

IDENTIFICATION AND MEFENOXAM SENSITIVITY OF OOMYCETE ROOT
PATHOGENS RECOVERED FROM ORNAMENTAL PLANTS IN GEORGIA

by

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(Under the Direction of JEAN L. WILLIAMS-WOODWARD)

ABSTRACT

Mefenoxam is a fungicide widely used in the greenhouse and ornamental industry to control *Pythium* and *Phytophthora* diseases. Mefenoxam insensitivity was reported in several states in the USA. The objectives of this study were to identify oomycete root pathogen species recovered from ornamental plant nurseries in Georgia, and to determine if any isolates were insensitive to mefenoxam fungicide. Isolates were collected from 42 plant species and identified based upon morphological characteristics and rDNA ITS region sequencing. The 117 isolates recovered included seven species of *Phytophthora*, nine species of *Pythium*, and four species of *Phytopythium*. Mefenoxam sensitivity screening was conducted in vitro. Mefenoxam insensitivity was identified in 45.3% of the 117 isolates corresponding to 7.7%, 54.3%, and 70% of the *Phytophthora*, *Pythium*, and *Phytopythium* spp. isolates, respectively. This is the first report of *Phytopythium* spp. recovery from ornamental plants and of mefenoxam insensitivity in *Pythium* spp. and *Phytopythium* spp. in Georgia.

INDEX WORDS: *Pythium*, *Phytophthora*, *Phytopythium*, fungicide resistance, ornamentals, mefenoxam, Subdue Maxx, ITS sequencing

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CHAPTER 1

INTRODUCTION

Purpose of the Study

The majority of root and crown diseases on ornamental crops are caused by Oomycete pathogens, including species of *Pythium* Pringsh. and *Phytophthora* de Bary. Both *Pythium* and *Phytophthora* cause root, crown, stem, and foliage blights. Symptoms often include root softening, sloughing, darkening of roots, crowns and stems, wilting, foliage chlorosis, leaf drop, stem dieback, and leaf and petiole blighting. Oomycete pathogens or “water molds” as they are commonly called, which also includes downy mildew causing pathogens, are unique and are not true fungi. They are more closely related to brown algae than fungi (Gunderson et al. 1987). One of the major differences between Oomycetes and true fungi is in their cell wall components. Oomycete cell walls are composed of a β -1,3 and β -1,6 glucans, whereas true fungi cell walls are composed of chitin (Gunderson et al. 1987). This is an important distinction because the mode of action of many fungicides is to act on and inhibit chitin cell wall biosynthesis. Since Oomycete cell walls do not contain chitin, these products have no activity on these pathogens. This has resulted in a limited number of commercially available fungicides with activity against *Pythium*, *Phytophthora* and downy mildew diseases.

The predominant fungicide used against *Pythium* and *Phytophthora* diseases in ornamentals has been the phenylamide systemic fungicide, metalaxyl, which was replaced by mefenoxam (the R-enantiomer of metalaxyl), and marketed under the trade names of Subdue 2E® and Subdue Maxx® (Syngenta Crop Protection, Inc., Greensboro, NC), respectively

(Taylor et al. 2002). Metalaxyl was registered for use in the United States in 1980 and within four years fungicide resistance in *Pythium* causing turf blight was identified (Sanders 1984).

Mefenoxam fungicide resistance, or rather insensitivity, has been noted in several states in *Pythium* and *Phytophthora* species causing root and crown rots of ornamental plants. In Pennsylvania, 32.5% of the 120 *Pythium* isolates recovered from infected plants were insensitive to mefenoxam (Moorman et al. 2002). Eleven species of *Pythium* were identified from the 120 isolates. The most common species were *P. irregulare* and *P. aphanidermatum* of which 36.8% and 37.5% of these species, respectively, were insensitive to mefenoxam. In North Carolina, three species of *Phytophthora* (*P. nicotianae*, *P. cryptogea*, and *P. palmivora*) were recovered as the predominant species infecting floriculture crops (Hwang and Benson 2005). Although, all isolates of *P. palmivora* were still sensitive to mefenoxam, 100% of the *P. cryptogea* and 21% of *P. nicotianae* isolates were insensitive. In a more recent North Carolina study, *P. nicotianae*, *P. drechsleri*, *P. cryptogea*, and *P. tropicalis* were the most commonly recovered *Phytophthora* species from floriculture crops, of which 66% of these *Phytophthora* isolates were insensitive or intermediate in resistance to mefenoxam (Olson and Benson 2011).

These studies would suggest that mefenoxam insensitivity is widespread within floriculture production. However, another study involving multiple states in the southeastern USA concluded that across six states and 488 isolates that only 6% of the *Phytophthora* isolates were insensitive to mefenoxam (Olson et al. 2013). The viability of mefenoxam as a valuable tool in managing *Pythium* and *Phytophthora* root diseases is of great concern. The objectives of this study were to 1) identify species of *Pythium* and *Phytophthora* from symptomatic plants within both floriculture and woody ornamental crops in Georgia and 2) determine if mefenoxam insensitivity is present within the recovered oomycete isolate population.

CHAPTER 2

LITERATURE REVIEW

The ornamental plant industry in Georgia had a farm gate value of \$462.95 million in 2013 (Wolfe and Stubbs 2014). The industry has seen a reduction of 20% since its peak in 2007, where it had a \$ 606.23 million farm gate value and ranked third in the state of all agricultural commodities (Boatright and McKissick 2008). With the high value and large number of plant material throughout the state, losses due to disease can be costly. Crop losses due to disease in ornamental plant production in Georgia was estimated at 9.1% in 2012. This equated to \$42.12 million in damage losses and costing \$25.7 million in control costs for a total disease loss estimate of \$67.82 million in 2013, of which \$27.32 million was lost in damage and cost of control for root and crown rot disease (Williams-Woodward *in press*). In 2014, there were 346 ornamental plant samples submitted to the University of Georgia Extension Plant Pathology Plant Disease Clinic in Athens, GA (Maultsby and Jogi *in press*). The overwhelming majority of disease loss for these samples occurred via root and crown rot diseases due to the Oomycete pathogens, *Pythium* spp. and *Phytophthora* spp.

Oomycete root pathogens

Oomycetes are classified in the kingdom Straminipila, class Oomycota, order Peronosporales, family Pythiaceae (Alexopoulos et al. 1996; Dick 2001). Oomycetes are known as ‘water molds’ (Margulis and Schwartz 2000). Most of the species in the Pythiaceae are soil

and water inhabitants, with approximately 60% of the oomycete species being plant pathogenic (Thines and Kamoun 2010).

Since the mid-1960s, researchers have reported evidence that species belonging to Oomycota are not related to true fungi (kingdom Eumycota) (Tyler 2002; Whittaker 1969). Instead, they are more closely related to golden-brown algae (Thines and Kamoun 2010). Morphological characteristics that set the Oomycota apart from true fungi have cellulose cell walls, a diploid life cycle, coenocytic mycelia, the inability to synthesize sterols, the inability to deposit polyphosphate as metachromatic granules, tubular cristae within mitochondria, and small-subunit ribosomal DNA sequences (Alexopolous et al. 1996; Baldauf and Palmer 1993; Gunderson et al. 1987; Latijnhouwers et al. 2003). The most common oomycete pathogens are *Phytophthora* and *Pythium*. However, several other genera have been described including *Phytopythium* and *Halophytophthora*.

Phytophthora

Phytophthora is a genus of plant pathogens that play a major role in the growing and production of many food and nursery crops. Members of this genus cause serious crown and root rot diseases, including most famously, *Phytophthora infestans*, cause of late blight of potato. There are 60 species of *Phytophthora* described at this time, most of which invade healthy plant tissue. Some species have a very limited ability to survive as saprobes (Cooke et al. 2000). Traditional classification of *Phytophthora* was based on morphology, primarily the morphology of the sporangia, arrangement of the anteridia and oogonia, and whether the species was homothallic or heterothallic (Cooke et al. 2000; Erwin and Ribeiro 1996). These key characteristics of morphology are still used today; however, identification has have shifted

toward the molecular level due to the lack of distinguishing morphological differences in some species.

Morphologically, *Phytophthora* species have hyphae that lacks septa (coenocytic) and produce ovoid to elliptical sporangia that may be nonpapillate, semipapillate, or papillate. Sporangia are often produced at the terminus of a hypha; however, within several species the production of intercalary sporangia is more common (Erwin and Ribeiro 1996). Biflagellate zoospores are formed inside the sporangium and are released directly from the sporangium through an apical pore. Released zoospores often encyst before host contact and infection.

Asexual chlamydospores may also develop from hyphal tips or by swellings of hyphal tubes in some *Phytophthora* species. The chlamydospores may be produced intercalary singly or in chains and range in size (Erwin and Ribeiro 1996). Most chlamydospores are spherical but some may vary in shape. Once the spores mature and are fully expanded, the walls thicken to varying degrees depending on environmental conditions and the species in question. The main function of a chlamydospore is to act as a resting spore in the life cycle of *Phytophthora* spp. (Erwin and Ribeiro 1996).

Oospores are the sexual spores that are formed when gametes are introduced into an oogonium either by directly transferring the oogonium through the antheridium (amphigyny) or by the antheridium attaching to the lower half of the oogonium (paragyny) and the resulting mating producing oospores (Erwin and Ribeiro 1996).

Pythium species

Pythium is an economically important pathogen found within the Oomycete group. *Pythium* is the most common root pathogen associated with greenhouse and ornamental nursery plants (Daughtrey et al. 1995). The pathogen is ubiquitous in soils around the world. Disease

often depends on the condition of the host, amount of moisture present, temperature, and species of *Pythium* present (Daughtrey et al. 1995).

Within the genus of *Pythium*, there are approximately 125 species. Of these 125 species, most are not host specific. Some species such as *P. irregulare* and *P. aphanidermatum* are commonly associated with potted plants (Daughtrey et al. 1995). *Pythium* is similar to *Phytophthora*, but very distinct as well. *Pythium* species differ from *Phytophthora* in morphology of the sporangia, hyphae, and sexual structures (anteridia and oogonia), as well as the nature of zoospore release and maturation (Waterhouse 1967). In *Pythium*, zoospores form when protoplasm from sporangia is emptied into a thin-walled vesicle (Waterhouse 1967). *Pythium* sporangia may be globose, filamentous, or swollen and oogonia are mostly spherical and smooth with a few instances of lobed oospores. Zoospores of *Pythium* spp. are motile and biflagellate similar to *Phytophthora*.

Pythium survives in the soil as chlamydospores and oospores until favorable conditions cause the pathogen to germinate and infect. Survival is not limited to just the soil; *Pythium* can survive on flats and pots that have debris left within, as well as in dust left on greenhouse floors. Germination of *Pythium* occurs with germinating seeds or roots release exudates. The pathogen detects these substances and oospores or chlamydospores germinate. Studies show that oospores can be induced to germinate within 1.5 hours of detecting exudates (Daughtrey et al. 1995). Not only does the susceptible host have to be present, a favorable environment is needed as well. Increased soil moisture along with adverse temperatures for plant growth can be conducive to disease development (Daughtrey et al. 1995). In some cases, high fertility rates can increase a plants susceptibility to *Pythium* root rots, while in other cases, low fertility can cause increased disease pressure.

Pythium can spread in the ornamental plant industry by workers, tools, contaminated plant materials and water. Nursery workers spread the disease on their gloves and tool as they work within the nursery. Zoospores can be spread by rain or splashing irrigation water. Some fungus gnats and shore flies have been shown to spread *Pythium aphanidermatum* through ingestion and egestion of mature oospores (Goldberg and Stanghellini 1990).

