofamid (Segway), and dimethomorph (Stature). Frequency distribution of sensitive, moderately resistant, and resistant isolates is shown in Figure 3. More than 80% of isolates were sensitive to mefenoxam, Adorn, and Stature (Fig. 3). Although a majority of isolates were inhibited by each of these fungicides, several isolates displayed variable resistance levels to at least one of these fungicides. Less than

50% growth inhibition was displayed by 1 (Adorn), 9 (Aliette), 10 (Heritage), and 11 (Segway) isolates (Fig. 3). The remaining isolates either displayed moderate resistance or were sensitive to these fungicides. Ornamental facilities, where the isolates reported in this study originated, often apply fungicides with different modes of action on the same crop multiple times. This led us to speculate that some isolates might display resistance to multiple fungicides. With the exception of a very low-level and statistically insignificant co-resistance between mefenoxam and Adorn ($\rho = 0.31$, $P = 0.09$) and between mefenoxam and Aliette ($\rho = 0.30$, $P = 0.09$), none of the other fungicides showed resistance to multiple isolates. All isolates which were resistant to one fungicide were inhibited by at least one other fungicide with a different mode of action. For example, isolates 05-215, 05-217, and 05-222 showed moderate resistance to Adorn but were sensitive to Aliette, Heritage, and Stature.

No correlation was found between genetic diversity of isolates and their sensitivity to fungicides (Table 2; Figs. 2 and 3; Spearman's rank correlation $P > 0.1$). For example, isolates 05-297 (resistant to mefenoxam) and 12-5 (sensitive to mefenoxam), belong to the same group *PpV* in the phylogenetic tree (Fig. 1). Isolates 05-297, 05-215, and 11-38, which were mefenoxam-resistant, displayed single-nucleotide polymorphism (SNP) at different sites and belonged to different phylogenetic groups (Fig. 1), suggesting that mefenoxam insensitivity is not necessarily associated with overall genetic variation.

Discussion

Florida is renowned for its ornamental plant industry and it ranks second only to California in total trade. Altogether, thousands of ornamental plant species are grown throughout Florida in greenhouses and nurseries. Ornamental plant producers in Florida often face heavy pressure from pathogens and insects which, due to a hot and humid climate, can be a year-round challenge. In addition, every year, millions of plants are traded through Florida, which can serve as an unintentional conduit for the spread of pathogens to new areas, where they can infect resident plants. Conglomeration of so many diverse plants and their associated pathogens in confined greenhouses can also serve as a potential breeding ground for the evolution

Table 3. Estimates of average evolutionary divergence over sequence pairs between groups^a

Group	Within-group distance
<i>Pp I</i>	0.00017
<i>Pp II</i>	0.00010
<i>Pp III</i>	0.00009
<i>Pp IV</i>	0.00216
<i>Pp V</i>	0.01073
<i>Phytophthora palmivora</i>	0.02053
<i>P. tropicalis</i>	0.00433
<i>P. katsurae</i>	0.00197

^a *PpI*, *PpII*, *PpIII*, *PpIV*, and *PpV* are subgroups of *P. nicotianae*. The number of base substitutions per site from averaging over all sequence pairs within each group are shown. Analysis involved 128 nucleotide sequences.

Table 2. Estimates of average evolutionary divergence over sequence pairs between groups^a

	<i>Pp II</i>	<i>Pp III</i>	<i>Pp IV</i>	<i>Pp V</i>	<i>P. palmivora</i>	<i>P. tropicalis</i>	<i>P. katsurae</i>	<i>P. cinnamomi</i>
<i>Pp I</i>	0.0002	0.0009	0.0081	0.0016	0.0629	0.0503	0.0534	0.0611
<i>Pp II</i>	...	0.0009	0.0079	0.0015	0.0627	0.0503	0.0533	0.0611
<i>Pp III</i>	...	...	0.0088	0.0024	0.0638	0.0492	0.0522	0.0600
<i>Pp IV</i>	...	...	...	0.0094	0.0712	0.0591	0.0627	0.0703
<i>Pp V</i>	...	...	...	...	0.0636	0.0513	0.0550	0.0625
<i>P. palmivora</i>	...	...	...	...	...	0.0434	0.0484	0.0583
<i>P. tropicalis</i>	...	...	...	...	...	...	0.0429	0.0510
<i>P. katsurae</i>	...	...	...	...	...	...	...	0.0511

