ABSTRACT

LOOKABAUGH, EMMA CHRISTINE. Understanding the Impact of Pythium Root Rot on Floriculture Crops in North Carolina. (Under the direction of Barbara Brewer Shew.)

Herbaceous ornamentals exhibiting symptoms of Pythium root rot were collected from 26 greenhouses in 21 counties in NC. Symptoms ranged from mild stunting to severe wilting, root rot, and death. Roots were assayed on selective media and 360 isolates of *Pythium* were recovered from 36 host species. Selected isolates were identified by sequencing of the ITS rDNA region. Fifteen species were identified, with *P. aphanidermatum*, *P. irregulare,* and *P. myriotylum* comprising 76% of the 289 isolates sequenced. Twelve greenhouses had more than one species present. To assay sensitivity to mefenoxam, all 360 isolates were grown in wells of 48-well micro-titer plates containing clarified V8 agar amended with 100 ppm mefenoxam. Colonization was scored after 24 to 48 h on a scale of 0 (no growth) to 5 (entire well colonized). Fifty-two percent of the isolates were resistant to mefenoxam (mean score ≥ 4). All 29 isolates of *P. myriotylum* were sensitive, whereas sensitivity varied among isolates of *P. aphanidermatum* and *P. irregulare*. Resistant and sensitive isolates of the same species were found within the same greenhouse. The aggressiveness of *P. aphanidermatum* and *P. irregulare* was evaluated on poinsettia, Gerbera daisy, and petunia. In greenhouse pathogenicity trials, *Pythium aphanidermatum* was more aggressive than *P. irregulare* on poinsettia and petunia. Sensitivity to mefenoxam was not related to aggressiveness. The results of this study will be used to evaluate the need and feasibility of offering mefenoxam resistance screening as a diagnostic service at the Plant Disease and Insect Clinic.

Understanding the Impact of Pythium Root Rot on Floriculture Crops in North Carolina

by
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BIOGRAPHY

Emma Christine Lookabaugh was born in Sumter, SC. As the daughter of an air force pilot, she had the opportunity to live all over the world. She spent her childhood on the beautiful island of Oahu, Hawaii. Growing up on an island, Emma developed a fascination with nature and a desire to understand the intricate processes that govern the natural world. She attended North Carolina State University, where she was introduced to the field of plant sciences. Emma took a summer job at the Plant Disease and Insect Clinic and fell in love with Plant Pathology and diagnostics. She graduated valedictorian, summa cum laude in May, 2010, with Bachelor of Science degrees in Biological Sciences and Plant Biology with a Minor in Genetics. Her time working in the PDIC led her to pursue a Master's degree in Plant Pathology under the direction of Dr. Barbara Shew.

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INTRODUCTION

The greenhouse and nursery industry ranks fourth in agricultural sales in North Carolina and brings in over $700 million dollars annually. Floriculture sales accounted for an estimated wholesale value of $250.5 million of the total, placing North Carolina fifth in floricultural production the United States in 2011 (NCDA, 2012). The major floricultural crops produced in North Carolina include garden chrysanthemum, poinsettia from cuttings, and geranium from cuttings. North Carolina produces more than four million pots of poinsettias annually, with an estimated wholesale value of over $17 million (NCDA, 2012). Given the importance of the industry and the diversity of crops grown, it is not surprising that commercially-produced ornamentals represent more than 20% of the samples submitted to the Plant Disease and Insect Clinic (PDIC) at NC State University. Root rots and other diseases caused by *Pythium* are the most frequently diagnosed problems from floriculture samples submitted to the PDIC (B. Shew, *unpublished*). However, the extent and severity of *Pythium* diseases in the State's floriculture industry is not well characterized.

Pythium spp. are very common soilborne plant pathogens that cause stem rots, root rots, and damping-off on many ornamental host species (Martin and Loper, 1999). *Pythium* belongs in the Kingdom *Straminipila* and the class *Oomycota*, which distinguishes them from true fungi (Kingdom *Eumycota*). Oomycetes are phylogenetically related to diatoms and brown algae. They contain cellulose and ß-gulcans in their cell walls, have coenocytic hyphae, produce biflagellate zoospores, with tinsel and whiplash flagella, and have diploid vegetative states. *Pythium* belongs to the same order as the genus *Phytophthora* (Peronosporales), whose members are heterotrophic, filamentous, multicellular organisms. The genus is highly diverse, comprising at least 106 species (Lévesque and de Cock, 2004; Schroeder et al., 2013).

In general, *Pythium* spp. are thought of as necrotrophic, opportunistic, non-specific plant pathogens with broad host ranges, but some species infect only a narrow range of plant hosts. For example, *P. aphanidermatum*, *P. ultimum*, and *P. irregulare* infect dozens of plant species, whereas *P. graminicola* and *P. arrehenomanes* are restricted to certain taxa (Poaceae) (Farr and Rossman, 2013). *Pythium* spp. are considered necrotrophic pioneer colonizers that attack young feeder roots and germinating seeds and seedlings. They cause pre- and post-emergence damping-off diseases that result in reductions in plant stand counts and transplant rates. On mature plants, *Pythium* infections in the roots result in reductions in root biomass, fine feeder roots, and root hairs, which causes stunting and yield reductions in the aboveground plant parts (Weller and Cook, 1986). Under wet conditions, *Pythium* spp. can also infect succulent fruits causing watery rots of melons, peppers, and cucumbers (cottony leak of cucurbits).

Some species of *Pythium* are aggressive plant pathogens, whereas others exist mainly as saprophytes or weak root pathogens. For example, *Pythium vexans* is readily isolated from Fraser fir roots. Ivors et al. (2008) demonstrated that isolates of *P. vexans* colonized the roots of Fraser fir, but the isolates failed to kill roots or incite root rot symptoms. They concluded that it is likely that *P. vexans* survives saprophytically on Fraser fir roots. In contrast, *Pythium undulatum* has been reported to cause severe root disease on Noble fir (*Abies* *procera*) in Ireland (Shafizadeh and Kavanagh, 2005). Some plant genera are known hosts to several species of *Pythium*, for example thirty species of *Pythium* have been identified on creeping bentgrass samples in North Carolina (Abad et al., 1994) and over thirty species of *Pythium* have been identified on wheat worldwide (Farr and Rossman, 2013). Multiple *Pythium* species can be present on one plant or in a particular site (Abad et al., 1994). Paulitz and Adams (2003) sampled soil and wheat roots from 80 wheat field sites and recovered anywhere from one to six *Pythium* species, which were found in 46 different species combinations. High species diversity could prove problematic from a management perspective because species have been shown to vary greatly in their aggressiveness on certain hosts (Shafizadeh and Kavanagh, 2005) or certain cultivars (Higginbotham et al., 2004). Furthermore, isolates within a given species can vary in aggressiveness (Abad et al., 1994). Species associated with certain common *Pythium*-incited diseases also vary. Pythium root dysfunction (PRD) on creeping bentgrass (*Agrostis palustris* Huds.) was first described by Hodges and Coleman in 1985 and they associated *P. aristosporum* and *P. arrhenomanes* as the primary causal agents (Hodges and Coleman, 1985). Since then, Feng and Dermoeden (1999) found *P. aristosporum* to be the most important causal agent of PRD in the mid-Atlantic region of the United States, whereas Kerns and Tredway (2008) found *P. volutum* to be the cause of PRD in the Southeastern United States. Additionally, *Pythium* species associated with Pythium root rot disease vary greatly. *P. aphanidermatum* has been shown to be particularly aggressive on poinsettia (*Euphorbia pulcherima*); however, *P. sylvaticum* and *P. ultimum* were more aggressive than *P. aphanidermatum* on chrysanthemum (*Chrysanthemum morifolium;* Bolton, 1978; Pettitt et al., 2011). Isolates of *P. irregulare* from bermudagrass were aggressive on *Poa trivialis*, but not on *Lolium perenne,* both of which are cool season turfgrass species used to overseed bermudagrass (Stiles et al. 2007). This indicates that *Pythium* species may survive from season to season on bermudagrass, and under favorable conditions, could cause disease on overseeded cool-season grasses. Host and cultivar susceptibility are important factors to consider when developing management strategies.