Phytopythium species

The genus, *Phytopythium*, is a relatively new genus that was first described in 2010 with the type species, *Phytopythium sindhum* Lodhi, Shahzad & Lévesque (Lodhi et al. 2010).

Phytopythium are morphologically and phylogenetically between *Pythium* and *Phytophthora* (Bala et al. 2010). Morphologically, *Phytopythium* sporangia are papillate and globose to ovoid in shape similar to *Phytophthora*; however, sporangia show internal proliferation, which is uncharacteristic of papillate sporangia of *Phytophthora* species (de Cock et al. 2015). Zoospore discharge is *Pythium*-like, with the sporangium forming a discharge tube and vesicle from which biflagellate zoospores are discharged (Bala et al. 2010). In addition, most species have smooth, thick-walled oospores and lobed antheridia. Species of *Phytopythium* comprise what was formerly known as *Pythium* clade K species according to molecular phylogeny presented by Lévesque and de Cock (2004).

Other *Pythium* species in clade K include *P. vexans*, *P. cucurbitacearum*, *P. chamaehyphon*, *P. helicoides*, *P. litorale*, and *P. mortanum* (Lévesque and de Cock 2004). Most clade K species have been renamed *Phytopythium* based upon rDNA and mitochondrial DNA analysis including *Phytopythium vexans* (= *Pythium vexans*), *P. chamaehyphon*, *P. litorale*, and *P. helicoides* (de Cock et al. 2015).

Phytophthora species are pathogenic to various plants. Yang et al. (2011) described a severe root and stem rot associated with begonias in a field trial. They were able to isolate a *Pythium*-like species from infected roots. The isolates all produced distinct single-stranded conformation polymorphism (SSCP) fingerprints that were unlike any other oomycete pathogen isolated from begonia. Morphological examination of these isolates revealed that the species matched the description of *Phytophthora helicoides*. Confirmation was made by sequencing the rDNA internal transcribed spacer (ITS) 1 and 2 regions (Yang et al. 2011). Pathogenicity of the isolates was confirmed by re-inoculation onto begonia plants with similar symptoms to the original infected plants observed.

Oomycete Pathogen Isolation and Detection

Symptoms of oomycete root pathogens are often nondescript and may also be confused with other root rot causing pathogens. Oomycete infected plants often show symptoms of chlorosis, wilting, crown rot, and dying back from leaf petioles. To begin diagnosis and identification of oomycete pathogens often the infected tissue is grown on selective media amended with antibiotics (pimaricin, ampicillin, rifampicin) to reduce bacterial growth and a fungicide (PCNB) to reduce other soilborne pathogens such as *Fusarium* and *Rhizoctonia* (Jeffers and Martin 1986). Once isolated, morphological characteristics can help aid in identifying oomycete species.

Enzyme-linked Immunosorbant Assays (ELISA) tests are also available to test for the presence of *Phytophthora*, and to a lesser degree *Pythium*, in diseased plant material and irrigation water (Alishtayeh et al. 1991; MacDonald et al. 1994). Agdia Inc. (Elkhart, IN) produces a double antibody sandwich (DAS) ELISA kit for *Phytophthora* detection, which has been used as part of the United States Department of Agriculture (USDA) *P. ramorum* detection

protocol (Jones 2006). Potential problems with commercial ELISA kits include the accuracy of species delineation, detection of non-viable propagules and potential false results (Alishtayeh et al. 1991; Osterbauer and Trippe 2005).

Molecular techniques and DNA sequencing is increasingly utilized to identify oomycete species due to the difficulty in distinguishing some species (Cooke and Duncan 1997). Techniques such as restriction fragment length polymorphisms (RFLP) (Bonants et al. 2000), amplified fragment length polymorphisms (AFLP) (Bonants et al. 2000), isozyme analysis (Cooke et al. 2000), single strand conformation polymorphisms (SSCP) (Kong et al. 2003; Kong et al. 2004; Kong et al. 2005), and DNA sequence analysis (Cooke and Duncan 1997; Lee and Taylor 1992) have been used to aid in identification. The most frequently used genetic loci is the highly repetitive internal transcribed spacers (ITS1 and ITS2) between the 18S and 28S genes on the ribosomal RNA gene (Cooke and Duncan 1997; Cooke et al. 2000; Levesque and de Cock 2004). However, mitochondrial cytochrome oxidase (*cox*) I and II gene is increasingly being used to distinguish species that are not easily differentiated by ITS sequencing (Martin 2000; Martin and Tooley 2003; Jung and Burgess 2009).

Surveying for and identifying *Phytophthora* species from ornamental nurseries has been conducted across the United States in response to the introduction and occurrence of *Phytophthora ramorum*, cause of sudden oak death. In Indiana, 106 *Phytophthora* isolates were collected and 13 species were identified using rDNA sequencing of the ITS region (Leonberger et al. 2013). *Phytophthora citricola* was identified as the predominant species comprising of 35.9% of the isolates. *P. citrophthora* accounted for 27.4% with the rest of the isolates being identified as *P. cactorum*, *P. cactorum* × *hedraiaandra*, *P. cambivora*, *P. capsici*, *P. drechsleri*, *P.*

hedraiandra, *P. nicotianae*, *P. nicotianae* × *cactorum*, *P. palmivora*, and *P. syringae* (Leonberger et al. 2013).

Direct sequencing of the ITS region and examination of morphological characters of *Phytophthora* isolates collected from leaves in woody ornamental nurseries in Tennessee resulted in the identification of six known species (*P. cactorum*, *P. citricola*, *P. citrophthora*, *P. nicotianae*, and *P. tropicalis*), as well as on newly described species *P. foliorum* (Donahoo and Lamour 2008).

Direct sequencing of the ITS rDNA, β -tubulin, and mitochondrial *cox1* genes were used to identify *Phytophthora* species recovered from ornamental nurseries in Minnesota (Schwingle et al. 2007). Species recovered included *P. cactorum*, *P. cambivora*, *P. citrophthora*, *P. hedraiandra*, *P. megasperma*, *P. nicotianae*, and the undescribed taxon *P. taxon Pgchlamydo*. The most common species encountered were *P. cactorum*, *P. citricola*, and *P. citrophthora*. Additionally, two isolates did not match known species.

Oomycete Disease Management

Management of oomycete pathogens revolves around sanitation and exclusion practices. Common practices include discarding and removal of infected plants from the growing area; improving water management including avoiding overwatering, growing plants in well-drained rooting substrates, and treating recirculating water to reduce pathogens; avoiding contaminated media, tools, containers; and preventing standing water or raising containers off the ground (Parke and Grunwald 2012). Another necessary disease management tool is using fungicides to reduce disease development. One of the most common fungicides for oomycete management is mefenoxam (Subdue Maxx®, Syngenta, Greensboro, NC). In 2009, 19% of growers in six states reported using mefenoxam (USDA 2009).

Fungicide Resistance

The predominant fungicide used against *Pythium* and *Phytophthora* diseases in ornamentals has been the phenylamide systemic fungicide, metalaxyl, which was replaced by mefenoxam (the R-enantiomer of metalaxyl), and marketed under the trade names of Subdue 2E® and Subdue Maxx® (Syngenta Crop Protection, Inc., Greensboro, NC), respectively (Taylor et al. 2002). Metalaxyl was registered for use in the United States in 1980 and within four years fungicide resistance in *Pythium* causing turf blight was identified (Sanders 1984).

Mefenoxam resistance has been studied previously on a wide range of *Pythium* and *Phytophthora* species. Moorman et al. (2002) examined *Pythium* species recovered from samples submitted to the Pennsylvania State University Plant Disease Clinic. Sensitivity of isolates to mefenoxam was evaluated in vitro by placing agar plugs on fungicide-amended agar medium containing 100 µg a.i./ml of mefenoxam. In this study, 11 species of *Pythium* from 120 samples were isolated. *Pythium irregulare* was the most abundant with 57 isolates obtained. Of the 57 isolates identified, 21 were found to be resistant to mefenoxam (Moorman et al. 2002). *Pythium aphanidermatum* was the next abundant species with 32 isolates identified and 12 found to be resistant to mefenoxam. In all of the 120 isolates identified and tested, 39 isolates were found to have resistance to mefenoxam.

In 2013, a six state study, which included Georgia, was conducted to investigate the diversity of *Phytophthora* species collected from root, plant tissue, and pond and irrigation water. Isolates were also tested to investigate the extent of mefenoxam resistance associated with the ornamental plant industry (Olson et al. 2013). Mefenoxam sensitivity was measured by placing a 5-mm piece of agar amended with 100 µg a.i./ml of Subdue Maxx® (Syngenta Crop Protection, Greensboro, NC) in 48-well tissue culture plates. Eight isolates were tested at a time with this

method. Plates were then placed in the dark and incubated at 25°C for 3-4 days. Mycelium growth was measured at a 0-5 scale. All wells then were evaluated for growth on a 0-5 scale, where 0 = no growth; 1 = hyphae visible only microscopically, with a few hyphae growing from the plug; 2 = hyphae visible only microscopically, with uniform growth around the plug; 3 = mycelium just visible macroscopically, with uniform growth around plug; 4 = mycelium visible macroscopically but growth less than growth in non-amended wells; and 5 = mycelium visible macroscopically and growth equal to that in non-amended wells. (Olson et al. 2013). Known mefenoxam-sensitive and -insensitive isolates of *P. nicotianae* were used for comparison.

Identification yielded 488 isolates of *Phytophthora* with 464 identified as 19 different species. In total, 1483 isolates were screened for mefenoxam sensitivity at 100 µg a.i./ml with 102 found to be insensitive. Of the insensitive isolates, 78% belonged to *P. nicotianae*, with 67% of those being recovered from herbaceous annuals. Other species found to be insensitive were *P. undulata* (four isolates), *P. palmivora* (one isolate), and *P. taxon 'Pgchlamydo'* (one isolate) (Olson et al. 2013).

Isolates from Georgia included in the Olsen et al. (2013) study were collected during *Phytophthora ramorum*, cause of sudden oak death, trace-forward nursery surveys, as well as from forest streams and nursery irrigation retention pond surveys using rhododendron leaf baits. The majority of the *Phytophthora* isolates recovered from plants in Georgia were *P. ramorum*, which were all sensitive to mefenoxam. Other species included *P. nicotianae*, *P. citrophthora*, *P. drechsleri*, *P. nicotianae*, *P. plamivbora*, *P. pini*, and *P. undulata* (syn. *Pythium undulatum*). *Phytophthora* isolates recovered from irrigation water and natural streams included *P. aquimorbida*, *P. citrophthora*, *P. gonapodyides*, *P. hydropathica*, *P. nicotianae*, *P. pini*, *P. tropicalis*, and *P. undulata* (syn. *Pythium undulatum*). Mefenoxam insensitivity was identified

among isolates of most species; however, the percentage of isolates originating from Georgia was not reported (Olsen et al. 2013).

Similar studies have been conducted on both *Pythium* and *Phytophthora* species. Garzon et al. (2011) evaluated six isolates of *Pythium* to determine if disease incidence was increased by sublethal doses of mefenoxam. Known mefenoxam sensitive and insensitive isolates of *Pythium aphanidermatum* and *Pythium cryptoirregulare* were used in this study. Results from this study showed that all isolates grew faster at rates lower than 1 µg a.i./ml for sensitive isolates and 100 µg a.i./ml for resistant isolates. An increase between 1% and 22% was noted for isolates grown at concentrations lower than their respected reference concentrations. Garzon et al. (2011) also evaluated disease severity on geranium plants of isolates following sub-lethal mefenoxam doses and found that disease severity increased.