^a *PpI*, *PpII*, *PpIII*, *PpIV*, and *PpV* are subgroups of *Phytophthora nicotianae*. The number of base substitutions per site from averaging over all sequence pairs within each group are shown. Analysis involved 128 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6.

of more aggressive pathogens through intra- and interspecific cross hybridizations. Therefore, it is important to investigate population structure and genetic diversity of plant pathogens on ornamentals in Florida. *Phytophthora* spp. are one of the most frequently isolated pathogens from symptomatic ornamental crops in Florida (Merritt et al. 2012). Based on symptoms, the type of host species infected, and previous etiology, these isolates were tentatively assigned to previously described *Phytophthora* spp. Beyond these initial assessments, little was known about the genetic variation and population structure of this *Phytophthora* collection.

Cooke et al. (2000) conducted a phylogenetic study of *Phytophthora* spp. using ITS sequences. Moreover, a large number of ITS sequences of *Phytophthora* spp. are available to compare in GenBank. Phylogenetic analysis was based on the ITS sequences, a commonly used molecular marker, and a barcode for oomycetes, which provided sufficient resolution to interpret phylogenetic trees and to distinguish various *Phytophthora* spp. (Kroon et al. 2012; Martin et al. 2012; Robideau et al. 2011). Based on these analyses, this collection was separated into five groups (Fig. 1). Group 1 consisted of *P. nicotianae*, with the majority of isolates displaying very low SNP. Based on SNP data, the *P. nicotianae* group can be further divided into additional subgroups. These groups were not associated with a particular group of hosts, ruling out any strong host specificity among the isolates. Moreover, the tree branches defining these groups were not supported by strong bootstrap values (<50%) and, therefore, each ITS sequence type has a broad host range. Our analyses, however, focused on species identification and it is likely that variation at other loci important for pathogenicity and aggressiveness, such as effector genes, could correlate with host range in *P. nicotianae*. With the ever-increasing ease and feasibility of sequencing entire genomes, this issue can be addressed by sequencing the entire genomes of select sets of isolates in this collection in the future. Analyses based on entire genome sequences will also

allow full-genome concatenated SNP-based analysis, which provides in-depth resolution in differentiating even closely related pathogen strains (Bart et al. 2012).

Evolutionary diversity estimates, which were as high as 7.5%, indicate that this *P. nicotianae* population is highly diverse. The high divergence suggests that most of the isolates are not a single clonal lineage and that they were probably introduced along with their hosts into Florida. One of the most divergent isolates, 08-168, and several less divergent isolates were isolated from the same host (a *Spathiphyllum* sp.). This kind of host overlap can potentially provide a favorable breeding ground for hybridization and the emergence of new isolates containing novel complements of virulence factors, which can further expand their already wide host range. It is interesting to note that the first natural hybrid between two *Phytophthora* spp. was also isolated from a *Spathiphyllum* sp. (Man in 't Veld et al. 1998), suggesting that this plant and probably other tropical foliage plants are susceptible to multiple species of *Phytophthora*. Similarly, occurrence of several different *Phytophthora* spp. with so much variation within a small geographic location can also potentially lead to the emergence of novel hybrid species with adaptability to new environments and hosts (Leonberger et al. 2013). Several emerging pathogenic *Phytophthora* spp. such as *P. andina* and *P. alni* have been shown to be hybrids of different *Phytophthora* spp., which most likely coinhabited the same environment or even the same plant species (Brasier et al. 1999; Érsek and Nagy 2008; Goss et al. 2011a; Ios et al. 2006). Similarly, hybrids between *P. nicotianae* (*nicotianae*) and *P. cactorum*, which possess an expanded host range, are likely due to a combination of novel virulent factors (Bonants et al. 2000; Hurtado-Gonzales et al. 2009; Man in 't Veld 2001; Man in 't Veld et al. 1998; Nirenberg et al. 2009). All these examples suggest that, if brought together, *Phytophthora* spp. can potentially hybridize, resulting in development of aggressive strains. This possibility is supported by the fact that both mating types A1 and A2 were represented in the isolates analyzed in this study. In our *Phytophthora* collection, we did not see any evidence of natural intra- or interspecific *Phytophthora* hybrids despite the fact that many isolates were recovered from a *Spathiphyllum* sp. A possible explanation could be incompatibility among *Phytophthora* spp. cohabiting with the same individuals of a host plant species. For example, we recovered *P. nicotianae* and *P. palmivora* from *Spathiphyllum* spp. and, assuming that these two *Phytophthora* spp. are sympatric, their chances of hybridization might have been obscured by their genetic divergence. This is because *P. nicotianae* belongs to clade 1 and *P. palmivora* belongs to clade 4 according to recent molecular classification (Blair et al. 2008; Kroon et al. 2012). Similarly, *P. tropicalis* and *P. palmivora* both infect *Hedera* spp. and, thus, can potentially occur concurrently on the same individual plant and belong to distinct clades—clade 2 (*P. tropicalis*) and clade