Species and isolates within *Pythium* vary in their response to environmental factors, including soil moisture, temperature, and soil pH (Martin and Loper, 1999). Typically, *Pythium*-caused diseases are favored by wet, cool, conditions, although some species are more commonly found in warmer conditions or drier conditions. Disease caused by *P. irregulare* tends to be severe at cool temperatures (20°C -24°C) (Sippell and Hall, 1982; Ben-Yephet and Nelson, 1999), whereas disease caused by *P. aphanidermatum* tends to be more severe at warmer conditions (above 27°C) (Littrell and McCarter, 1970). Hancock and Grimes (1990) documented seasonal variation in *Pythium* species prevalence on alfalfa, with *P. ultimum* being more prevalent in the summer and *P. irregulare* in the cooler spring. Conclusions about temperature influences on pathogen aggressiveness and disease severity should be made with caution because intraspecific variation also has been reported (Abad et al., 1994; Nelson and Craft, 1991; Hodges and Campbell, 1994; Kerns and Tredway, 2008). On alfalfa, *P. ultimum* and *P. irregulare* were often isolated together and their isolation was positively correlated with average rainfall, whereas *P. sylvaticum* was negatively correlated with rainfall and seemed to be associated with higher temperatures and drier conditions

(Larkin et al., 1995). Soil moisture fluctuations also affect the type of reproductive structures produced; for example, high soil moisture favors oospore production in *P. ultimum*, whereas low moisture soils favor sporangium development (Bainbridge, 1970). In addition to soil temperature and moisture, soil nutrients have been shown to impact aggressiveness and disease severity (Moorman, 1986; Gladstone and Moorman, 1989; Grunwald et al., 2000). Morphological characteristics used to differentiate *Pythium* spp. include size and shape of sporangia, formation of hyphal swellings, size of the oogonium, the extent to which the oospore fills the oogonium, oogonium ornamentation, and the origin, mode of attachment, and number of antheridia per oogonium. Identification of *Pythium* to species based on morphology can be difficult, laborious, and requires a great deal of skill. Most species of *Pythium* are homothallic, but some species are heterothallic (*P. sylvaticum*, *P. splendens*, and *P. catenulatum*, *P. macrosporum*, *P. intermedium*, *P. heterothallicum*, and *P. splendens)* and require both mating types for production of sexual structures and thus for species identification (Hendrix and Campbell, 1973; Lévesque and de Cock, 2004). Making identifications based on morphology without sexual structures is difficult or impossible. Commonly used media such as cornmeal agar and potato dextrose agar may not be conducive to the mating reaction and the production of sexual structures used to identify species (Martin, 1992). Media containing hemp seed extract and sterols can be used to stimulate production of sexual structures and the mating reaction (Martin, 1992). Grass blade cultures may be used to produce sporangia, antheridia, oogonia and oospores for identification purposes (Abad et al., 1995). Because of the skill required to successfully identify and differentiate species based on morphology, most diagnostic clinics, including the PDIC, do not routinely identify *Pythium* to species (Moorman et al., 2002).

Recently, researchers have developed molecular tools to identify and characterize *Pythium* species and isolates, including isozyme analysis (Barr, 1998), nucleotide sequence of mitochondrial DNA (Martin, 2000), internal transcribed spacer (ITS) sequences (Moorman et al., 2002), restriction fragment length polymorphisms (RFLPs) of genomic and mitochondrial DNA (Harvey et al., 2000; 2001), amplified fragment length polymorphism (ALFP) fingerprinting (Garzon et al., 2005), and SSR or microsatellite markers (Lee and Moorman, 2008; Lee et al., 2010). Most molecular studies with *Pythium* species have focused on the ITS sequence for species differentiation because ITS regions within *Pythium* spp. are conserved within a species but variable between species (Chen, 1992; Chen et al., 1992; Lévesque and de Cock, 2004; Matsumoto et al., 1999). The ITS region in oomycetes varies from 750-1050 bp as compared to 300-700 bp in Eumycota, which provides more characters for analysis. Lévesque and de Cock (2004) analyzed the ITS region of more than 100 *Pythium* species and the large ribosomal subunit for 60% of them to investigate diversity in the genus. Their results showed that *Pythium* is highly heterogeneous. They placed species into 11 distinct clades labeled A to K. *Pythium* species with filamentous or globose sporangia formed two monophyletic groups. Heterothallism and oogonium ornamentation were clearly polyphyletic. Unfortunately, there are cases in which ITS sequences for different species are nearly identical, making identification difficult. Some species (*P. irregulare* and *P. vexans*) form species complexes and morphological differentiation within groups has been inconclusive due to overlapping characteristics or insignificant differences between putative species.

While many molecular tools are very sensitive and capable of separating many species of *Pythium*, they are not designed for quantitative identification directly from soil or plant samples. Traditional methods of studying population dynamics of *Pythium* species involve dilution plating onto selective media followed by observation of morphological characteristics to identify individual isolates to species (Conway, 1985; Ali-Shtayeh et al., 1986). The results of dilution plating may not provide an adequate representation of the species found in soil or plants because the process does not discriminate against nonpathogenic species, while some slow-growing pathogenic species may be underrepresented or overgrown by faster growing species (Paulitz and Adams, 2003). In efforts to solve this problem, Schroeder et al. designed species-specific primers for the detection and quantification of nine *Pythium* species from soils with real-time PCR (Schroeder et. al., 2006). Their real-time assay provided a rapid and accurate means of identifying and quantifying *Pythium* species from soil.

Another trait that varies among species and isolates of *Pythium* is sensitivity to common fungicides, including mefenoxam and metalaxyl (Moorman et al. 2002; Moorman and Kim, 2004; Schroeder et al., 2013). Resistance to metalaxyl was first reported in *Pythium* from turf in 1984, after only three years of use on the application site (Sanders, 1984). Mefenoxam-resistant strains or species of *Pythium* are now found in many locations and crops, including floriculture crops (Aegerter et al., 2002; Moorman et al., 2002; Moorman and Kim, 2004). Studies conducted in Pennsylvania showed that almost 40% of *P. aphanidermatum* and *P. irregulare* isolates were resistant to mefenoxam, which was significant because these two species accounted for 74% of all *Pythium* cases collected between 1996 and 2001 (Moorman et al., 2002). Treating resistant strains with mefenoxam increases the cost of production without the benefit of reducing disease. Recent research suggests that treating resistant strains with mefenoxam can even stimulate growth of *Pythium*, resulting in higher rates of damping-off in geranium (Garzon et al., 2011). *Pythium* populations in greenhouses may also exhibit phenotypic and genotypic changes over time (Al-Sadi et al., 2012). The frequency of isolation of metalaxyl-sensitive *P. aphanidermatum* increased between 2006 and 2011, suggesting that resistant isolates were lost from the population and replaced by new highly sensitive isolates (Al-Sadi et al., 2012).

Pythium is commonly isolated from greenhouse floriculture crops. Historical data on species associations within the greenhouse industry may not reflect the current situation due to changes in the industry over time. In the 1940s, *P. ultimum* was the most commonly isolated *Pythium* species on poinsettia cuttings from greenhouse locations in California; *P. debaryanum*, *P. perniciosum*, and *P. irregulare* were also recovered (Tompkins and Middleton, 1950). In the 1970s, *P. splendens* was the most aggressive species found on chrysanthemums and begonias in Canadian greenhouses, followed by *P. aphanidermatum* and *P. irregulare*. These species were also recovered most often from Ohio greenhouses in studies conducted from 1974 to 1975 (Stephens and Powell, 1982). Later, studies conducted in Pennsylvania greenhouses over a five-year period from 1996 to 2001 found 11 identified species and two unidentified species of *Pythium* from 110 plant samples, five potting soil tests, and five tests of irrigation water. *P. irregulare* was the most commonly isolated species from plants, accounting for 45% of the total isolates from plant samples, and was the most commonly isolated species from irrigation water and soil samples. *P. aphanidermatum* accounted for 29% of the total isolates from plant samples and comprised 77% of the isolates from poinsettia samples. Other species identified included *P. cylindrosporum*, *P. dissotocum*, *P. heterothallicum*, *P. myriotylum*, *P. sylvaticum*, *P. splendens*, and *P. ultimum* (Moorman et al., 2002). Several species of *Pythium* were present within a greenhouse and a given species was present on multiple host species within the same greenhouse (Moorman et al., 2002; Moorman and Kim 2004). In 2001, significant losses due to Pythium root rot were reported from greenhouses in California. *P. irregulare* was isolated from Gerbera daisy and lisianthus, with reported losses of 15 and 75%, respectively (Aegerter et al., 2002).

In most of these studies, isolates were collected from symptomatic plant samples. Studies have shown that *Pythium* can be isolated from plant samples without visible symptom expression (Farvin et al. 1988, Stanghellini and Kronland, 1986, Sanogo and Moorman 1993). Stanghellini and Kronland (1986) demonstrated that "subclinical" infection by *Pythium* may be relatively common. Yield reductions as high as 54% were reported in hydroponically grown lettuce not showing visible symptoms. Microscopic examination of infected roots revealed fungal structures within healthy-appearing epidermal cells (Stanghellini and Kronland, 1986). These types of losses would be much harder to detect in ornamental species which are not sold by weight as is lettuce. Olson and Benson consistently isolated *Phytophthora* species from non-symptomatic inoculated plants, indicating that growers could unknowingly spread *Phytophthora* to other growers or consumers on non-symptomatic infected ornamentals (Olson and Benson, 2013). Infection of plants exhibiting mild symptoms, such as minor stunting, may be underrepresented in studies that evaluate isolates from symptomatic plants or diagnostic samples.