Mefenoxam resistance studies have primarily evaluated *Phytophthora* isolates. Hu et al. (2010) examined mefenoxam sensitivity in *P. cinnamomi* isolates in Virginia. Sixty five isolates were collected and tested for the presence of mefenoxam resistance. Of the sixty five isolates, thirty seven were collected from ornamental nurseries and twenty eight were received from neighboring states. In this study, all isolates were exposed to 0 and 100 µg a.i./ml of mefenoxam. Isolates of *P. cinnamomi* were all sensitive to mefenoxam at 100 µg a.i./ml. Mycelia were inhibited by the presence of mefenoxam. Percentage of inhibition ranged from 82%-100% inhibition of growth on fungicide-amended plates, with 14 of the isolates expressing no growth. There were variations of sensitive growth based on host plants but not enough to draw any correlation. Decreased resistance was contributed to the nature of the pathogen and fewer applications needed for control in the field. Researchers were alerted by intermediately sensitive isolates and the possibility of resistance building.

Hwang and Benson (2005) evaluated 483 *Phytophthora* spp. isolates collected from various floriculture crops in North Carolina for mefenoxam sensitivity and compatibility type. Of the total number of isolates tested for resistance to mefenoxam, 248 (51%) were sensitive at either 1 or 100 µg a.i./ml. All isolates of *P. cryptogea* showed either intermediate sensitivity or were insensitive to mefenoxam at 1 µg a.i./ml. In all, 57 isolates were intermediately sensitive to mefenoxam at 100 µg a.i./ml and 122 isolates were insensitive to mefenoxam at 100 µg a.i./ml. Of the 217 *P. nicotianae* isolates collected in this study, 79% were sensitive to mefenoxam. All 26 isolates of *P. palmivora* were sensitive to mefenoxam. Isolates that showed growth at 1 µg a.i./ml concentrations were further tested to determine their EC₅₀. EC₅₀ values ranged from 0.1-549.5 µg a.i./ml. Isolates were also looked at based on their host and year collected. *P. nicotianae* isolates collected from African violet (*Saintpaulia ionantha*) at the same location in 2001 and 2002 had the same level of sensitivity. In contrast, isolates of *P. cryptogea* collected from dusty miller (*Senecio cineraria*) in 2001 had an EC₅₀ value of 407.4 µg a.i./ml, and isolates collected in 2002 had EC₅₀ values of either 0.1 or 27.5 µg a.i./ml. This showed that there was variability in some species from year to year.

Hu et al. (2008) collected 95 isolates from ornamentals and irrigation water in the state of Virginia. Of the isolates from ornamental plants tested, all were either resistant or intermediately resistant, whereas the isolates tested from irrigation water were found have a 40% resistance rate when tested with 100 µg a.i./ml of mefenoxam. There was a noticeable increase however in sporangia and zoospore production in mefenoxam resistant isolates. When researchers looked at their competitive ability, mefenoxam-resistant isolates were more competitive compared with mefenoxam-sensitive isolates. It was noted in this study that all but one isolate tested was found to be sensitive to mefenoxam.

Olson and Benson (2011) reassessed mefenoxam resistance in *Phytophthora* spp. recovered from floriculture crops in North Carolina. Of the 163 isolates tested, 107 of them (66%) were either resistant or intermediately resistant at 1 µg a.i./ml of mefenoxam, and 102 of the 107 isolates were also resistant or intermediately resistant at 100 µg a.i./ml. It was determined by ITS region sequencing and RFLP analysis that 59% of the isolates were *Phytophthora nicotianae*, 23% *P. drechsleri*, 9% *P. cryptogea*, 4% *P. tropicalis*, and less than 1% were *P. citrophthora*. Of the 163 isolates, 58% of *P. nicotianae* isolates were resistant at 100 µg a.i./ml. *Phytophthora drechsleri* was resistant at both 1 and 100 µg a.i./ml and had EC₅₀ values between 340 and 910 µg a.i./ml of mefenoxam. Other isolates were either sensitive or showed intermediate resistance.

Mefenoxam resistance has been studied in other areas of production agriculture. Dunn et al. (2010) studied four vegetable growing regions in New York, screening for *Phytophthora capsici* and later testing for mefenoxam resistance. A total of 257 isolates were collected and tested in-vitro using 0, 5, and 100 µg a.i./ml of mefenoxam. Results of this study were varied by district. In one district, 66% of isolates screened were found to be resistant to mefenoxam. In another district, 25% of isolates were either intermediately resistance or resistant to mefenoxam. In the last two regions, all isolates were sensitive to mefenoxam.

Mefenoxam resistance has also been evaluated within *P. capsici* isolates from bell pepper in North Carolina and New Jersey (Parra and Ristaino 2001). A total of 150 isolates were collected dating back to 1997. Of those, 30% were sensitive to mefenoxam, 10% were intermediately resistant, and 59% were resistant to mefenoxam. In total, 82% of fields sampled had isolates that were resistant to mefenoxam. Of the resistant isolates, 90% showed growth greater than the control at concentration 100 µg a.i./ml. The mean EC₅₀ value for resistant

isolates was 366.5 $\mu\text{g a.i./ml}$. Researchers looked at the field where samples were taken and found different methods of disease control. In fields where mefenoxam was used alone, higher resistance was noted. However in some of those fields where mefenoxam was used singularly, resistance was reduced indicating inconsistencies with the disease. There was lower resistance incidence across the board for fields where mefenoxam was used in conjunction with other disease controlling methods.

CHAPTER 3

OOMYCETE ROOT PATHOGENS RECOVERED FROM ORNAMENTAL PLANTS FROM GREENHOUSES AND NURSERIES IN GEORGIA¹

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ABSTRACT

A total of 117 oomycete isolates was recovered from 168 plant samples collected from 16 ornamental plant production nurseries in Georgia in 2010 and 2011. Samples collected showed root and stem rot symptoms including plant stunting, wilting, and chlorosis, shoot blighting, root discoloration and plant death. Oomycetes recovered included 26 *Phytophthora* isolates representing seven species; 81 *Pythium* isolates representing nine species; and 10 *Phytopythium* isolates representing four species. Species identification was based upon morphology and ITS rDNA sequencing. The predominant *Phytophthora* species recovered was *P. nicotianae*, which accounted for 38.4% of the *Phytophthora* isolates followed by *P. pini*. The most abundant *Pythium* spp. included *P. irregulare*, *P. myriotylum*, *P. aphanidermatum* and *P. undulatum*. Ten *Phytopythium* spp. isolates were also recovered including *P. helicoides*, *P. vexans*, and *P. litorale*.

INTRODUCTION

The oomycete pathogens, *Pythium* and *Phytophthora*, cause considerable losses within ornamental nurseries. Symptoms often include root softening, sloughing, darkening of roots, crowns and stems, wilting, foliage chlorosis, leaf drop, stem dieback, and leaf and petiole blighting. Numerous studies have reported *Phytophthora* spp. recovered from ornamental plant nurseries in the USA (Donahoo and Lamour 2008; Hwang and Benson 2005; Leonberger et al. 2013; Olsen and Benson 2011; Olsen et al. 2013; Schwingle et al. 2007). Fewer have reported on *Pythium* spp. diversity (Moorman et al. 2002; Stephens and Powell 1982).

Identification of oomycete pathogen species can aid in diagnostics, identifying potential new threats to an ecosystem or production facility, and refine disease management recommendations that could include host specificity and sensitivity to fungicides. Some *Pythium*

species are recognized as highly pathogenic, whereas others are weakly pathogenic or saprobic (Csinos and Hendrix 1978; Ivors et al. 2008). *Pythium* and *Phytophthora* spp. within plant disease diagnostic laboratories are often not identified to species, which could lead to an over-dependence on fungicide management strategies that may or may not be directed at a particular pathogenic species. Pathogen populations also may change over time as production practices change. In the past *P. ultimum* was the predominant *Pythium* species associated with floriculture crops (Scheffer and Haney 1956; Stephens and Powell 1982); however, in more recent surveys, the predominant *Pythium* species have included *P. irregulare* and *P. aphanidermatum*, with *P. ultimum* recovered at a much lower frequency (Lookabaugh 2013; Moorman et al. 2002).

Species identification has moved from morphological characters to a molecular approach due to the difficulty of distinguishing among closely related species. Direct sequencing of the internal transcribed spacer regions (ITS-1 and ITS-2) of ribosomal DNA (rDNA), beta-tubulin gene, and mitochondrial encoded cytochrome c oxidase (*cox*) I and II genes have been utilized in multiple studies to differentiate *Pythium* and *Phytophthora* species (Bhat and Browne 2007; Donahoo and Lamour 2008; Leonberger et al. 2013; Martin and Tooley 2003; Moorman et al. 2002; Olsen et al. 2013; Schwingle et al. 2007). Single strand-conformation polymorphism of rDNA (PCR-SSCP) is another utilized method of differentiating among species (Kong et al. 2003; Kong et al. 2005).

Although *Phytophthora* isolates from woody ornamental plant samples, irrigation water sources, and natural waterways have been collected and identified in Georgia (Olsen et al. 2013), a survey for oomycete pathogens, particularly *Pythium* spp., on herbaceous ornamental plants has not been conducted. The objective of this study was to determine the presence and identity of

oomycete root pathogens causing disease on ornamental plants in on commercial production nurseries Georgia.

MATERIALS AND METHODS

Isolate Collection. Plant samples exhibiting symptoms of root and crown rot disease including stem dieback, wilting, chlorosis, and root discoloration were collected from 16 wholesale ornamental plant production nurseries within 11 counties in Georgia (nine specializing in container-grown woody shrubs and seven specializing in floricultural or herbaceous crops) in 2010 and 2011. In a few instances, leaf samples exhibiting petiole death and lesions were collected in addition to root samples. Symptomatic tissue sections were washed with tap water, blotted dry on sterile filter paper and cut into 1 to 10 mm sections. Tissue sections were embedded into V8-PARP medium (15 g Bacto agar [Becton, Dickerson and Co., Sparks, MD]; 50 ml clarified V8 juice [Campbells, Camden, NJ]; 400 μ l pimaricin [Sigma-Aldrich, St. Louis, MO]; 250 mg ampicillin [Sigma-Aldrich]; 10 mg rifampicin [Sigma-Aldrich]; , 67 mg pentachloronitrobenzene (PCNB) [Terraclor; Chemtura, Middlebury, CT]; in 950 ml of deionized water and) (Jeffers and Martin 1986). Plates were incubated in the dark at 22°C for up to 10 days. Suspect Oomycete colonies were transferred onto fresh V8-PARP or non-amended V8 juice agar medium (15 g Bacto agar; 100 ml clarified V8 juice; 900 ml deionized water) until a pure culture was obtained.

Three isolates were obtained from recirculated irrigation water within one woody ornamental production nursery in GA. *Rhododendron maximus* leaves were enclosed in mesh bags and floated in the water sources for one week. Leaves were rinsed with tap water and symptomatic tissue (discolored, watersoaked lesions) was excised from the leaves and plated onto V8-PARP medium as described previously.