Table 4. Results of mating type testing^a

Species	A1	A2	Sterile	A1A2
<i>Phytophthora nicotianae</i>	28	40	22	0
<i>P. palmivora</i>	1	5	17	0
<i>P. tropicalis</i>	2	2	2	0
<i>P. katusae</i>	0	0	0	3
<i>P. cinnamomi</i>	0	1	0	0
Total	31 (25.2%)	48 (39.0%)	41 (33.3%)	3 (2.4%)

^a Mating types were determined using known tester mating types A1 and A2; each isolate was also tested with itself for homothallism. Sterile isolates are defined as those that did not display any evidence of oospores, antheridia, or oogonia; A1A2 represents homothallic isolates.

Fig. 2. Sensitivity of *Phytophthora* isolates to mefenoxam. **A**, Dose-response curves of *Phytophthora nicotianae* isolates 05-297 (resistant) and 05-217 (sensitive) to mefenoxam on V8 juice media. Each data point is a mean of four replications; error bars are standard error. **B**, Representative photographs of isolates 05-297 and 05-217 on mefenoxam-containing and control plates 5 days after the start of experiment.

4 (*P. palmivora*) (Blair et al. 2008; Kroon et al. 2012)—suggesting that they also may be too genetically diverse to cross-hybridize. Evolutionary diversity estimates, which were as high as 7.5%, indicate that this *P. nicotianae* population is highly divergent. The high divergence suggests that most of the isolates are not a single clonal lineage and that they were probably introduced along with their hosts into Florida. In our study, most isolates collected from 1993 to 2012 belong to mating type A2 whereas most isolates collected in 2013 belong to mating type A1. Further, most isolates collected in 2014 belong to mating type A2. Such a change in mating type year by year was evident in Europe for mating type A2 (Gisi and Cohen 1996). This pattern might involve epidemiological properties of the mating type, which is less competitive than the other mating type.

Assessing fungicides sensitivity of *Phytophthora* spp. is an essential step in planning and employing proper disease management strategies. Occurrence of fungicide resistance is a recurring problem with most single-site fungicides, including mefenoxam resistance in *Phytophthora* spp. (Ferrin and Kabashima 1991; Hu et al. 2005; Hu et al. 2008, 2010; Hwang and Benson 2005; Jeffers et al. 2004; Timmer et al. 1998), which can severely impact disease management programs. In several cases, mefenoxam resistance has been suggested to be associated with repeated use of mefenoxam (Hu et al. 2008). The isolates reported in this study were collected over a longer time from nurseries that rely on chemicals for disease control, thus raising the probability of fungicide resistance development. In our analyses, a majority of isolates were sensitive to all fungicides, including mefenoxam. This may be due to growers practicing fungicide rotation and tank mixes, which is widely publicized and emphasized through extension and outreach efforts in Florida. Moreover, only a few isolates (1 to 7) were collected each year starting from 1993, with exceptions of years 2013 and 2014. Such a small number of isolates collected each year could be a reason for the narrow range of species. For example, four species were recovered from a total of 45 isolates in 1993 to 2012, whereas four species were recovered from a total of 78 isolates only from 2 years (2013 and 2014). However, there were notable exceptions of two isolates displaying insensitivity to mefenoxam. One isolate was recovered in 2005 and another isolate was recovered in 2014. In 2014, in total, 33 isolates were collected, which increased a chance of capturing resistant isolates. This was expected because mefenoxam has been used extensively in nurseries in Florida. The substantial variation in mefenoxam sensitivities in tested isolates suggests that they are still evolving. It is likely that these isolates could further evolve and become more resistant to mefenoxam, especially if mefenoxam is used exclusively. These results agree with those reported by Ferrin and Kabashima (1991), where 7.7% of the *P. nicotianae* isolates collected from nurseries in Southern California were mefenoxam resistant (Ferrin and Kabashima 1991). Our results differed substantially from those reported from *P. nicotianae* isolates collected from nurseries elsewhere, which reported 21% (North Carolina) and 26% (Virginia) mefenoxam-resistant isolates (Hu et al. 2008; Hwang and Benson 2005). Hu et al. (2008) collected 95 isolates of *P. nicotianae* from diverse nursery crops and irrigation water from Virginia, whereas Hwang and Benson (2005) recovered 483 isolates from seven different plant species from North Carolina. Based on our data, if we assume that approximately 8.8% *P. nicotianae* isolates present in Florida are resistant to mefenoxam and that mefenoxam is used frequently, we should have seen an increase of mefenoxam-resistant isolates over time. However, this is not what was observed in the analyses. The most resistant isolate (05-297) was isolated in 2005, whereas isolates collected after 2005 were more sensitive to mefenoxam. An explanation for this is that the plants could have been shipped elsewhere before *Phytophthora* spp. could become established in the nursery, or they could have been phased out from natural populations due to use of fungicides with modes of actions that are different from mefenoxam. In addition, mefenoxam has been in the market since 1996 for use on various crops but, as years have passed, more fungicides have become available which belong to different modes of action. Commercialization of new products may have reduced the exposure of *Phytophthora* spp. to mefenoxam. More intensive and extensive sampling