Pythium can come into contact with susceptible plants through a variety of ways. *Pythium* and other plant pathogens can be recovered from commercial peat-based products and soilless mixes. Kim et al. (1975) isolated *Pythium* spp., including *P. aphanidermatum* and *P. irregulare*, from 15 of 52 mixes sampled. Farvin et al. (1988) recovered *P. aphanidermatum*, *P. irregulare*, and *P. coloratum* from 20 out of 48 soilless mix samples. Recent studies have shown that *Pythium* is frequently recovered from irrigation water (Hong and Moorman, 2005). *Pythium* was consistently transmitted to healthy potted cucumber plants when grown in an ebb-and-flow subirrigation system when the irrigation system was artificially infested with *P. aphanidermatum*. Conversely, when inoculated pots were placed among non-inoculated pots, transfer of *P. aphanidermatum* was low and sporadic. This indicates that it is possible for *Pythium* to be spread through the irrigation system if the water becomes heavily infested (Sanogo and Moorman, 1993). Plant debris and infested media found on greenhouse floors and benches also serve as sources of potential inoculum (Stephens et al., 1983; Moorman et al., 2002). Shore flies and fungus gnats can spread *Pythium* from debris or potting media throughout a greenhouse facility when they feed (Goldberg and Stanghellini 1990, Hyder et al., 2009). Oospores of *P. aphanidermatum* retained their viability following ingestion of adult shore flies and larval stages of fungus gnats and shore flies, and hyphal swellings of *P. splendens*, *P. sylvaticum,* and *P. ultimum* maintained their viability after passage through the digestive tracts of shore fly and fungus gnat larval stages (Hyder et al., 2009). Insect transmission of *Pythium* and other plant pathogens may play a significant role in the initial introduction of pathogens into a greenhouse facility. These insect vectors may enter greenhouse operations on infested cuttings or substrates, thereby bringing *Pythium* with them. It is speculated that *Pythium* can move long distances between greenhouses on infected plant material but comprehensive studies evaluating this risk have not been completed. By this process, fungicide resistant strains have the potential to move long distances within the industry.

OBJECTIVES

The objectives of this research were to i) identify species of *Pythium* associated with important floriculture crops grown in North Carolina; ii) characterize populations of *Pythium* isolated from floriculture crops for their sensitivity to mefenoxam; and iii) determine the aggressiveness of predominant species on selected economically important floriculture crops in NC.

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CHAPTER 1. Understanding the Impact of Pythium Root Rot on Floriculture Crops in North Carolina

ABSTRACT

Herbaceous ornamentals exhibiting symptoms of Pythium root rot were collected from 26 greenhouses in 21 counties in NC. Symptoms ranged from mild stunting to severe wilting, root rot, and death. Roots were assayed on selective media and 360 isolates of *Pythium* were recovered from 36 host species. Selected isolates were identified by sequencing of the ITS rDNA region. Fifteen species were identified, with *P. aphanidermatum*, *P. irregulare*, and *P. myriotylum* comprising 76% of the 289 isolates sequenced. Twelve greenhouses had more than one species present. To assay sensitivity to mefenoxam, all 360 isolates were grown in wells of 48-well micro-titer plates containing clarified V8 agar amended with 100 ppm mefenoxam. Colonization was scored after 24 to 48 h on a scale of 0 (no growth) to 5 (entire well colonized). Fifty-two percent of the isolates were resistant to mefenoxam (mean score ≥ 4). All 29 isolates of *P. myriotylum* were sensitive, whereas sensitivity varied among isolates of *P .aphanidermatum* and *P. irregulare*. Resistant and sensitive isolates of the same species were found within the same greenhouse. The aggressiveness of *P. aphanidermatum* and *P. irregulare* was evaluated on poinsettia, Gerbera daisy, and petunia. *Pythium aphanidermatum* was more aggressive than *P. irregulare* on poinsettia and petunia. Sensitivity to mefenoxam was not related to aggressiveness. We will use the results of this study to evaluate the need for and the feasibility of offering mefenoxam resistance screening as a diagnostic service at the Plant Disease and Insect Clinic.

INTRODUCTION

The greenhouse and nursery industry ranks fourth in agricultural sales in North Carolina and brings in over $700 million dollars annually. Floriculture sales accounted for an estimated wholesale value of $250.5 million of the total, placing North Carolina fifth in floricultural production the United States in 2011 (NCDA, 2012). Major floricultural crops produced in North Carolina include garden chrysanthemum, geranium from cuttings, and poinsettia from cuttings. North Carolina produces more than four million pots of poinsettias annually, with an estimated wholesale value of over $17 million (NCDA, 2012). Given the importance of the industry and the diversity of crops grown, it is not surprising that commercially-produced ornamentals represent more than 20% of the samples submitted to the Plant Disease and Insect Clinic (PDIC) at NC State University. Root rots and other diseases caused by *Pythium* are the most frequently diagnosed problems from floriculture samples submitted to the PDIC (B. Shew, *unpublished*). However, the extent and severity of *Pythium* diseases in the State's floriculture industry is not well characterized.

Pythium spp. are very common soilborne plant pathogens that cause stem rots, root rots, and damping off on many ornamental host species (Martin and Loper, 1999). *Pythium* is a diverse genus comprising at least 106 species (Lévesque and de Cock, 2004; Schroeder et al., 2013). In general, *Pythium* spp. are thought of as necrotrophic, opportunistic, non-specific plant pathogens with broad host ranges, but some species infect only a narrow range of plant hosts. Some species of *Pythium* are aggressive plant pathogens, whereas others exist mainly as saprophytes or weak root pathogens. Species also vary greatly in their aggressiveness on certain hosts (Abad et al., 1994). In spite of these differences, most diagnostic clinics, including the PDIC, do not routinely identify *Pythium* to species because of the skill required to successfully identify and differentiate species based on morphology.

Environmental conditions and common production practices in greenhouses are favorable for the growth and spread of *Pythium*. Studies conducted in Pennsylvania showed that several species of *Pythium* can be present in a greenhouse and that a given species can be present on multiple host species within the same greenhouse (Moorman et al., 2002; Moorman and Kim, 2004). *Pythium* populations in greenhouses may also exhibit phenotypic and genotypic changes over time (Al-Sadi et al., 2012). Another trait that varies among species and isolates of *Pythium* is sensitivity to common fungicides, including mefenoxam or metalaxyl (Moorman et al. 2002; Moorman and Kim, 2004; Schroeder et al., 2013). Resistance to metalaxyl was first reported in *Pythium* from turf in 1984, after only three years of use on the application site (Sanders, 1984). Mefenoxam-resistant strains or species of *Pythium* are now found in many locations and crops, including floriculture crops (Aegerter et al., 2002; Moorman et al., 2002; Moorman and Kim, 2004). Treating resistant strains with mefenoxam increases the cost of production without the benefit of reducing disease. Recent research suggests that treating resistant strains with mefenoxam can even stimulate growth of *Pythium*, resulting in higher rates of damping off in geranium (Garzon et al., 2011). The extent of mefenoxam resistance in populations of *Pythium* commonly found in floriculture crops in North Carolina is unknown.

In a diagnostic setting, understanding the potential for damage and the likelihood that attempts at control will be successful may depend on identifying the *Pythium* present on a diseased specimen to the species level or below. Recently, researchers have developed molecular tools to identify and characterize *Pythium* species and isolates, including isozyme analysis (Barr et al., 1998), nucleotide sequence of mitochondrial DNA (Martin, 2000), internal transcribed spacer (ITS) sequences (Moorman et al., 2002), restriction fragment length polymorphisms (RFLPs) of genomic and mitochondrial DNA (Harvey et al., 2000; 2001), amplified fragment length polymorphism (ALFP) fingerprinting (Garzon et al., 2005), and SSR or microsatellite markers (Lee and Moorman, 2008; Lee et al., 2010). These tools could help to improve routine diagnosis and recommendations for control of diseases caused by *Pythium* in the floriculture industry.

The objectives of this study were to identify species of *Pythium* associated with important floriculture crops grown in North Carolina, characterize these populations of *Pythium* for their sensitivity to mefenoxam, and to characterize the aggressiveness of predominant *Pythium* spp. on petunia, poinsettia, and Gerbera daisy.

MATERIALS AND METHODS

Isolate collection. Herbaceous ornamentals exhibiting symptoms of Pythium root rot were collected from 26 greenhouses in 21 counties in North Carolina. Samples were collected over a period from March 2011 to February 2012. Greenhouses were selected for sampling based on diagnostic records of *Pythium*-positive plants submitted through the Plant Disease and Insect Clinic (PDIC). County extension agents identified other sample locations based on histories of disease problems or production of the target crops. In the case of six greenhouses, samples were collected more than once so that spring (for example, petunia) and fall (for example, poinsettia) crops could be sampled from the same greenhouse. The greenhouses sampled varied widely in production systems used, size, and level of management. Growers were asked to provide information on fungicide application practices, irrigation water sources, and sanitation practices (Table 1.1). Plants expressed a range of symptoms from slight stunting to severe wilting, root rot, and death. When possible, at least six symptomatic plants were collected from each block of symptomatic plants. A block consisted of a group of plants of a particular species, cultivar, planting stage, pot size, and/or location within the greenhouse facility. Symptomatic plants were removed from their original pots and placed into labeled plastic bags. Samples were transported and stored in coolers until samples were processed in the laboratory.

Isolations. Symptomatic roots were selected for direct isolation. Roots were rinsed under running water and blotted dry. Roots were cut into 1-cm pieces and three clumps of root pieces were directly plated on cornmeal agar (CMA; 17 g CMA agar [Becton, Dickinson, and Company], 1 L deionized water; autoclaved 30 minutes) amended with pimaricin, ampicillin, rifampicin, and PCNB (P_5ARP; Jeffers and Martin, 1986) and dispensed in 100-mm Petri plates. Plates were incubated in the dark for 24 to 72 h. Colonies resembling *Pythium* were transferred to fresh CMA-P_5ARP. After 24 to 48 h, pure cultures were obtained by transferring actively growing hyphal tips onto fresh water agar (18 g Bacto agar [Becton, Dickinson, and Company], 1 L deionized water; autoclaved 30 minutes) dispensed in 60-mm

Petri plates. If bacterial contamination occurred, actively growing hyphal tips were transferred to cornmeal media amended with rose Bengal disodium salt after autoclaving (0.15 g rose Bengal disodium salt [Fisher Scientific, Inc.], 17g CMA, 1 L deionized water). To obtain pure cultures, 5-mm CMA-P_5ARP agar plugs were placed on top of the actively growing culture. After 24 h hyphal tips growing out of the top of the CMA-PARP plugs were transferred to fresh water agar plates. Working cultures were maintained on 2% water agar plates.