Isolate Identification. Putative *Pythium* and *Phytophthora* isolates were grown for three days in the dark at 25°C on V8 juice agar medium at which time Three 5-mm-diameter agar plugs were flooded with non-sterile soil extract solution (NSES) in 60-mm plastic petri plates to observe sporangia formation and morphological characteristics. NSES was prepared by adding 15 g of a loamy field soil (Watkinsville, GA) to 1000 ml of distilled water that was stirred continuously for 4 hours and allowed to settle overnight. The supernatant was then decanted into 250 ml Nalgene bottles and centrifuged for 10 minutes at 8000 rpm. If needed, the solution was vacuum-filtered to remove any residual debris. Flooded agar plugs were examined after 24 hours at room temperature (22-24°C). Preliminary isolate identification was based on morphological characteristics (Erwin and Ribeiro 1996; van der Plaats-Niterink 1981; Waterhouse 1963; Waterhouse 1967).

Isolates were further identified based upon sequencing the ITS region (ITS1, 5.8S, and ITS2). Single line isolates were created by hyphal tip transfer of each isolate onto fresh V8 juice agar and grown at 22-24°C for 72 hours. Aerial hyphae was scraped or lightly touched with a sterile 200- μ l pipette tip. The tip was then placed into a 0.5-ml PCR tube containing a PuReTaq Ready-To-Go™ PCR Bead (GE Healthcare, Pittsburgh, PA) containing 1 μ l of 10 μ M ITS-1 primer (TCCGTAGGTGAACCTGCGG-3'), 1 μ l of 10 μ M ITS-4 primer (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) and 23 μ l of sterile nuclease-free water and mixed gently by pipetting up and down several times. Total PCR reaction volume was 25 μ l. Thermal cycling conditions consisted of an initial denaturation of 94°C for 5 min; followed by 34 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min; and a final extension step of 72°C for 5 min, followed by a 4°C hold (Moorman et al. 2002). Amplified rDNA product

recovery was confirmed by 1.0% agarose gel electrophoresis with an rDNA product range of 400-600 kb. Amplified rDNA products were purified using QIAquick Purification Kit (Qiagen, Inc., Valencia, CA) according to kit protocol. Purified isolate DNA was stored at -20°C until submission to the Georgia Genomics Facility (Athens, GA) for sequencing on an Applied Biosystems ABI 3730XL sequencer (Applied Biosystems Inc., Foster City, CA) using the same primer set that was used to amplify the DNA. DNA sequences were aligned and manually edited using Geneious software (Biomatters Ltd., Auckland, New Zealand). ITS sequences were BLAST analyzed in GenBank (National Center for Biotechnology Information, Bethesda, MD) and the *Phytophthora* Database (<http://www.phytophthoradb.org/>).

RESULTS

Of the 168 symptomatic samples collected from 42 ornamental plant taxa and three water sources, oomycete root pathogens were recovered from 70% of the samples. Either no pathogen or a non-oomycete isolate was recovered from the remaining samples. Of the 117 oomycete isolates recovered, 26 were identified as *Phytophthora* spp., 81 as *Pythium* spp., and 10 as *Phytopythium* spp. (Table 3.1).

Nine *Pythium* species recovered included *P. acanthophoron*, *P. aphanidermatum*, *P. cucurbitacearum*, *P. diclinum*, *P. irregulare*, *P. monospermum*, *P. myriotylum*, *P. undulatum* and *P. zingiberis* (Table 3.1). *Pythium irregulare* was the most prevalent and accounted for 22.2% of the identifiable species, followed by *P. myriotylum* and *P. undulatum* (Figure 3.1). Five isolates of *P. aphanidermatum* isolates were recovered from only one floricultural crop greenhouse facility from *Euphorbia pulcherrima* (poinsettia) plant samples (Table 3.1). The majority of the *Pythium* isolates recovered (44 isolates) could not be identified to the species level. Initial

identification was made microscopically using morphological characteristics seen in culture and after flooding with NSES. Species identity could not be confirmed with ITS rDNA sequencing either because sequences did not match any listed in GenBank or the isolate was lost or became contaminated. The most common contaminant in the *Pythium* isolates was *Mortiella* spp. Contamination was not prevalent within the *Phytophthora* or *Phytopythium* isolates.

Seven *Phytophthora* species were recovered including *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae*, *P. palmivora*, and *P. pini* with *P. nicotianae* being the most prevalent (38.5% of the *Phytophthora* isolates). Three of the *Phytophthora* isolates could not be identified to species based upon morphology or ITS rDNA sequencing (Figure 3.2).

Ten *Phytopythium* isolates were recovered from diverse symptomatic plants including *Camellia japonica*, *Coreopsis lanceolata*, *Hydrangea arborescens* ‘Annabelle’, *Leucanthemum* × *superbum* (Shasta daisy), *Gardenia jasminoides*, *Rhododendron* ‘Jennifer’ (Kurume azalea), *Rosmarinus officinalis*, *Sedum* sp., *Tagetes patula*, and *Thymus praecox* (creeping thyme) from six production nurseries (Table 3.1). *Phytopythium* is a relatively new taxonomic genus whose members were classified as clade K species of *Pythium*, and have more characteristics similar to *Phytophthora* than other *Pythium* species. *Pythium litorale*, *P. heliocoloides*, *P. chamaeophyon*, *P. vexans* are now classified as *Phytopythium* species (Robideau et al. 2011) and were recovered in this study. One isolate of *Phytopythium* could not be identified to species because its ITS sequence did not have a match in GenBank.

DISCUSSION

Pythium species were recovered in greatest abundance in this study. The water-loving nature of *Pythium* lends itself well to ornamental plant production settings where excessive

watering is a common problem. The most common *Pythium* species described as causing root and stem rot disease on ornamental plants are *P. ultimum* Trow, *P. aphanidermatum* (Edson) Fitzp., and *P. irregulare* Buisman (Daughtrey et al. 1995). Both *P. irregulare* and *P. aphanidermatum* were recovered in this study, with *P. irregulare* being the most abundant species. It was also recovered from diverse hosts at multiple production nurseries. In contrast, *P. aphanidermatum* was only recovered from one host, *Euphorbia pulcherrima* (poinsettia), from one plant production facility. The lack of recovery of *P. aphanidermatum* from other plant species and production nurseries indicates that host specificity may be a factor and that it is not common in Georgia greenhouses. Moorman et al. (2002) also found *P. irregulare* and *P. aphanidermatum* to be responsible for the majority of *Pythium* infections of ornamental plants in Pennsylvania. They are also noted the association of *P. aphanidermatum* and poinsettia samples and questioned whether the association that may be due to the interconnectedness of poinsettia propagators, pathogen virulence specific to poinsettia, or that cultural conditions of poinsettia production may selectively favor *P. aphanidermatum* over other *Pythium* species.

The second most abundant *Pythium* species recovered in this study was *P. myriotylum*, which was recovered from six herbaceous perennial and bedding plant species. This species has been found to be pathogenic on ornamental plants including caladium (Ridings and Hartman 1976) and *Antirrhinum majus* (snapdragon) (Gill 1970). However, its pathogenicity can vary with isolate (Csinos and Hendrix 1978). Pathogenicity has not been proven for any of the hosts from which this species was isolated in this study. Additional pathogenicity studies are needed to determine if *P. myriotylum* is of concern for the ornamental plant industry.

Several *Pythium* species, including *P. diclinum* and *P. monospermum*, were recovered that are not often associated with ornamental plants. Although the species were recovered from

symptomatic plants, the pathogenicity of these isolates is questionable. In contrast, *Pythium undulatum*, which was recovered from plant samples, as well as from rhododendron leaf baits floated in water sources in one production facility, has been reported to cause severe root disease on Noble fir (*Abies procera*) and Douglas fir (*Pseudotsuga menziesii*) in Ireland and Germany (Shafizadeh and Kavanagh 2005; Weber et al. 2004). Its pathogenicity on herbaceous ornamental host plants is unknown.

Pythium taxonomy and species identification based upon morphological characteristics can be difficult. Taxonomic changes further complicate species identification. For example, five isolates of *Pythium undulatum* were recovered in this study. However, based upon ITS sequence analysis and morphological descriptions, the isolates were initially identified as *Phytophthora undulata*, which was described as a new *Phytophthora* species by Dick (1989). *Phytophthora undulata* is currently considered a homotypic synonym of *P. undulatum* which was first described in 1909 (van der Plaats-Niterink 1981). This species was further reclassified as *Elongisporangium undulatum* (Uzuhashi et al. 2010); however, this designation is also currently considered a homotypic synonym according to MycoBank database (www.mycobank.org) (Crous et al. 2004).

The *Phytophthora* species recovered in this study are similar to those identified from other ornamental plant nurseries in multiple states (Donahoo and Lamour 2008; Hwang and Benson 2005; Leonberger et al. 2013; Olsen and Benson 2011; Olsen et al. 2013; Schwingle et al. 2007). *Phytophthora nicotianae* was the most common species recovered in this study. Others have identified *P. citricola* and *P. citrophthora* as the most abundant species in ornamental plant nurseries (Donahoo and Lamour 2008; Leonberger et al. 2013, Schwingle et al. 2007). This may be due to the type of samples collected. In those surveys, the majority of the samples consisted of

leaf lesions and samples were collected often as part of *Phytophthora ramorum* surveys (Stokstad 2004). Samples collected and plated in our survey were of roots and lower stems where *P. nicotianae* may be more prevalent.

The second most abundant *Phytophthora* species recovered in our survey was *P. pini*. This species was first described in 1925 (Leonian 1925); however, it was classified as *P. citricola* by Waterhouse (1963). Hong et al. (2011) formerly resurrected *P. pini* as a distinct species. *Phytophthora citricola* was known as a complex (Bhat and Browne 2007; Jung and Burgess 2009), and with the use of molecular markers several former *citricola* complex species have been described, including *P. multivora* (Scott et al. 2009), *P. plurivora* (Jung and Burgess 2009), *Phytophthora menzei* (Hong et al. 2009), and *P. pachypleura* (Henricot et al. 2014). In previous studies, isolates of *P. citricola*-complex were recovered from leaf lesions, roots, container substrates and recirculated irrigation and natural water sources (Bienapfl and Balci 2014; Donahoo and Lamour 2008; Hwang and Benson 2005; Leonberger et al. 2013; Olsen and Benson 2011; Olsen et al. 2013; Schwingle et al. 2007). In the present study, *P. pini* isolates were only recovered from rhododendron leaves as opposed to root and stem isolations that were the predominant sample source tissues. It is likely that *P. pini* and other *citricola*-complex species prefer an aerial environment rather than saturated rooting substrate environment and may have been detected in greater abundance if more leaf tissue samples were collected.

Of note in this study, is the identification of ten *Phytopythium* isolates recovered from diverse symptomatic plants from six production nurseries. *Phytopythium* is a relatively new taxonomic genus whose members were classified as clade K species of *Pythium*, and have more characteristics similar to *Phytophthora* than other *Pythium* species (Robideau et al. 2011). Pathogenicity of *Phytopythium* spp. isolates is also in question. Pathogenicity has not been

proven on the recovered *Phytophthium* spp. isolates. Pathogenicity may also vary among *Phytophthium* species. In recent studies, *Phytophthium helicoides* was found to be pathogenic to begonia in Virginia (Yang et al. 2013) and *P. litorale* was pathogenic to squash in Georgia (Parkunan and Ji 2013). Pathogenicity of *P. vexans* is questionable. Stephens and Powell (1982) determined that *P. vexans* caused only minor damping off on impatiens and little to no disease on a series of other annual flowering bedding plants and vegetable transplants. Isolates of *P. vexans* in North Carolina were determined to colonize roots of Fraser fir without inciting root rot symptoms or root death (Ivors et al. 2008). It was concluded that *P. vexans* survives saprophytically on Fraser fir roots.