might be needed to gain a true assessment of the prevalence of mefenoxam-resistant isolates in Florida.

Our analysis finds resistance in *Phytophthora* spp. to Adorn (fluopicolide), Aliette (fosetyl-Al), Heritage (azoxystrobin), and Segway (cyazofamid). To our knowledge, this is the first report of resistance in an isolate of *Phytophthora* to fluopicolide. The finding of an isolate resistant to Adorn is not surprising because fluopicolide has a single-site mode of action for efficacy against oomycetes. In fact, the product manufacturer recommends that Adorn is not to be applied alone. There are reports of reduced sensitivity in *Phytophthora* spp. to Aliette. For example, *P. cinnamomi* obtained from avocado trees continuously treated with phosphonates was less inhibited by Aliette (Duvenhage 1994). Similarly, all *P. capsici* isolates collected from diverse vegetable crops were found to have either resistance or intermediate sensitivity to Segway (Jackson et al. 2012).

Several groups have reported increased fitness of metalaxyl-resistant isolates as increased vegetative growth, oospore formation, and lesion sizes (Hu et al. 2008; Kadish and Cohen 1988a,b; Kadish et al. 1990; Porter et al. 2007). Interestingly, in our study, we found that, in the presence of lower mefenoxam doses, mefenoxam-resistant isolates displayed increased mycelial growth, suggesting that these isolates have adapted to exploit mefenoxam to their advantage through an unknown mechanism. Because mefenoxam is suggested to affect the activity of RNA polymerases (Davidse et al. 1983, 1988; Fisher and Hayes 1984), it is possible that the resistant isolates have accumulated mutations that, instead of inhibiting, enhance the activity of RNA polymerase for favorable mycelial growth. Mefenoxam has been popular for controlling *Phytophthora* diseases in ornamental nurseries. The frequencies of resistant *P. nicotianae* isolates may increase in the future if these isolates have greater fitness compared with sensitive isolates. Moreover, by cross hybridizing with other *Phytophthora* isolates or species, mefenoxam resistance can be transferred into hybrids with better fitness in natural ecosystems. Therefore, caution is advised in using mefenoxam where it has been found to be ineffective; under such situations, it is advisable to discontinue using mefenoxam altogether because this can lead to the selection and reappearance of mefenoxam-resistant populations, as was suggested for *P. infestans* (Hu et al. 2012). Resistance can also be managed by rotating fungicides with different modes of action such as fosetyl-Al and dimethomorph, which provided effective control against those isolates, which were resistant to mefenoxam. In addition, all other possible integrated disease management approaches should be practiced such as using clean potting mixes, clean water, and including biological and biorational pesticides with broad-spectrum activity. This work provides important information about the genetic structure of *Phytophthora* populations in Florida. These findings will help in formulating effective strategies and policies for preventing the spread of *Phytophthora* spp. to new niches and will mitigate risks of fungicide resistance development.

Fig. 3. Frequency distribution of resistant, moderately resistant, and sensitive *Phytophthora* isolates to different fungicides. Resistant, $\leq 50\%$ growth inhibition; Moderate, moderately resistant, with 51 to 79% growth inhibition; and Sensitive, $\geq 80\%$ growth inhibition.

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