For long term storage, four 5-mm agar plugs from an actively growing culture were placed into a 2-ml screwcap microcentrifuge tube containing 1 ml of sterile deionized water and two twice autoclaved hemp seeds (Raabe et al., 1973). Cultures were stored at 25°C in the dark.

Identification of *Pythium* species with ITS sequencing. The internal transcribed spacer region (ITS4, 5.8S, and ITS5) of the ribosomal DNA of the isolates was sequenced for DNA-based identification. Isolates were grown on potato dextrose agar (PDA; 35 g PDA [Becton, Dickinson, and Company], 1 L deionized water; autoclaved 30 minutes) dispensed in 60-mm Petri plates for 7 to 10 days at room temperature (20 to 25°C). Mycelium mats were harvested by stripping them from the agar surface, placed in a 1.5-ml microcentrifuge tube, and stored at -20°C. Genomic DNA was extracted from mycelia with the PUREGENE DNA Isolation Kit for 10-20mg of fungal tissue (Qiagen, Valencia, CA) with a protocol optimized for *Pythium* and *Phytophthora* (K.L. Ivors, *pers. comm.*).

PCR amplification of the ITS region was performed using ITS4 (5’ TCCTCCGCTTATTGATATGC) and ITS5 (5’ GGAAGTAAAAGTCGTAACAAGG) primers. Reactions were 25µl in volume and were composed of 5.0 µl of 5X GoTaq Colorless Flexi Buffer (Promega Corporation, Madison, WI), 1.5µl of 25mM $MgCl_2$, 2.5µl of 40mM dNTP mix (10mM of each dNTP; Promega Corporation), 0.25 µl of each 50µM primer, 0.25 µl of GoTaq DNA polymerase (5U/µl; Promega Corporation), 14.25 µl nuclease-free water, and 1µl of extracted DNA. Thermal cycling conditions consisted of an initial denaturation step of 95°C for 4 min; 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and a final extension step of 72°C for 10 minutes. Amplification products were confirmed and estimated visually with gel electrophoresis (1% agarose; 60V for 120 min). PCR products were purified with USB ExoSAP-IT PCR Product Cleanup (Affymetrix) and submitted to ETON Bioscience, Inc. for sequencing. DNA sequences were aligned and manually edited with CLC Main Workbench 6 (CLC Bio). Edited sequences of each isolate were subject to BLAST analysis using GenBank (NCBI). Isolates were identified to species based on highest percent similarity (>98% sequence homology) within the ITS region.

Mefenoxam sensitivity of *Pythium* isolates. Individual isolates were screened for mefenoxam sensitivity using a rapid mefenoxam screening assay (RMSA) developed by S.N. Jeffers at Clemson University and modified for use with *Pythium* isolates. The RMSA utilized media amended with 100 µg a.i./ml mefenoxam dispensed in 48-well micro-titer plates (VWR International, Inc.) (Olson et al., 2012). A 100X stock solution of mefenoxam was prepared by diluting Subdue Maxx 2EC (Syngenta Crop Protection) with sterile deionized water. The 48 wells of micro-titer plates used in this study were 4 mm in diameter and were arranged in eight columns x six rows of wells. We dispensed 0.5 ml of molten non-amended 5% clarified V8 juice agar (CV8A; 50 ml of clarified V8 juice, 15 g of Bacto agar [Becton, Dickinson, and Co.], and 950 ml of deionized water; autoclaved 30 minutes), into each well in the bottom three rows of the micro-titer plate and 0.5 ml of molten amended 5% CV8A (1 ml mefenoxam stock solution per 100 ml of molten media) into each well in the top three rows of the micro-titer plate for a final concentration of 100 µg a.i./ml. To obtain actively growing cultures, isolates were transferred to 60-mm Petri plates containing 5 ml 5% CV8A and cultures were incubated in the dark for 24 hours at 25°C. Six 1-mm plugs were cut from the advancing edge of the actively growing colony with a sterilized glass disposable Pasteur pipet. One plug was transferred into the center of each of the six wells of a freshly prepared test plate. A flame-sterilized hypodermic needle was used to pick up and transfer the plugs (Figure 1.1). Plates were incubated in the dark at 25°C for 48 hours or until mycelium in the control wells completely covered the agar surface. Each plate screened eight isolates (one isolate per column) with three observations in amended media and three observations in non-amended media.

Colonization was scored on a rating scale in which 0=no growth, 1= a few hyphae growing from plug but only visible microscopically, 2= hyphae growing uniformly around plug but visible only microscopically, 3= hyphae growing uniformly around plug and just visible macroscopically, 4= hyphae visible macroscopically but not completely covering agar surface, 5= agar surface completely covered by mycelium and growth equal to that in non-amended wells (Olson et al., 2012). Isolates with mean sensitivity scores of 4 were considered resistant to mefenoxam (Figure 1.2). Each round of testing included a known mefenoxam-resistant isolate of *Pythium aphanidermatum* and known mefenoxam-sensitive isolate of *Pythium aphanidermatum* obtained from G.W. Moorman at Pennsylvania State University. The entire procedure was completed twice for each isolate.

Pathogenicity and aggressiveness of *P. aphanidermatum* and *P. irregulare* on selected hosts. Pathogenicity and aggressiveness of two *Pythium* species, *P. aphanidermatum* and *P. irregulare*, were evaluated on three herbaceous ornamental plant species commonly grown in NC greenhouses. Seeds of petunia (*Petunia X hybrida*) 'Pretty Grand White' were obtained from Ball Seed Company, Inc. Rooted cuttings of Gerbera daisy (*Gerbera jamesonii*) 'Jaguar Yellow' (run 1) and 'Jaguar Orange Picotee' (run 2) were provided by Green Circle Growers. Cuttings of poinsettia (*Euphorbia pulcherima*) 'Angelica White' were taken from stock plants maintained by Kala Parker (D.M. Benson Laboratory; NCSU, Raleigh, NC). To produce rooted plugs of petunia, seeds were sown into new 80-cell plug trays containing Fafard 2 peat-based potting mix (Conrad Fafard Inc.) and trays were maintained on a mist bench until roots completely colonized the cell and white roots began to grow out of the bottom of the cell drainage hole. The mist system cycled on for 1 minute six times daily. To produce rooted cuttings of poinsettia, terminal cuttings (approx. 10-cm long, two to four leaves per cutting) were taken with a sterile scalpel. Cuttings were placed in Oasis Wedge growing media (Smithers-Oasis North America) and maintained under mist irrigation until roots began to grow out of the bottom of the foam wedge. Rooted plugs of petunia and Gerbera daisy were transferred to new 10-cm plastic pots containing Fafard 4P peat-based potting mix. Rooted plugs of poinsettia were transferred to new 15-cm plastic pots containing

Fafard 4P peat-based potting mix. Once transplanted, 5 g of Osmocote 19-6-12 (Scotts) was sprinkled evenly on the surface and watered in. Plants were hand-watered twice a day for the duration of the trial. To control whiteflies, poinsettia and petunia plants were treated with Marathon 15 G (OHP, Inc.) insecticide; 1 g / 10-cm pot, 1.4 g / 15-cm pot, prior to inoculation.

Six isolates of *P. aphanidermatum* and six isolates of *P. irregulare* were chosen from the isolate collection obtained through the survey of NC greenhouses for the presence of *Pythium*. Selected isolates of *P. aphanidermatum* were originally obtained from poinsettia. Selected isolates of *P. irregulare* were originally obtained from Gerbera daisy, petunia, and snapdragon (Table 1.2). The six isolates of each species included three species resistant to mefenoxam and three species sensitive to mefenoxam as determined above. Inoculum was prepared by transferring four 4-mm-diameter agar disks from an actively growing culture into 125-ml flasks of twice-autoclaved long-grain rice (25 g rice and 18 ml deionized water) and cultures were incubated for 5 days at 25°C (Holmes and Benson, 1994). Flasks were shaken twice daily to promote uniform colonization of rice grains and prevent clumping.

Poinsettia, petunia, and Gerbera daisy were inoculated in July 2012. Petunias and Gerbera daisies growing in 10-cm pots were inoculated by placing three colonized rice grains in a triangular pattern around the plant base (1-cm deep and 2.5-cm from the stem base) in the potting media. Poinsettias growing in 15-cm pots received six rice grains per pot arranged in a circular pattern around the plant base. Saucers were placed under the pots for 48 hours post inoculation and then removed for the duration of the trial. Non-inoculated plants of each host were included as controls. The experiment was arranged in a split-plot design with four randomized blocks of the three plant species as the whole plot factor and the 12 isolates as the subplot factor. The average temperature throughout the duration of trial one was 26°C (maximum temperature of 42°C and minimum temperature of 21°C). The experiment was repeated once in December 2012. The average temperature throughout the duration of trial two was 24°C (maximum temperature of 31°C and minimum temperature of 16°C).