The study is the first report of *Phytophthium chamaeophon*, *P. helicoides* and *P. litorale* recovered from ornamental plants in Georgia. Without confirmation through pathogenicity testing, the role of *Phytophthium* spp. in oomycete disease development is unknown at this time.

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Table 3.1. Oomycete species identified from symptomatic plant samples collected from ornamental plant production nurseries in Georgia in 2010 and 2011.

Plant genera collected	<i>Pythium</i> species and number of isolates*	<i>Phytophthora</i> species and number of isolates	<i>Phytopythium</i> species and number of isolates
<i>Begonia</i>	1- <i>Pythium</i> sp.		
<i>Buxus</i>	1- <i>Pythium</i> sp.		
<i>Calibrachoa</i>	1- <i>P. irregulare</i> 2- <i>P. myriotylum</i> 1- <i>P. zingiberis</i>		
<i>Camellia</i>	2- <i>Pythium</i> sp.		1- <i>P. chamaehyphon</i>
<i>Catharanthus</i>		5- <i>P. nicotianae</i>	
<i>Coriandrum</i>	1- <i>Pythium</i> sp.		
<i>Coreopsis</i>			1- <i>Phytopythium</i> sp.
<i>Cornus</i>	6- <i>Pythium</i> sp.		
<i>Cryptomeria</i>	1- <i>Pythium</i> sp.		
<i>Daphne</i>	3- <i>Pythium</i> sp.		
<i>Delosperma</i>	1- <i>Pythium</i> sp.		
<i>Dianthus</i>	1- <i>P. irregulare</i>		
<i>Euonymus</i>	1- <i>Pythium</i> sp.		
<i>Euphorbia</i> , <i>Poinsettia</i>	5- <i>P. aphanidermatum</i> 1- <i>P. myriotylum</i>		
<i>Gardenia</i>	1- <i>Pythium</i> sp.	3- <i>P. nicotianae</i>	1- <i>P. chamaehyphon</i>
<i>Geranium</i>	1- <i>P. cucurbitacearum</i>	1- <i>P. drechsleri</i>	
<i>Hedera</i>	1- <i>Pythium</i> sp.		
<i>Helichrysum</i>	1- <i>P. irregulare</i>		
<i>Hydrangea</i>	1- <i>P. irregulare</i>	2- <i>P. cinnamomi</i> 1- <i>P. nicotianae</i>	1- <i>P. helicoides</i>
<i>Iberis</i>	1- <i>P. irregulare</i> 1- <i>P. myriotylum</i>		
<i>Impatiens</i>	1- <i>P. irregulare</i> 1- <i>P. myriotylum</i> 1- <i>Pythium</i> sp.		
<i>Juniperus</i>	2- <i>P. undulatum</i> 1- <i>Pythium</i> sp.		
<i>Lamium</i>	1- <i>P. acanthophoron</i>	1- <i>P. cryptogea</i>	
<i>Lavandula</i>	1- <i>Pythium</i> sp.		
<i>Leucanthemum</i>	1- <i>P. irregulare</i>		1- <i>P. helicoides</i>
<i>Lysimachia</i>	1- <i>Pythium</i> sp.		

<i>Matthiola</i>	2- <i>Pythium</i> sp.		
<i>Nandina</i>	1- <i>Pythium</i> sp.		
<i>Nemesia</i>	1- <i>P. irregulare</i>		
<i>Pachysandra</i>	1- <i>Pythium</i> sp.		
<i>Pelargonium</i>	1- <i>P. myriotylum</i>		
<i>Phlox</i>	2- <i>P. irregulare</i>	1- <i>Phytophthora</i> sp.	
	1- <i>Pythium</i> sp.		
<i>Plectranthus</i>	1- <i>P. myriotylum</i>		
<i>Rhododendron</i>	3- <i>P. monospermum</i>	1- <i>P. citrophthora</i>	1- <i>P. vexans</i>
	1- <i>P. undulatum</i>	7- <i>P. pini</i>	
	9- <i>Pythium</i> sp.	1- <i>Phytophthora</i> sp.	
<i>Rosa</i>	1- <i>Pythium</i> sp.		
<i>Rosmarinus</i>	3- <i>Pythium</i> sp.		1- <i>P. litorale</i>
<i>Sedum</i>	1- <i>P. undulatum</i>	1- <i>P. palmivora</i>	1- <i>P. vexans</i>
	1- <i>P. diclinum</i>	1- <i>P. nicotianae</i>	
<i>Senecio</i>	1- <i>P. diclinum</i>		
<i>Tagetes</i>			1- <i>P. helicoides</i>
<i>Thymus</i>	1- <i>Pythium</i> sp.		1- <i>P. helicoides</i>
<i>Veronica</i>	1- <i>Pythium</i> sp.	1- <i>Phytophthora</i> sp.	
<i>Vinca</i>	1- <i>Pythium</i> sp.		
<i>Pond/Stream Water</i>	1- <i>P. diclinum</i>		
	2- <i>P. undulatum</i>		
Total No. Isolates	81	26	10

* Oomycete identification was based upon morphological characteristics and rDNA ITS sequencing. Numbers preceding the species designation are the number of isolates recovered from that host.

Table 3.2. Reference *Pythium*, *Phytophthora*, and *Phytopythium* species used for ITS12 sequence comparison.

<i>Oomycete species</i> ¹	GenBank Accession no. ²
<u><i>Pythium</i> species</u>	
<i>P. acanthophoron</i>	AF216652
<i>P. aphanidermatum</i>	HM008882
<i>P. cucurbitacearum</i>	HQ237483
<i>P. diclinum</i>	GU233301
<i>P. irregulare</i>	AF382820
	GQ410398
	JN630483
	JN630488
<i>P. monospermum</i>	AY598621
	JN630508
<i>P. myriotylum</i>	HQ237488
<i>P. undulatum</i>	EU240049
<i>P. zingiberis</i>	HQ643973
<u><i>Phytophthora</i> species</u>	
<i>P. cinnamomi</i>	GU799638
	HM041805
<i>P. citrophthora</i>	JN605987
<i>P. cryptogea</i>	FJ801967
<i>P. drechsleri</i>	HQ261553
<i>P. nicotianae</i>	JF792541
<i>P. palmivora</i>	FJ801962
<i>P. pini</i>	GQ324989
<u><i>Phytopythium</i> species</u>	
<i>P. chamaehyphon</i>	GU266220
<i>P. helicoides</i>	HQ643383
	GU133575
	HQ643383
<i>P. liorale</i>	NZFS3441
<i>P. vexans</i>	GU931701

¹Species were identified by direct sequencing of the ITS 1 and 2 rDNA region using ITS1 and ITS4 universal primers and a BLAST search of the sequence in GenBank

² GenBank accession number of representative isolates that provided a 99-100% similarity match.

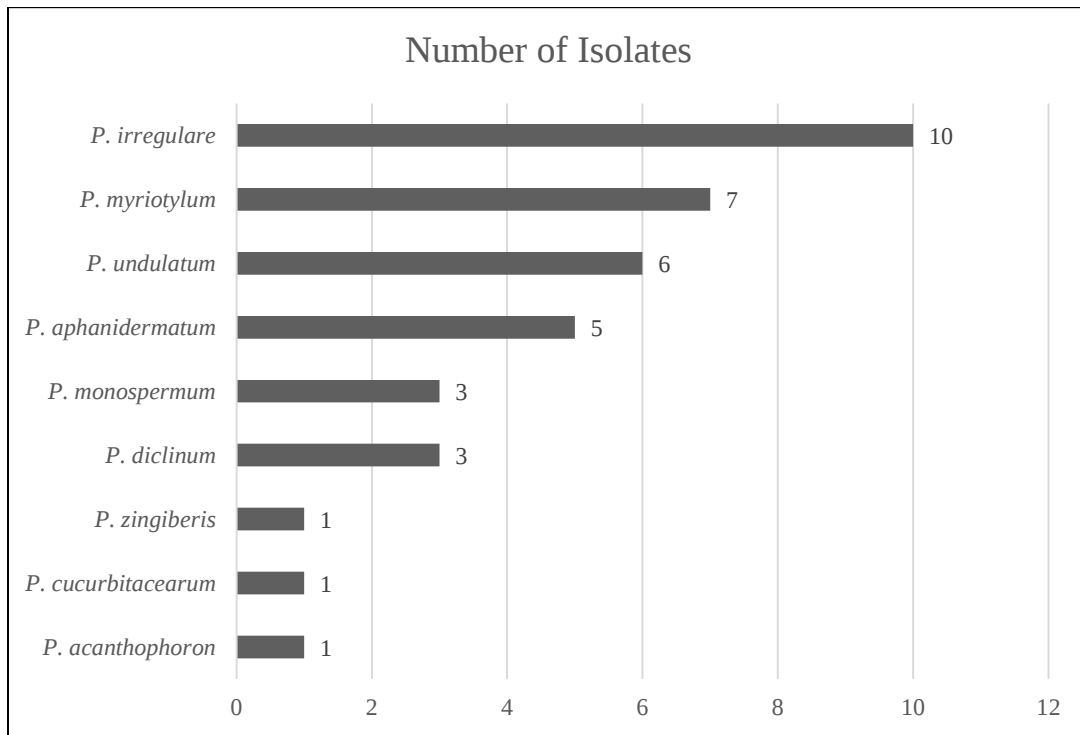


Figure 3.1: Number of isolates and known species identity of *Pythium* isolates recovered from commercial nurseries and greenhouses in Georgia. An additional 44 isolates were classified as either unable to identify to species or unknown species identity based upon ITS rDNA sequencing.

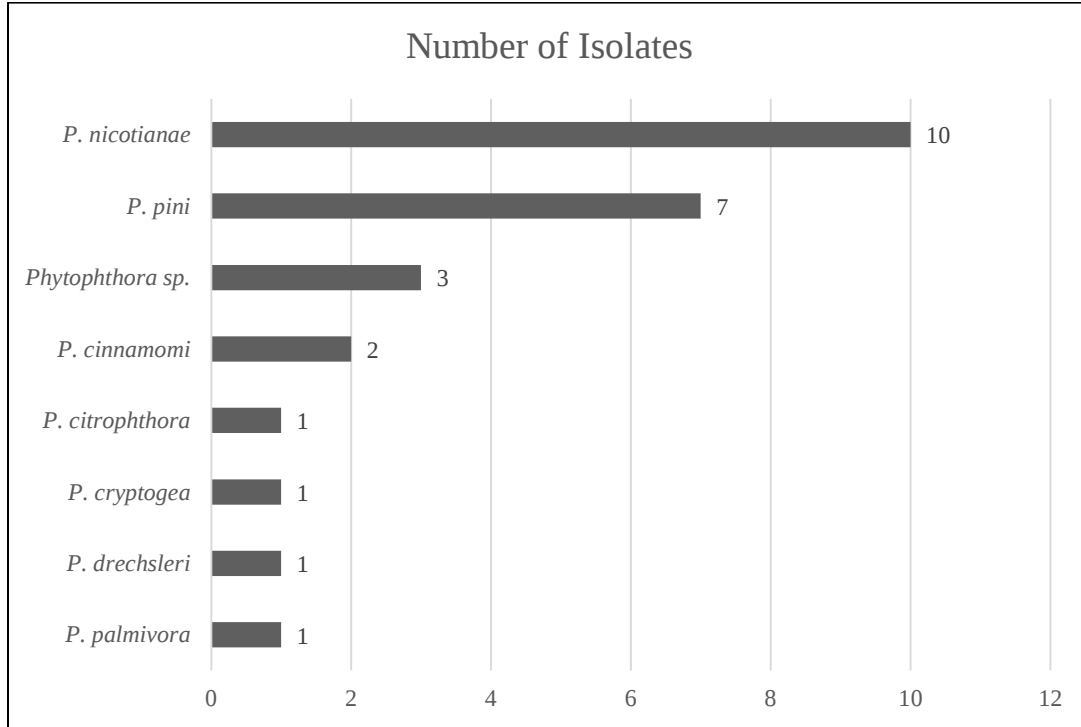


Figure 3.2: Number of isolates and species identity of *Phytophthora* isolates recovered from commercial nurseries and greenhouses in Georgia.

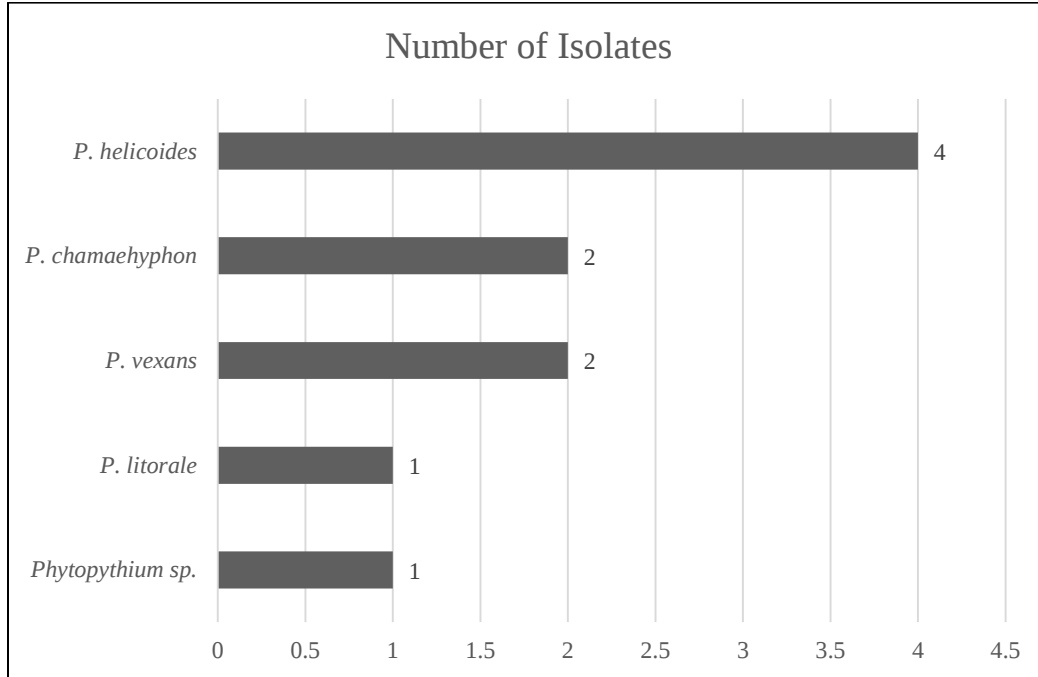


Figure 3.3: Number of isolates and species identity of *Phytophthium* isolates recovered from commercial nurseries and greenhouses in Georgia.

CHAPTER 4

MEFENOXAM SENSITIVITY OF OOMYCETE ROOT PATHOGENS RECOVERED FROM ORNAMENTAL PLANTS IN GEORGIA¹

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ABSTRACT

Mefenoxam is a fungicide widely used in the greenhouse and ornamental industry to control disease caused by *Pythium* and *Phytophthora* species. Resistance has been identified. Isolates collected from 16 ornamental plant production nurseries were screened in vitro against 100 µg a.i./ml mefenoxam. Isolates that grew > 50% of the control non-amended plates were considered insensitive. The 117 isolates evaluated included seven species of *Phytophthora*, nine species of *Pythium*, and four species of *Phytopythium*. Mefenoxam insensitivity was identified in 45.3% of the 117 isolates corresponding to 7.7%, 54.3%, and 70% of the total number of *Phytophthora*, *Pythium*, and *Phytopythium* spp. isolates, respectively.

INTRODUCTION

Root and crown rot diseases caused by species of the oomycete pathogens cause considerable damage to ornamental plants. Disease management relies on exclusion of the pathogen, following strict sanitation practices, water and fertilizer management, and judicious use fungicides (Daughtrey et al. 1995). Mefenoxam (metalaxyl-M) is often used to manage oomycete root pathogens. In 2009, approximately 19% of the ornamental plant producers from six states (CA, FL, MI, OR, PA, TX) reported applying mefenoxam for root disease management and ranked mefenoxam third in use behind chlorothalonil and thiophanate methyl fungicides (USDA 2009).

Mefenoxam fungicide insensitivity has been noted in several states in populations of *Pythium* and *Phytophthora* species causing root and crown rots of ornamental plants. In Pennsylvania, 32.5% of the 120 isolates of *Pythium* spp. recovered from infected plants were insensitive to mefenoxam (Moorman et al. 2002). Eleven species of *Pythium* were identified

from the 120 isolates. The most common species were *P. irregulare* and *P. aphanidermatum* of which 36.8% and 37.5% of these species, respectively, were insensitive to mefenoxam. In North Carolina, three species of *Phytophthora* (*P. nicotianae*, *P. cryptogea*, and *P. palmivora*) were recovered as the predominant species infecting floriculture crops (Hwang and Benson 2005). Although, all isolates of *P. palmivora* were still sensitive to mefenoxam, 100% of the *P. cryptogea* and 21% of *P. nicotianae* isolates were insensitive. In a more recent North Carolina study, *P. nicotianae*, *P. drechsleri*, *P. cryptogea*, and *P. tropicalis* were the most commonly recovered *Phytophthora* species from floriculture crops, of which 66% of these *Phytophthora* isolates were insensitive or intermediate in resistance to mefenoxam (Olson and Benson 2011). These studies would suggest that mefenoxam insensitivity is widespread within floriculture production. However, in another study involving six states in the southeastern USA concluded that across six states and 488 isolates that only 6% of the *Phytophthora* isolates were insensitive to mefenoxam (Olson et al., 2013). The viability of mefenoxam as a valuable tool in managing *Pythium* and *Phytophthora* root diseases is of great concern. The objective of this study was to evaluate soilborne oomycete pathogens recovered from ornamental plants in Georgia for mefenoxam sensitivity.

MATERIALS AND METHODS

Isolates Evaluated. A total of 117 oomycete root pathogen isolates recovered from 42 symptomatic ornamental plant taxa collected in 2010 and 2011 from 16 commercial production nurseries (nine specializing in woody ornamental plants and seven specializing in herbaceous plants) and three water sources within one production facility were used in this study. Recovery and identification of the oomycete isolates was described previously (Chapter 3).

The isolates evaluated contained both identified and unidentified species of *Pythium*, *Phytophthora*, and *Phytopythium*. The *Pythium* isolates (81 total) consisted of 37 isolates of known species including *P. acanthophoron*, *P. aphanidermatum*, *P. cucurbitacearum*, *P. diclinum*, *P. irregulare*, *P. monospermum*, *P. myriotylum*, *P. undulatum*, *P. zingiberis* and 44 unidentified *Pythium* sp. isolates. The *Phytophthora* isolates (26 total) included 23 isolates of known species including *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae*, *P. palmivora*, and *P. pini* and three unidentified *Phytophthora* sp. isolates. The *Phytopythium* isolates (10 isolates) included *P. chamaehyphon*, *P. helicoides*, *P. litorale*, and *P. vexans* and one isolate of an unidentified *Phytopythium* sp.

Fungicide Sensitivity. Sensitivity to mefenoxam (Subdue Maxx®; Syngenta Crop Protection, Greensboro, NC) was screened in vitro by amending V8 juice agar (15 g Bacto agar, 50 ml clarified V8 juice, and 950 ml deionized water) with 100 µg a.i./ml of mefenoxam. Subdue Maxx (22% mefenoxam) was diluted in sterile deionized water and distributed into molten agar prior to dispensing into 60-mm plastic petri plates. Non-amended V8 juice agar plates were used as a control. Agar plugs (7-mm-diameter) were cut from the leading edge of a 3-to 4-day-old isolate culture and inverted onto the center of mefenoxam-amended and non-amended plates. Two non-amended and two mefenoxam-amended plates for each isolate per trial were incubated at 22°C in the dark for 24 to 72 h depending upon isolate growth rate. Each isolate was evaluated in at least two trials. Plates were evaluated macroscopically and mycelial growth was measured from the edge of the plug to the edge of the colony along two perpendicular radii per plate. For each isolate, relative growth was determined as the percentage of mycelial growth of the mefenoxam-amended agar plates compared to the growth on non-amended medium. Isolates with growth $\geq 50\%$ of the non-amended medium control ($EC_{50} > 100$ ug a.i./ml) were considered

insensitive (I). Isolates that grew < 50% as compared to the control were considered to be sensitive (S) (Moorman et al. 2002).

Isolates determined to be insensitive at 100 µg a.i./ml mefenoxam were further evaluated at 500 and 1000 µg a.i./ml mefenoxam. The higher concentration mefenoxam-amended V8 juice agar medium was prepared and the isolate evaluations were conducted as previously described above.

RESULTS

Including all oomycete isolates in this study, 45.3% were mefenoxam insensitive (Table 1). Insensitivity was identified in 7.7% of the *Phytophthora* spp., 54.3% of the *Pythium* spp. and 70% of the *Phytophthium* spp. isolates (Table 4.1). A total of 57 and 60 oomycete isolates were recovered from production nurseries that specialized in herbaceous and woody ornamentals, of which 19 and 35 isolates, respectively, were identified as mefenoxam insensitive.

Mefenoxam insensitivity was identified in 54.3% of the *Pythium* isolates evaluated. Of the 37 isolates identified to species, six were found to be mefenoxam insensitive. Four of these isolates recovered from symptomatic plant samples from three different nurseries were identified as *P. undulatum* (Table 4.2). The remaining two mefenoxam-sensitive isolates were baited from water sources within one nursery using *Rhododendron maximum* leaves. It is likely that the water-derived isolates have never been previously exposed to mefenoxam fungicide and therefore have remained sensitive to it. Only one *P. irregulare* and one *P. myriotylum* isolates were mefenoxam-insensitive (Table 4.2). The majority of the insensitivity was found within the unidentified *Pythium* sp. Eight of the 44 unidentified *Pythium* sp. isolates (18.2%) were sensitive to mefenoxam and six of these isolates originated from one nursery (Table 4.2).

Only two of the 26 *Phytophthora* spp. isolates were insensitive to mefenoxam. The isolates originated from two different nurseries and included the only *P. palmivora* isolate recovered from *Sedum telephium* and an unidentified *Phytophthora* sp. recovered from a *Rhododendron* hybrid (Table 4.3). The unidentified *Phytophthora* sp. isolate rDNA ITS sequence did not closely match a sequence in GenBank and may be a new species or hybrid. All *P. nicotianae* and *P. pini* isolates, which accounted for over 65% of the total number of *Phytophthora* isolates recovered, were sensitive to mefenoxam.

Seven of the 10 of the *Pythopythium* isolates recovered from diverse crops and nurseries were insensitive to mefenoxam. The three sensitive isolates included two *P. chamaeophyon* and one *P. vexans* that were recovered from woody ornamental plant species from three different nurseries (Table 4.4.).