Plants were monitored weekly for the development of above-ground symptoms (wilting, stunting, chlorosis) of Pythium root rot. Poinsettia plant heights were recorded at day zero (day of inoculation) and once a week for the remainder of the trial. Heights (from plant base to shoot apex) were measured with a ruler to the nearest centimeter. Plants were maintained in the greenhouse until no new plants developed symptoms (48 days for trial 1 and 63 days for trial 2). At the end of the experiment, the health of each plant was evaluated on a visual scale of 1= healthy, 2= slightly stunted, 3= chlorosis, moderate stunting and/or defoliation, 4= wilting and/or severe stunting, 5= dead (Figure 1.3; *D.M. Benson, pers. comm.*). Plants were carefully inverted, the pot was removed, and the roots were observed with the growth media intact. Visual root rot ratings were assessed based on the size and integrity of the root ball and root color. The rating scale used was 1= healthy white roots with root ball completely intact, 2= 25% root rot, some root discoloration present, 3= 50% root rot, brown discoloration evident throughout root system, root ball integrity fairly weak, root cortex sloughs off easily, 4= 75% root rot, brown dead roots evident throughout root system, root ball integrity severely compromised, very few white roots, 5= 100% brown dead roots, root ball lost all integrity (Figure 1.4). The tops of the plants were cut from the roots at the soil line and the fresh tops were weighed to the nearest gram.

Each plant was assayed for the presence of *Pythium* in the roots. Roots were rinsed with running water, and blotted dry. Roots were cut into 1-cm pieces and three clumps of roots were directly plated on to CMA-PARP media. Cultures were incubated in the dark at room temperature for 24 to 48 h. Non-inoculated control plants were also assayed. Recovered isolates were transferred to water agar and observed. After incubation, isolates were tentatively separated into two groups; group 1 comprising isolates resembling *P. aphanidermatum* and group 2 comprising isolates resembling *P. irregulare*. Isolates were grown in grass-blade culture to confirm morphological distinctions between the two species. Isolates growing on water agar were transferred to grass-leaf cultures to induce production of sporangia, antheridia, oogonia, and oospores (Abad et al., 1994). Four 5-mm agar plugs were placed in a sterile 60-mm Petri dish containing 15 ml of sterile deionized water and 4-cm pieces of autoclaved (30 min) tall fescue leaves. The grass-leaf cultures were incubated at room temperature under continuous fluorescent light for 3 to 5 days. *Pythium* species were confirmed using keys and descriptions of Van der Plaats- Neterink (1981). Isolates were then screened for mefenoxam sensitivity as described above.

Isolate aggressiveness on poinsettia, Gerbera daisy, and petunia was evaluated based on root rot rating scores, plant health rating scores, and final top weight measurements. The difference in initial and final plant height measurements was also used to evaluate aggressiveness on poinsettia. Isolate effects on root rot rating scores and plant health rating scores were analyzed with PROC Glimmix (SAS 9.3; SAS Institute). Host, isolate, and the interaction were treated as fixed effects and trial and rep were treated as random effects. Data from both trials were combined. The host-isolate interaction was significant; therefore, the least-squares means were calculated for the interactions and the simple effects were analyzed by host to determine significant isolate differences. Single-degree-of-freedom linear contrasts were performed to determine differences in isolates of *P. aphanidermatum* and *P. irregulare* and differences in resistant isolates and sensitive isolates.

Isolate effects on top weight measurements were analyzed with PROC Glimmix (SAS 9.3; SAS Institute). Due to inherent differences between host sizes, data were sorted by host before further analysis. Single-degree-of-freedom linear contrasts were performed to determine differences in isolates of *P. aphanidermatum* and *P. irregulare* and differences in resistant isolates and sensitive isolates.

Isolate effects on poinsettia plant heights were analyzed with PROC Mixed (SAS 9.3; SAS Institute). Data from both trials were analyzed separately due to differences in isolate effects across runs. Single-degree-of-freedom linear contrasts were performed to determine differences in isolates of *P. aphanidermatum* and *P. irregulare* and differences in resistant isolates and sensitive isolates.

Estimated cost of mefenoxam rapid screening assay. We evaluated the cost of screening samples for mefenoxam sensitivity in order to determine whether a resistance screening assay would be beneficial to growers. Costs to perform the screening assay were estimated based on current market prices of ingredients and supplies as of May 2013. Labor was estimated based on current labor rates for temporary workers in the Plant Disease and Insect Clinic. Fungicide costs were estimated based on current market prices of Subdue Maxx 2EC (Syngenta; Syngenta Crop Protection), Mefenoxam 2 AQ (Quali-Pro), Terrozole 35% WP (OHP, Inc.), and Truban 30% WP (Everris NA, Inc.) and standard application rates for 15-cm pots (*Blake Edwards, Southern Agricultural Inc., pers. comm.*). Fungicide application costs were estimated based on labor costs associated with hand-drenching poinsettia plants. Figures were determined based on how many tracts of plants are treated by one worker in one eight hour workday (*Greenhouse grower, pers. comm.).*

RESULTS

Isolate collection. The plants sampled expressed a range of symptoms from slight stunting to severe wilting, root rot, and death. The most severe symptoms were seen on poinsettia, garden mums, Gerbera daisy, and snapdragons. The most common symptoms observed on poinsettia plants were severe root rot, root sloughing, upward cupping of the foliage, wilting, stem lesions, severe stunting, and eventual death. Symptoms associated with Gerbera daisy included root discoloration, root sloughing, wilting, stunting, and delayed flower development. On garden mums, *Pythium* was often isolated from plants co-infected with *Fusarium oxysporum* or those infested with insects (including cutworms, army worms, and noctuid moths). Severe root rot and wilting were observed. In contrast, symptoms on petunia were mild, with minor stunting the only symptom observed. Infected roots appeared white and healthy. On snapdragon, the most common symptoms were wilting and root discoloration, whereas on *Fuchsia*, delayed flowering and stunting were observed. On other bedding plants (*Vinca* and pansy) the most common symptoms were root discoloration and stunting.

A total of 360 isolates of *Pythium* were recovered from 36 plant species and one or more isolates of *Pythium* were recovered from plants collected in 26 of the 28 greenhouses sampled. *Pythium* was recovered most often from poinsettia, garden mum (*Chrysanthemum* spp.*)*, and snapdragon (*Antirrhinum majus)* (Table 1.3). Fifteen species of *Pythium* were identified from sequencing of 289 isolates in the collection (Table 1.4). *Pythium myriotylum*, *P. aphanidermatum*, and *P. irregulare* comprised 76% of the isolates sequenced. Sixteen isolates, putatively identified as *Pythium* sp., did not match any sequences listed in Genbank and could not be identified based on morphological characteristics. *Pythium irregulare* was recovered from plants in greenhouses at 14 locations, *P. aphanidermatum* at 10, and *P. myriotylum* at 5. Plants from 12 locations had more than one species present (Table 1.5). In general, there was not a strong association of a given *Pythium* species with a particular greenhouse location, with the exception of greenhouse A, in which *Pythium irregulare* comprised 94% of the total isolates collected. Multiple species of *Pythium* were recovered from 13 host types. In general, *Pythium* species were not associated with any particular host species with the exception of poinsettia, in which *Pythium aphanidermatum* comprised 91% of the total isolates from this host (Figure 1.5). *Pythium aphanidermatum* was recovered only from poinsettia and garden mum. Among other frequently isolated species, *P. myriotylum* was recovered from poinsettia, garden mum and fairy fanflower (*Scaevola* *aemula*) and *P. irregulare* was recovered from 19 hosts, including petunia, snapdragon, Gerbera daisy, and geranium (*Pelargonium* spp.).

Mefenoxam sensitivity. Mefenoxam sensitivity ratings for individual isolates were highly consistent within samples and across runs, with isolates scoring either 0 (no growth) or 5 (mycelium completely covering the well) in all cases. Of the 360 isolates tested, 52.4% were resistant to mefenoxam at 100 µg a.i./ml. Both resistant and sensitive isolates were recovered from plants collected from 12 greenhouses (Table 1.5) and 12 host plants (Figure 1.6). All isolates from Gerbera daisy (21) were resistant to mefenoxam regardless of the greenhouse sampled and 99% of isolates from garden mum were sensitive to mefenoxam. In addition, resistant and sensitive strains of the same *Pythium* species were found within the same greenhouse. Sensitivity to mefenoxam varied within isolates of *P. aphanidermatum* and *P. irregulare*, whereas all 29 isolates of *P. myriotylum* were sensitive to mefenoxam. Two additional species had both resistant and sensitive isolates (*P. catenulatum* and *P. dissotocum*). All isolates (2) of *P. mamillatum* were sensitive to mefenoxam. All 12 isolates of *P. segnitium* and all three isolates of *P. rostratifingens* were resistant to mefenoxam (Table 1.6).