Twelve mefenoxam-insensitive isolates (at 100 µg a.i./ml) were also evaluated in vitro at mefenoxam concentrations of 500 µg a.i./ml and 1000 µg a.i./ml. Eight isolates (75%) were found to be insensitive at 500 µg a.i./ml (Table 4.5). Four isolates (*Pythium irregulare*, two *Pythium undulam*, and *Pythopythium helicoides*) continued to be insensitive at 1000 µg a.i./ml.

DISCUSSION

The seemingly high occurrence (45.3%) of mefenoxam-insensitive *Phytophthora*, *Pythium*, and *Pythopythium* isolates recovered in this study suggests that the usefulness of mefenoxam to manage oomycete root diseases is questionable. The percentage of insensitive isolates is similar to what has been identified in *Pythium* spp. and *Phytophthora* spp. isolates collected from ornamental production nurseries in other states (Hwang and Benson 2005; Hu et al. 2008; Moorman et al. 2002; Olsen and Benson 2011). A difference in this study is that the

percentage of mefenoxam-insensitive *Phytophthora* spp. isolates is considerably lower than reported in other studies (7.7% compared to 21% and 66% in North Carolina and 26.5% in Virginia) (Hu et al 2008; Hwang and Benson 2005; Olsen and Benson 2011). Perhaps if a larger number of *Phytophthora* spp. isolates were collected from additional samples, the percentage of mefenoxam insensitivity may be affected. The frequency of mefenoxam insensitivity for *Phytophthora* spp. recovered from 488 plant samples from six southeastern states was 9.8% when insensitive and intermediate isolates were combined (Olsen et al. 2013). If *P. ramorum* isolates are excluded from the previous data set because it is not currently found in ornamental plant production nurseries in the southeastern USA, then the incidence of mefenoxam insensitivity would increase to 11.3% for *Phytophthora* spp. commonly associated with ornamental plant nurseries, which is closer to the incidence percentage found in this study.

The highest percentage of mefenoxam-insensitivity in this study (70%) was identified in *Phytopythium* isolates. *Phytopythium* is a relatively new genus that was first described in 2010 with the type species, *Phytopythium sindhum* Lodhi, Shahzad & Lévesque (Bala et al. 2010). *Phytopythium* are morphologically and phylogenetically between *Pythium* and *Phytophthora* (Bala et al. 2010). Morphologically, *Phytopythium* sporangia are papillate and globose to ovoid in shape similar to *Phytophthora*; however, sporangia show internal proliferation, which is uncharacteristic of papillate sporangia of *Phytophthora* species (de Cock et al. 2015). Zoospore discharge is *Pythium*-like, with the sporangium forming a discharge tube and vesicle from which biflagellate zoospores are discharged (Bala et al. 2010). In addition, most species have smooth, thick-walled oospores and lobed antheridia. Species of *Phytopythium* comprise what was formerly known as *Pythium* clade K species according to molecular phylogeny presented by

Lévesque and de Cock (2004) and include *P. vexans*, *P. chamaehyphon*, *P. helicoides*, *P. litorale*, and *P. mortanum*.

Plant pathogenicity of several *Phytophthora* spp., including *P. helicoides* and *P. litorale* has been confirmed (Parkunan and Ji 2013; Yang et al. 2013). However, Ivors et al. (2008) failed to incite disease on Fraser fir (*Abies fraseri*) following inoculation with *P. vexans*. Pathogenicity of *Phytophthora* spp. isolates recovered here on ornamental plants still needs to be confirmed. Several *Phytophthora* spp. isolates were recovered that were insensitive to mefenoxam at a 1000 µg a.i./ml in vitro concentration. Mefenoxam insensitivity of *P. vexans* has been reported previously (Kato et al. 1990). Fungicides do not affect all pathogen species equally. It is possible that what has been identified as mefenoxam insensitivity may actually be due to the product being ineffective on these species rather than as a result of fungicide resistance development from over-exposure.

Mefenoxam insensitivity was identified in 54.3% of the *Pythium* spp. isolates in this study, but only in 8% of the isolates identified to species. Isolates of *P. undulatum* comprised the majority of the mefenoxam-insensitive isolates. This species was formerly classified as *Phytophthora undulata* (Dick 1989) and isolates of this species were also found to be mefenoxam-insensitive (Kato et al. 1990; Olsen et al. 2013). A large number of unidentified *Pythium* sp. isolates (44) were evaluated in this study. These isolates were identified as *Pythium* sp. based upon morphological characteristics including sporangial size and shape, zoospore production and discharge, antheridia and oogonia development, and hyphal growth characteristics; however, either their rDNA ITS sequence did not match closely to known sequences in GenBank or the isolates were lost or contaminated and a clean rDNA sequence was not able to be obtained. It is plausible that some of the unidentifiable *Pythium* sp. isolates, of

which the majority were mefenoxam-insensitive, may be new species. Some of the isolates may also be saprobic and not plant pathogenic. Until pathogenicity is proven, the high occurrence of mefenoxam insensitivity within *Pythium* isolates, may be misleading.

Of the 69 oomycete isolates with known species identity, 12 (19.4%) were mefenoxam-insensitive. This study proves that mefenoxam insensitivity does exist in Georgia ornamental plant production nurseries. Most, but not all, of the insensitive isolates were recovered from nurseries that routinely use mefenoxam (Subdue Maxx®) to manage root disease. Several insensitive isolates were recovered from nurseries that do not have a history of mefenoxam use. Ornamental plant nurseries often purchase propagated material that originates from other, often distant, nurseries. It is possible that mefenoxam-insensitive populations could be introduced on propagative material. Fungicide-insensitive isolates do not appear to lose competitive advantage (Café-Filho and Ristaino 2008; Chapara et al. 2011; Hu et al. 2008) and, therefore, may become established in new locations.

This and previously reported studies prove that mefenoxam insensitivity exists within ornamental plant production nurseries at varying levels, which may be a consequence of production practices and fungicide use history of individual plant nurseries. This study is the first report of mefenoxam insensitivity within *Pythium* and *Phytophythium* spp. from ornamental plants in Georgia. Mefenoxam insensitivity had been reported in *Phytophthora* isolates included in a regional study by Olsen et al. (2013). However, the relative amount of mefenoxam insensitivity attributed to *Phytophthora* isolates originating within Georgia as compared to isolates from the other southeastern states included in the study was not stated. The incidence of mefenoxam insensitivity should concern growers and instill the importance of implementing strict sanitation and other cultural disease management practices to reduce the reliance on fungicides.

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Table 4.1. Number of isolates and percentage of mefenoxam insensitivity identified within 117 oomycete pathogen isolates recovered from 16 ornamental plant production facilities in Georgia in 2010 and 2011.

Nursery ¹	Total # Samples ²	Oomycete spp.			<i>Phytophthora</i> spp.			<i>Pythium</i> spp.			<i>Phytopythium</i> spp.		
		Total ³	# I ⁴	% I ⁵	Total	# I	% I	Total	# I	% I	Total	# I	% I
A	10	7	1	14.3				7	1	14.3			
B	10	7	4	57.1	1	0	0	6	4	66.7			
C	28	20	8	40.0	3	0	0	14	5	35.7	3	3	100
D	8	7	1	14.3				7	1	14.3			
E	8	6	4	66.7	2	1	50.0	3	2	66.7	1	1	100
F	5	5	0	0				5	0	0			
G	5	5	0	0	5	0	0						
H	7	5	5	100				3	3	100	2	2	100
I	7	5	4	80.0	1	1	100	3	2	66.7	1	0	0
J	4	3	3	100				3	3	100			
K	18	8	5	62.5	2	0	0	4	4	100	2	1	50
L	6	1	0	0				1	0	0			
M	10	7	1	14.3	5	0	0	1	1	100	1	0	0
N	3	1	1	100				1	1	100			
O	9	6	6	100				6	6	100			
P	30	24	11	45.8	7	0	0	17	11	64.7			
Total	168	117	53	45.3 ⁶	26	2	7.7 ⁶	81	44	54.3 ⁶	10	7	70.0 ⁶

¹ Nursery designation for 16 ornamental plant production nurseries in Georgia. Nurseries A-G specialize in herbaceous ornamental taxa and H-P specialize in woody ornamental taxa.

² Total number of samples that were collected from the nursery showing disease symptoms of wilting, chlorosis, root decay, leaf lesions or plant death.

³ Total number of isolates recovered on V8-PARP medium from plant samples separated displayed as total number of Oomycete pathogens (*Pythium*, *Phytophthora*, or *Phytopythium* spp. combined) and number of isolates of *Phytophthora*, *Pythium* and *Phytopythium* separately.

⁴ The number of isolates that were determined to be insensitive at 100 µg a.i./ml mefenoxam fungicide.

⁵ Percentage of isolates that were insensitive to mefenoxam fungicide out of the total number of oomycete pathogen isolates recovered.

⁶ Column is not additive. It is the percentage of mefenoxam –insensitive isolates out of the total number of oomycete isolates recovered.

Table 4.2. *Pythium* species recovered from ornamental plant samples collected in Georgia in 2010 and 2011 and their sensitivity to mefenoxam fungicide.

<i>Pythium</i> Isolate ¹	No. Isolates ²	Sensitivity Class ³	Nursery ⁴	Host Taxa ⁵
<i>P. acanthophoron</i> (1)	1	S	I	<i>Lamium maculatum</i>
<i>P. aphanidermatum</i> (5)	5	S	F	<i>Euphorbia pulcherrima</i>
<i>P. cucurbitacearum</i> (1)	1	S	C	<i>Geranium sanguineum</i>
<i>P. diclinum</i> (3)	1	S	C	<i>Senecio cineraria</i>
	1	S	E	<i>Sedum telephium</i>
	1	S	P	Irrigation pond
<i>P. irregulare</i> (10)	1	S	B	<i>Phlox subulata</i>
	1	S	B	<i>Iberis sempervirens</i>
	1	S	C	<i>Dianthus gratianopolitanus</i>
	1	S	C	<i>Helichrysum thianschanicum</i>
	1	S	C	<i>Leucanthemum</i> × <i>superbum</i>
	1	I	C	<i>Nemesia fruticans</i>
	1	S	C	<i>Phlox subulata</i>
	1	S	D	<i>Calibrachoa</i> hybrid
	1	S	D	<i>Impatiens hawkerii</i>
	1	S	L	<i>Hydrangea arborescens</i>
<i>P. monospermum</i> (3)	1	S	P	<i>Rhododendron</i> ‘ <i>chiroides</i> ’
	2	S	P	<i>Rhododendron maximum</i>
<i>P. myriotylum</i> (7)	1	S	B	<i>Iberis sempervirens</i>
	1	S	D	<i>Pelargonium germaun</i>
	2	S	D	<i>Calibrachoa</i> hybrid
	1	S	D	<i>Impatiens hawkerii</i>
	1	I	D	<i>Plectranthus scutellarioides</i>
	1	S	F	<i>Euphorbia pulcherrima</i>
<i>P. undulatum</i> (6)	1	I	E	<i>Sedum telephium</i>
	2	I	K	<i>Juniperus conferta</i>
	1	I	P	<i>Rhododendron catawbiense</i>
	2	S	P	Irrigation Pond/Stream
<i>P. zingiberis</i> (1)	1	S	D	<i>Calibrachoa</i> hybrid
<i>Pythium</i> sp. (44)	1	I	A	<i>Coriandrum sativum</i>
	2	S	A	<i>Matthiola incana</i>
	2	S	A	<i>Rosmarinus officianalis</i>
	1	S	A	<i>Impatiens hawkerii</i>
	1	S	A	<i>Thymus vulgaris</i>
	1	I	C	<i>Begonia coccinea</i> hydrid
	1	I	C	<i>Euonymus fortunei</i>

1	I	C	<i>Hedera helix</i>
1	I	C	<i>Lavandula angustifolia</i>
1	I	C	<i>Lysimachia nummularia</i>
1	I	C	<i>Pachysandra</i>
1	S	C	<i>Phlox paniculata</i>
1	I	C	<i>Rosmarinus officinalis</i>
1	S	C	<i>Veronica spicata</i>
1	I	C	<i>Vinca minor</i>
1	I	E	<i>Delosperma cooperi</i>
1	I	H	<i>Cryptomeria japonica</i>
1	I	H	<i>Nandina domestica</i>
1	I	H	<i>Rosa hybrid</i>
2	I	I	<i>Camellia japonica</i>
3	I	J	<i>Daphne odora</i>
1	I	K	<i>Buxus sempervirens</i>
1	I	K	<i>Juniperus conferta</i>
1	I	M	<i>Gardenia jasminoides</i>
6	I	O	<i>Cornus florida</i>
5	I	P	<i>Rhododendron catawbiense</i>
4	I	P	<i>Rhododendron maximum</i>

¹ Isolate species was identified based upon rDNA sequencing of the ITS1 and ITS2 regions and conducting a BLAST search in GenBank. The number in parentheses is the total number of isolates of that species that was recovered from symptomatic ornamental plant samples or water source.