Pathogenicity and aggressiveness. Inoculations resulted in production of Pythium root rot symptoms, but disease, as indicated by root rot rating, plant health rating, top weight, and plant height, was more severe in run 1 than in run 2. For example, the mean root rot rating across hosts was 2.4 in run 1 and 2.1 in run 2. In both runs, poinsettia expressed more severe symptoms than Gerbera daisy and petunia. Typical symptoms observed on poinsettia included stunting, downward cupping of the leaves, wilting, defoliation, root discoloration, severe root rot, and in some cases, complete loss of root ball integrity. Symptoms on gerbera daisy and petunia were less severe. On Gerbera daisy, symptoms included delayed flowering, minor stunting, root discoloration and root rot. Inoculated petunia plants were difficult to distinguish from non-inoculated control plants based on above ground symptoms. Root rot was less pronounced than in Gerbera daisy and poinsettia. Symptoms included minor losses in root ball integrity and root sloughing. Root discoloration was not a common symptom and most of the root systems remained white in color. In all hosts, isolations from inoculated roots consistently yielded the inoculated species and *Pythium* was not recovered from non-inoculated control plants.

Root rot ratings. Averaged across isolates, the three hosts tested did not differ in root rot rating (P = 0.2380); however, host response depended on the isolate as indicated by a significant isolate by host interaction (P = 0.0082). Linear contrasts indicated that *P. aphanidermatum* caused more severe root rot symptoms (mean rating of 2.5) than *P. irregulare* (mean rating of 2.0; P = 0.0008), but there was no difference between root rot ratings in plants inoculated with mefanoxam sensitive compared to resistant isolates (2.3 vs. 2.2; Table 1.7). When inoculated with *P. aphanidermatum*, poinsettia had a mean root rot rating of 3.5, whereas mean ratings were 2.0 on petunia and 2.1 on Gerbera daisy (Table 1.9). Isolate PAS2 (*P. aphanidermatum;* sensitive) was the most aggressive isolate on poinsettia, causing an average root rot rating of 4.1 (Table 1.9). Root rot also was greater than on the non-inoculated control on poinsettia inoculated with isolates PAS1 (*P. aphanidermatum*;

sensitive), PAS3 (*P. aphanidermatum*; sensitive), and PAR4 (*P. aphanidermatum*; resistant). Isolate PAR6 (*P. aphanidermatum*; resistant) was the most aggressive on petunia as measured by root rot rating, with an average rating of 2.4 (Table 1.9). Within Gerbera daisy, inoculation with isolates PAS1 (*P. aphanidermatum*; sensitive) and PIR12 (*P. irregulare*; resistant) resulted in the highest root rot ratings, with an average rating of 2.5 for each; no other isolates caused significant root rot relative to the control. Linear contrasts within hosts indicated that isolates of *P. aphanidermatum* caused more root rot than isolates of *P. irregulare* on petunia and poinsettia (Table 1.8; Table 1.9).

Plant health ratings. Averaged across isolates, the three hosts tested did not differ in plant health rating ($P = 0.3602$); however, host response depended on the isolate as indicated by a significant isolate by host interaction ($P = 0.0024$). The interaction was attributed to differences in isolate aggressiveness on poinsettia (Table 1.10). Linear contrasts indicated that *P. aphanidermatum* caused more root rot symptoms than *P. irregulare* ($P = 0.0022$); however, there was no difference between plants inoculated with sensitive compared to resistant isolates (Table 1.7). Isolate PAS1 (*P. aphanidermatum*; sensitive) resulted in the poorest plant health rating with an average rating of 3.5. Isolate PAS2 (*P. aphanidermatum*; sensitive) also differed significantly from the non-inoculated control. There were no significant differences in plant health among isolates on petunia or Gerbera daisy. Linear contrasts indicate that *P. aphanidermatum* caused poorer plant health ratings on petunia and poinsettia than *P. irregulare* (Table 1.8).

Differences in top weights. Averaged across isolates, the three hosts tested did not differ in top weight measurements (P=0.6278); however, host response depended on the isolate as indicated by a significant host by treatment interaction (P=0.05). The interaction was attributed to isolate aggressiveness on Gerbera daisy (Table 1.11). Averaged across isolates, hosts responded differently to isolate groups (Table 1.8). Top weights of petunia inoculated with *P. aphanidermatum* were 33.5 g compared to a weight of 39 g on plants inoculated with *P. irregulare* (P=0.0028; Table 1.8; Table 1.11). On poinsettia, *P. aphanidermatum* infections resulted in lower top weight measurements than did *P. irregulare* (P=0.0442) and inoculation with sensitive isolates resulted in lower top weight measurements than with resistant isolates (P=0.0093) (Table 1.8; Table 1.11). There were no effects of isolate groups on Gerbera daisy.

Plant heights. Isolate effects differed across runs, so runs were analyzed separately. In runs one and two, inoculation with *P. aphanidermatum* isolates resulted in lower plant height measurements than inoculation with isolates of *P. irregulare* (Table 1.7; Table 1.12). There were no significant isolate effects in run one, but isolate differences were observed in run two (Table 1.12). Plants inoculated with isolates PAS1 (*P. aphanidermatum*; sensitive) and PAS3 (*P. aphanidermatum*; sensitive) had significantly lower plant heights than plants inoculated with isolate PIS7 (*P. irregulare*; sensitive).

Cost of the mefenoxam rapid screening assay. The total cost to test eight isolates for mefenoxam sensitivity was estimated as $49.16 (Table 1.13). The cost, in chemicals, to treat 10,000, 15-cm pots with Subdue Maxx 2EC (Syngenta; Syngenta Crop Protection) was an estimated $12.34 or $10.16 for the generic mefenoxam product (Mefenoxam 2AQ; Quali-Pro) (Table 1.14). Factoring in labor costs, the cost to treat 10,000 15-cm pots with Subdue Maxx 2EC was estimated as $72.37 or $70.19 for the generic mefenoxam product. Applications of other products recommended for control of Pythium root rot (Terrazole 35% WP (OHP, Inc.) and Truban 30% WP (Everris NA, Inc.) were more costly.

DISCUSSION

Composition and diversity of *Pythium* spp. varied with and among hosts and greenhouses sampled in North Carolina. The presence of two or more *Pythium* spp. within many greenhouses probably indicates multiple sources of inoculum. A variety of problems with production practices were observed in some greenhouses (Table 1.1). Poor sanitation, particularly between crops, and poor choice of fungicides were the most common problems. Both could have increased the likelihood of multiple introductions and persistence of problems over time. In other cases, there was no apparent explanation for the diversity of species found in a greenhouse. *Pythium* can enter and spread throughout a greenhouse through long-or short distance transport of infected cuttings and transplants, through contaminated irrigation water and media, and can even be spread by certain insects (El-Hamalawi, 2008; Hong and Moorman, 2005; Hyder et al., 2009; Sanogo and Moorman, 1993).

Previous studies identified *Pythium aphanidermatum* and *Pythium irregulare* as being responsible for the majority of *Pythium* cases submitted to clinics in Pennsylvania (Moorman et al., 2002). Unfortunately, species that cause minor disease symptoms may be underrepresented in clinic samples, especially in cases of co-infections with other pathogens. Specimens submitted to clinics for diagnosis are selected based on existing disease symptoms. Sampling plants with severe root rot may underrepresent species that cause minor symptoms. In contrast, at the NC State Plant Disease and Insect clinic, all greenhouse samples are routinely assayed for *Pythium*. The importance of a Pythium root rot diagnosis is not well understood, especially when given in combination with abiotic stresses, other diseases, and insect problems. In order to address this issue, our collection consisted of both clinic samples and isolates collected through the systematic sampling of greenhouse plants.

We found that *P. irregulare*, *P. aphanidermatum*, and *P. myriotylum* are the most commonly isolated species from greenhouse floriculture crops in North Carolina. We found a strong association of *P. aphanidermatum* with poinsettia and *P. myriotylum* with garden chrysanthemums. The association of *P. aphanidermatum* and poinsettia is especially interesting due to the nature of the poinsettia production system. In this study, all of the poinsettia producers propagated cuttings in-house, which limits the opportunity for new isolates to enter the greenhouse on cuttings from outside sources. Similar studies have also found a strong association of *P. aphanidermatum* with poinsettia (Moorman et al., 2002). It is not known whether the isolates of *P. aphanidermatum* obtained from poinsettia were specifically adapted to poinsettia or if poinsettia production practices favor *P. aphanidermatum* infection and symptom development. It is important to note that the main propagation of poinsettia plants occurs in summer when temperatures inside the greenhouse are high, which correlates with growth of *P. aphanidermatum,* which is also favored by warm temperatures (Littrell and McCarter, 1970). Additionally, run one had higher average temperatures than run two and we observed more severe disease symptoms in run one than in run two. In our pathogenicity trials, isolates of *P. aphanidermatum* originally collected from poinsettia plants were able to infect Gerbera daisy and petunia and cause symptoms typical of Pythium root rot. We did not have any isolates of *P. aphanidermatum* originally collected from Gerbera daisy or petunia to test on poinsettia.

P. irregulare was the most frequently collected species in our survey, comprising approximately 39% of the isolates obtained. In the pathogenicity trials, isolates of *P. irregulare* originally collected from snapdragon, geranium, petunia, and Gerbera daisy, were all able to infect and cause symptoms on poinsettia, Gerbera daisy, and petunia. Isolates of *P. irregulare* from Gerbera daisy were originally collected from plants with severe symptoms including severe root rot, wilting, stem rot, and plant death. However, these dramatic symptoms could not be reproduced on Gerbera daisy in the greenhouse trials. It is likely that cultivars within a species differ in resistance to infection and symptom development (Higginbotham et al., 2004). It is also possible that specific environmental or cultural factors are required for severe symptom development and disease epidemics to occur. Inoculum levels, inoculum source, plant stage at infection, original site of infection, and type of potting media used could all influence severity of infection and symptom development. In a previous study, spread and subsequent disease development was more likely when inoculum was placed in irrigation water than when pots of infested plants were placed within the same ebb-and-flow irrigation system with non-inoculated pots (Sanogo and Moorman, 1993). In our study, all of the isolates of *P. irregulare* from petunia were originally collected from plants with subtle stunting symptoms. In the pathogenicity trial, petunia expressed minor symptoms when inoculated with either *P. aphanidermatum* or *P. irregulare*. Other studies have shown that *Pythium* species were consistently isolated from necrotic as well as apparently healthy roots (Stanghellini and Kronland, 1986; Mircetich, 1971; Ivors et al., 2008; Funck-Jenson and Hockenhull, 1983). This could indicate that petunia or other symptomless hosts harbor *Pythium* in a greenhouse.

Mefenoxam resistance was widespread among the greenhouses sampled in this study. Histories of mefenoxam use and production practices were not consistently associated with mefenoxam sensitivity or resistance of the *Pythium* isolates collected at some sites. Mefenoxam-resistant isolates were found in some greenhouses with no known history of mefenoxam or metalaxyl use. In the case of under-resourced producers, poor record-keeping may have been partially to blame. In other instances, it is likely that resistant isolates were introduced on propagative materials or potting mixes or other materials purchased from outside sources. Studies in Pennsylvania greenhouses reported a lower incidence of resistant isolates, with only 33% of isolates screened being resistant to mefenoxam as compared to over 50% in our study (Moorman et al., 2002). In contrast to our study, mefenoxam resistance in *Phytopthora* isolates collected from horticultural plants and irrigation water showed a relatively low incidence of mefenoxam resistance (Olson et al., 2013). In this study, *P. myriotylum* isolates collected from three host plants in five greenhouse locations were all were sensitive to mefenoxam. Additionally, over 99% of isolates collected from garden chrysanthemums were sensitive to mefenoxam. The majority of chrysanthemum growers sampled obtained propagative materials from outside sources. The reason for the lack in mefenoxam resistance in these two groups is unclear. It is possible that production practices associated with chrysanthemums do not favor resistance development or frequent fungicide applications in the propagation stage. Studies to determine sources and pathways of *Pythium* inoculum in the floriculture industry could lead to better control of Pythium root rot, and could be used as a model for tracing pathways of pathogen introduction in the floriculture industry in general.

The results suggest that identification of *Pythium* to genus may not provide sufficient guidance to growers who submit floriculture samples to the PDIC. The presence of two or more species in a particular greenhouse location or host plant makes forming generalizations about *Pythium* distributions and supplying informative diagnoses difficult. With the exception of *P. aphanidermatum* on poinsettia, there were not clear associations between host and greenhouse locations and particular *Pythium* species. Greenhouse producers could obtain additional value from diagnosis of *Pythium* to species, testing for mefenoxam resistance, or both.

The method used to screen for mefenoxam resistance was simple, reproducible, and rapid, requiring only 48 to 72 hours for results after initial isolations are made. Large commercial growers in North Carolina have expressed interest in mefenoxam testing as a diagnostic service offered by the Plant Disease and Insect Clinic. Greenhouse applications of Subdue Maxx 2EC and the generic form of mefenoxam (Mefenoxam 2AQ) are significantly less expensive than the current industry alternatives (Terrazole 35% WP and Truban 30% WP), which require significantly higher rates for potting media high in peat and other organic amendments. Additionally, fungicide applications to mature poinsettia plants must be hand-drenched to prevent interference with color change, which corresponds to added labor costs. Higher chemical costs associated with alternative chemicals could account for the industry's continued reliance on mefenoxam to treat oomycete diseases despite recommendations to rotate fungicides and to discontinue mefenoxam use after resistance is established in a particular greenhouse. The rapid mefenoxam sensitivity assay costs approximately $49.16, including labor and supplies, to screen eight *Pythium* isolates. The cost to treat 10,000 15-cm pots with Subdue Maxx 2EC is approximately $72.37 per application, and typically, poinsettias receive two to three applications during production. In North Carolina, the largest poinsettia producer has over 115 acres of poinsettia plants at the season's peak, meaning that it would cost over $79,000 to treat every plant one time. Treating resistant strains with mefenoxam increases the cost of production without the benefit of reducing disease. The rapid mefenoxam sensitivity assay would provide growers with timely fungicide application recommendations based on their specific *Pythium* populations, potentially saving them time and money.

It may also be possible to develop relatively rapid screens to differentiate the predominant *Pythium* species found in the North Carolina floriculture industry. In cases where *Pythium* is associated with damping-off diseases, identification to genus may be sufficient information to develop effective management strategies. In contrast, identification to genus may not offer adequate information when Pythium root rot is diagnosed on mature plants, and further identification and quantification may be required to offer disease diagnosis and management recommendations. Previous work in Washington State used real-time PCR to characterize predominant species from wheat (Schroeder et al., 2006). Real-time PCR offers several benefits including high sample throughput and increased sensitivity, and requires less specialized knowledge and skill as compared to traditional identification based on morphological characteristics. Molecular methods of identification and quantification of *Pythium* species will help researchers understand the epidemiology and ecology of *Pythium* within a greenhouse as well as how *Pythium* species move within the industry.

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Table 1.1. Greenhouse practices associated with positive *Pythium* diagnoses identified through the Plant Disease and Insect Clinic or sampling trips conducted during 2011-2012 in North Carolina

	Greenhouse																									
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
Chemical History																										
Mefenoxam						X			X				X			X	X		X							
Terrazole	X					X			X							X										
Truban						X	X												X							
Other			X					X	X		X		X													
Water Source																										
Well			X						X		X		X			X			X							
Pond																X										
Recirculating system						X													X							
Irrigation Practice																										
Flooding						X													X							
Overhead	X					X													X							
Drip													X													
Poor Sanitation																										
Reuse pots or media					X		X									X										
Failure to sanitize between crops					X																					
Drainage problem																	X									
Contact with native soil	X								X		X		X				X									
Sample Type																										
Clinic	X			X		X	X	X	X	X		X	X	X	X	X			X	X	X	X	X	X	X	X
Survey	X	X	X		X	X	X	X	X		X	X	X			X	X	X	X							

Table 1.2. Isolates of *Pythium* tested for pathogenicity and aggressiveness on petunia, Gerbera daisy, and poinsettia in greenhouse trials

Isolate	Mefenoxam Sensitivity	Original Host	Greenhouse[/a]
		P. aphanidermatum	
PAS 1	Sensitive	Poinsettia	F
PAS 2	Sensitive	Poinsettia	S
PAS 3	Sensitive	Poinsettia	F
PAR 4	Resistant	Poinsettia	P
PAR 5	Resistant	Poinsettia	F
PAR 6	Resistant	Poinsettia	F
		P. irregulare	
PIS 7	Sensitive	Snapdragon	E
PIS 8	Sensitive	Geranium	C
PIS 9	Sensitive	Petunia	G
PIR 10	Resistant	Gerbera	A
PIR 11	Resistant	Snapdragon	P
PIR 12	Resistant	Geranium	P

[/a] Original greenhouse location of isolate; letters correspond to specific greenhouses

Table 1.3. Host sources of *Pythium* isolated from floricultural crops surveyed in North Carolina greenhouses from 2011-2012

Host	Percentage of Isolates[a/]
Poinsettia	21%
Mum	20%
Snapdragon	9%
Petunia	7%
Geranium	7%
Gerbera	6%
Lantana	6%
Fuchsia	4%
Vinca	3%
Verbena	3%
Others, less than 1% each	

[a/]Percentage of 360 isolates

Table 1.4. Species of *Pythium* isolated from floriculture crops grown in 26 greenhouses in North Carolina from 2011-2012

Species	Percentage[a]	Number	Reference Accession[b]
Pythium aphanidermatum	26%	73	AY598622.2
		1	DQ059572.1
		1	JN695768.1
		1	JA898455.1
Pythium catenulatum	1%	3	HQ643494.1
		1	HQ237487.1
Pythium dissotocum	9%	22	AY598634.2
		3	KC689900.1
		3	KC689901.1
Pythium inflatum	< 1 %	1	AY598626.2
Pythium intermedium	< 1 %	1	HQ643577.1
Pythium irregulare	39%	83	HQ643646.1
		11	HQ643595.1
		4	HQ643642.1
		4	HQ643600.1
		3	HQ643650.1
		2	HQ643636.1
		1	HQ643647.1
		1	HQ643601.1
		1	GQ410393.1
Pythium litorale	< 1 %	1	JX985744.1
Pythium mamillatum	< 1 %	2	HQ643688.1
Pythium myriotylum	11%	22	HQ237488.1
		9	HQ643702.1
		1	HQ643703.1
Pythium rostratifingens	1%	2	JN630500.1
		1	FJ415927.1
Pythium segnitium	4%	12	HQ643772.1
Pythium splendens	< 1 %	1	GU983648.1
Pythium ultimum	< 1 %	1	KC689906.1
Pythium vexans	< 1 %	1	GU13378.1
Pythium sp.	5%	16	No Match