² Number of isolates recovered from the pathogen-host combination

³ Sensitivity class is based on mycelial growth of the isolate on 100 µg a.i./ml of mefenoxam (Subdue Maxx, Syngenta, Greensboro, NC) amended V8 juice agar medium compared to non-amended V8 juice agar medium. Isolates that grew <50 % compared to non-amended medium were classified as sensitive (S). Isolates that grew ≥ 50% of the non-amended medium were classified as insensitive (I). All isolates were screened in two separate trials.

⁴ Designation of the ornamental plant production nurseries where samples were collected. Nurseries A-G specialize in herbaceous floricultural crops; nurseries H-P specialize in woody ornamental crops.

⁵ Host from which the isolate was recovered. All plants were symptomatic and displayed wilting, chlorosis, root decay, foliage blighting, or plant death. Three isolates were recovered from *Rhododendron maximum* leaf baits that were floated in mesh bags for 7 days.

Table 4.3. *Phytophthora* species recovered from ornamental plant samples collected in Georgia in 2010 and 2011 and their sensitivity to mefenoxom fungicide.

<i>Phytophthora</i> Isolate ¹	No. Isolates ²	Sensitivity Class ³	Nursery ⁴	Host taxa ⁵
<i>P. cinnamomi</i> (2)	2	S	M	<i>Hydrangea arborescens</i>
<i>P. citrophthora</i> (1)	1	S	K	<i>Rhododendron catawbiense</i>
<i>P. cryptogea</i> (1)	1	S	C	<i>Lamium maculatum</i>
<i>P. drechsleri</i> (1)	1	S	C	<i>Geranium sanguineum</i>
<i>P. nicotianae</i> (10)	1	S	E	<i>Sedum telephium</i>
	2	S	M	<i>Gardenia jasminoides</i>
	1	S	M	<i>Hydrangea macrophylla</i>
	5	S	G	<i>Catharanthus roseus</i>
	1	S	K	<i>Gardenia jasminoides</i>
<i>P. palmivora</i> (1)	1	I	E	<i>Sedum telephium</i>
<i>P. pini</i> (7)	6	S	P	<i>Rhododendron catawbiense</i>
	1	S	P	<i>Rhododendron</i> ‘Nova Zembla’
<i>Phytophthora</i> sp. (3)	1	S	B	<i>Veronica spicata</i>
	1	S	C	<i>Phlox subulata</i>
	1	I	I	<i>Rhododendron</i> hybrid (azalea)

¹ Isolate species was identified based upon rDNA sequencing of the ITS1 and ITS2 regions and conducting a BLAST search in GenBank. The number in parentheses is the total number of isolates of that species that was recovered from symptomatic ornamental plant samples or water source.

² Number of isolates recovered from the pathogen-host combination

³ Sensitivity class is based on mycelial growth of the isolate on 100 µg a.i./ml of mefenoxam (Subdue Maxx, Syngenta, Greensboro, NC) amended V8 juice agar medium compared to non-amended V8 juice agar medium. Isolates that grew <50 % compared to non-amended medium were classified as sensitive (S). Isolates that grew ≥ 50% of the non-amended medium were classified as insensitive (I). All isolates were screened in two separate trials.

⁴ Designation of the ornamental plant production nurseries where samples were collected. Nurseries B-G specialize in herbaceous floricultural crops; nurseries H-P specialize in woody ornamental crops.

⁵ Host from which the isolate was recovered. All plants were symptomatic and displayed wilting, chlorosis, root decay, foliage blighting, or plant death.

Table 4.4. *Phytophthium* species recovered from ornamental plant samples collected in Georgia in 2010 and 2011 and their sensitivity to mefenoxom fungicide.

<i>Phytophthium</i> Isolate ¹	No. Isolates ²	Sensitivity Class ³	Nursery ⁴	Host taxa ⁵
<i>P. chamaeophyon</i> (2)	1	S	K	<i>Camellia japonica</i>
	1	S	M	<i>Gardenia jasminoides</i>
<i>P. litorale</i> (1)	1	I	H	<i>Rosmarinus officinalis</i>
<i>P. helicoides</i> (4)	1	I	K	<i>Hydrangea macrophylla</i>
	1	I	C	<i>Thymus praecox</i>
	1	I	C	<i>Tagetes patula</i>
	1	I	C	<i>Leucanthemum</i> × <i>superbum</i>
<i>P. vexans</i> (2)	1	S	I	<i>Rhododendron</i> (Kurume hybrid)
	1	I	E	<i>Sedum telephium</i>
<i>Phytophthium</i> sp. (1)	1	I	H	<i>Coreopsis lanceolata</i>

¹ Isolate species was identified based upon rDNA sequencing of the ITS1 and ITS2 regions and conducting a BLAST search in GenBank. The number in parentheses is the total number of isolates of that species that was recovered from symptomatic ornamental plant samples or water source.

² Number of isolates recovered from the pathogen-host combination

³ Sensitivity class is based on mycelial growth of the isolate on 100 µg a.i./ml of mefenoxam (Subdue Maxx, Syngenta, Greensboro, NC) amended V8 juice agar medium compared to non-amended V8 juice agar medium. Isolates that grew <50 % compared to non-amended medium were classified as sensitive (S). Isolates that grew ≥ 50% of the non-amended medium were classified as insensitive (I). All isolates were screened in two separate trials.

⁴ Designation of the ornamental plant production nurseries where samples were collected. Nurseries C-G specialize in herbaceous floricultural crops; nurseries H-M specialize in woody ornamental crops.

⁵ Host from which the isolate was recovered. All plants were symptomatic and displayed wilting, chlorosis, root decay, foliage blighting, or plant death.

Table 4.5. Sensitivity of mefenoxam-insensitive isolates evaluated in vitro with 500 and 1000 µg a.i./ml mefenoxam concentrations.

Oomycete Species ¹	Host	Sensitivity Class ² at 500 µg a.i./ml	Sensitivity Class ² at 1000 µg a.i./ml
<i>Pythium irregulare</i>	<i>Nemesia fruticans</i>	I	I
<i>Pythium undulatum</i>	<i>Sedum telephium</i>	I	I
	<i>Rhododendron catawbiense</i>	I	I
	<i>Juniperus conferta</i>	S	S
	<i>Juniperus conferta</i>	S	S
<i>Phytophythium litorale</i>	<i>Rosmarinus officinalis</i>	S	S
<i>Phytophythium helicoides</i>	<i>Thymus praecox</i>	I	S
	<i>Tagetes patula</i>	I	S
	<i>Leucanthemum × superbum</i>	I	I
	<i>Hydrangea macrophylla</i>	I	S
<i>Phytophythium vexans</i>	<i>Sedum telephium</i>	I	S
<i>Phytophythium</i> sp.	<i>Coreopsis lanceolata</i>	I	S

¹ Isolate that was determined to be mefenoxam-insensitive at 100 µg a.i./ml of mefenoxam (Subdue Maxx, Syngenta, Greensboro, NC) amended V8 juice agar medium compared to non-amended V8 juice agar medium.

² Sensitivity class is based on mycelial growth of the isolate on 500 and 1000 µg a.i./ml of mefenoxam (Subdue Maxx) amended V8 juice agar medium compared to non-amended V8 juice agar medium. Isolates growing <50 % compared to non-amended medium were classified as sensitive (S). Isolates that grew ≥ 50% of the non-amended medium were classified as insensitive (I). All isolates were screened in two separate trials.

CHAPTER 5

CONCLUSION

A total of 117 oomycete isolates collected from 16 ornamental plant production nurseries were identified including nine species of *Pythium*, seven species of *Phytophthora* and four species of *Phytopythium*. Mefenoxam insensitivity was documented in 45.3% of all oomycete isolates. Of the *Pythium* isolates identified, *P. irregulare* was the predominant species. This was not unexpected since *P. irregulare* has been identified all around the world on over 200 host species (Farr et al. 2004). These pathogens are favored by abundant moisture which can be found in ornamental plant production nurseries. They are commonly dispersed via contaminated irrigation water in which zoospores infect new host plants. In this study, only one isolate (recovered from *Nemesia fruticans* ‘Bluebird’) out of 10 isolates was found to be insensitive to mefenoxam. In total, 54% of the *Pythium* spp. isolates had some degree of mefenoxam sensitivity. Not all of these isolates could be identified to species molecularly using rDNA ITS region sequencing. This suggests that some could be new species. Other isolates became contaminated with *Mortierella* spp. that could not be separated from the *Pythium* in culture and prevented the use of direct sequencing to aid in identification.

Phytopythium spp. isolates were identified in this study including *P. chamaehyphon*, *P. heliocolides*, *P. litoriole*, *P. vexans* and potentially a new species. A high percentage (70%) of the isolates recovered were insensitive to mefenoxam, which could be a concern for the ornamental

plant production industry in Georgia. Plant pathogenicity needs to be confirmed on these isolates.

The most prevalent *Phytophthora* spp. recovered from ornamental plant samples in this study was *P. nicotianae*. One of 10 *P. nicotianae* isolates insensitive to mefenoxam. Overall, 7.7% of the *Phytophthora* spp. identified were insensitive to mefenoxam, which is less than has been found on ornamental plants in other states (Hu et al 2008; Hwang and Benson 2005; Olsen et al. 2011).

Mefenoxam insensitivity does exist in the ornamental plant industry in the state of Georgia. This study is the first report of mefenoxam insensitivity within *Pythium* and *Phytophthora* spp. from ornamental plants in Georgia. Mefenoxam insensitivity had been reported in *Phytophthora* isolates included in a regional study by Olsen et al. (2013). However, the relative amount of mefenoxam insensitivity attributed to *Phytophthora* isolates originating within Georgia as compared to isolates from the other southeastern states included in the study was not stated.

More studies are needed to determine the extent of the mefenoxam insensitivity across Georgia and in additional nurseries. Pathogenicity tests need to be conducted on the mefenoxam-insensitive isolates, as well as the unknown species to determine if the presence of these isolates is of concern. There is some cause for concern with this and other studies identifying mefenoxam-insensitive oomycete pathogens across the United States. Mefenoxam is one of the few options available for oomycete root and stem rot disease management. This study reinforces the importance of growers following fungicide resistance management guidelines in order to preserve the efficacy of this product for as long as possible.

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