[a/] Percentage of 289 isolates identified to species

[/b] GenBank accession number for closest match; > 98%

Table 1.5. Distribution of mefenoxam-resistant isolates of *Pythium* from floriculture crops grown in North Carolina greenhouses 2011-2012

Greenhouse[a]	Isolates resistant (%)[b]	*Pythium* species (number)[c]	Isolates (number)[d]	Hosts sampled
A	96.3%	4	54	Fuchsia, Gerbera, impatiens, lantana, petunia
B	84.2%	4	19	Begonia, lantana, verbena
C	63.2%	2	19	Bacopa, geranium, Gerbera, Heuchera, jamesbrittania, lantana, verbena, vinca
D	100%	1	1	Jasmine
E	11.8%	2	17	Gomphrena, petunia, snapdragon, vinca
F	34.7%	5	49	Erysimum, Gerbera, licorice plant, mum, petunia, poinsettia, pothos, scaevola, pericallis
G	56.4%	5	39	Gerbera, pansy, petunia, poinsettia, vinca
H	75.0%	3	12	Geranium, lantana, marjoram, thyme, verbena
I	46.7%	3	15	Snapdragon, mum
J	100%		1	Fuchsia
K	0%	2	2	Mum
L	0%	1	4	Mum
M	4.2%	6	24	Mum
N	0%	1	2	Mum

Table 1.5. continued

O	0%	1	1	Mum
P	89.1%	6	46	Snapdragon, poinsettia, Gerbera, geranium, fern
Q	0%	1	4	Mum
R	0%	4	22	Mum
S	16.7%	5	18	Verbena, poinsettia, kalanchoe, bacopa
T	100%	1	1	Begonia
U	40.0%	3	5	Polygonatum, oregano, euphorbia, choisya
V	0%		2	Dahlia
W	100%	1	1	Geum
X	0%	1	1	Pansy
Y	100%		1	Rosemary
Z	0%	1	1	Buddleia

[a/] Letters correspond to specific greenhouse locations
[b/] Resistant to mefenoxam (Subdue Maxx 2EC; Syngenta Crop Protection) at 100 µg a.i/ml
[c/] Number of *Pythium* species collected from a specific greenhouse location
[d/] Number of *Pythium* isolates collected from a specific greenhouse location

Table 1.6. Prevalence of resistance to mefenoxam in populations of *Pythium* recovered from floriculture crops grown in 26 greenhouses in North Carolina from 2011-2012

Species	**Number of Isolates**	**Percentage resistant**[/a]
Pythium irregulare	113	69.0%
Pythium aphanidermatum	74	45.9%
Pythium myriotylum	32	0%
Pythium dissotocum	24	87.5%
Pythium segnitium	12	100%
Pythium catenulatum	4	25.0%
Pythium rostratifingens	3	100%
Pythium mamillatum	2	0%

[/a] Resistant to mefenoxam (Subdue Maxx 2EC; Syngenta Crop Protection) at 100 µg a.i/ml

Table 1.7. Linear contrasts between isolate groups averaged across poinsettia, Gerbera daisy, and petunia inoculated with *Pythium* in greenhouse evaluations of pathogenicity and aggressiveness

	Root rot rating[/b]		Plant health rating[/c]		Top weight[/d]		Plant height[/e] (run 1)		Plant height (run 2)	
Contrasts between Groups[/a]	Estimate	P > | t |	Estimate	P > | t |	Estimate	P > | t |	Estimate	P > | t |	Estimate	P > | t |
P. aphan. vs. *P. irregulare*	0.5	0.0008	0.5	0.0022	4.3	0.0554	-2.8	0.0482	-14.2	<.0001
Mefenoxam sensitive vs. resistant	0.1	0.2770	0.1	0.3651	4.4	0.0541	-2.5	0.0712	0.5	.8388

[/a] Data from two trials was combined and analyzed using PROC GLIMMIX in SAS (Version 9.3); single-degree-of-freedom linear contrasts between groups

[/b] Rating scale with 1= healthy white roots and 5= brown, dead roots; higher root rot ratings correspond to more disease symptoms

[/c] Rating scale with 1= healthy plant and 5= dead plant; higher plant health ratings correspond to more disease symptoms

[/d] Top weight measurements (to the nearest gram) taken at the end of the trial

[/e] Difference between initial plant height (to the nearest cm) and final plant height on poinsettia

Table 1.8. Linear contrasts between isolate groups within hosts inoculated with *Pythium* in greenhouse evaluations of pathogenicity and aggressiveness

		Root Rot Ratings[/b]		Plant Health Ratings[/c]		Top Weights[/d]	
Contrasts between groups[/a]	Host	Estimate	P > | t |	Estimate	P > | t |	Estimate	P > | t |
P. aphan. vs. *P. irregulare*	Petunia	0.4	0.0068	0.3	0.0062	-5.5	0.0028
P. aphan. vs. *P. irregulare*	Poinsettia	1.2	< .0001	1.1	< .0001	-8.8	0.0442
P. aphan. vs. *P. irregulare*	Gerbera Daisy	0.03	1	0.1	0.4324	1.3	0.1638
Mefenoxam sensitive vs. resistant	Petunia	0.2	0.2697	0.1	0.5758	-1.8	0.3171
Mefenoxam sensitive vs. resistant	Poinsettia	0.2	0.3369	0.3	0.0968	-11.5	0.0093
Mefenoxam sensitive vs. resistant	Gerbera Daisy	0.03	0.7935	-0.05	0.6943	0.2	0.8010

[/a] Data from two trials was combined and analyzed using PROC GLIMMIX in SAS (Version 9.3); single-degree-of-freedom linear contrasts between groups
[/b] Rating scale 1-5 with 1= healthy white roots and 5= brown, dead roots; higher root rot ratings correspond to more disease symptoms
[/c] Rating scale 1-5 with 1= healthy plant and 5= dead plant; higher plant health ratings correspond to more disease symptoms
[/d] Top weight measurements (to the nearest gram) taken at the end of the trial

Table 1.9. Root rot ratings on petunia, Gerbera daisy, and poinsettia inoculated with mefenoxam sensitive and resistant isolates of *Pythium aphanidermatum* and *P. irregulare* in the greenhouse

Species	Isolate[/a]	Petunia[/b,c]	Poinsettia	Gerbera	Mean[/d]
P. aphanidermatum	PAS1	1.9 ab	3.9 ab	2.5 a	2.8 A
P. aphanidermatum	PAS2	1.9 ab	4.1 a	1.8 ab	2.6 A
P. aphanidermatum	PAS3	2.3 ab	3.6 abc	2.3 ab	2.7 A
P. aphanidermatum	PAR4	1.6 ab	3.4 abc	2.3 ab	2.4 A
P. aphanidermatum	PAR5	1.8 ab	3.0 abcd	2.0 ab	2.3 AB
P. aphanidermatum	PAR6	2.4 a	3.1 abcd	1.8 ab	2.4 A
***P.aphanidermatum* mean**		**2.0**	**3.5**	**2.1**	**2.5**
P. irregulare	PIS7	1.8 ab	2.1 cd	2.1 ab	2.0 AB
P. irregulare	PIS8	1.8 ab	2.4 bcd	2.1 ab	2.1 AB
P. irregulare	PIS9	1.5 ab	2.1 cd	1.9 ab	1.8 AB
P. irregulare	PIR10	1.4 ab	2.6 abcd	1.8 ab	1.9 AB
P. irregulare	PIR11	1.3 ab	2.4 bcd	2.1 ab	1.9 AB
P. irregulare	PIR12	1.6 ab	2.5 abcd	2.5 a	2.2 AB
***P. irregulare* mean**		**1.6**	**2.4**	**2.1**	**2.0**
Non-inoculated Control	NEG	1.0 b	1.6 d	1.0 b	1.2 B
Mean[/e]		1.7 A	2.8 A	2.0 A	
Pr > F[/f]		*0.0278*	*<0.0001*	*0.0268*	

[/a] Isolate designations PAS = *P. aphanidermatum* sensitive to mefenoxam; PAR = *P. aphanidermatum* resistant to mefenoxam; PIS = *P. irregulare* sensitive to mefenoxam; PIR = *P. irregulare* resistant to mefenoxam

[/b] Data from two trials was combined and analyzed using PROC GLIMMIX in SAS (Version 9.3); least squares means shown with Tukey-Kramer grouping (alpha = 0.05) within host type.

[/c] Rating scale 1-5 with 1= healthy white roots and 5= brown, dead roots; higher root rot ratings correspond to more disease symptoms

[/d] Mean isolate effect across hosts. Isolate differences are indicated by upper case letters.

[/e] Mean host effect across isolates. Host differences are indicated by upper case letters.

[/f] Isolate effect within host. Differences within host are indicated by lower case letters.

Table 1.10. Plant health ratings on petunia, Gerbera daisy, and poinsettia inoculated with mefenoxam sensitive and resistant isolates of *Pythium aphanidermatum* and *P. irregulare* in the greenhouse

Species	Isolate[/a]	Petunia[/b,c]	Poinsettia	Gerbera	Mean [/d]
P. aphanidermatum	PAS1	1.4 a	3.5 a	1.6 a	2.2 A
P. aphanidermatum	PAS2	1.1 a	3.4 ab	1.4 a	2.0 A
P. aphanidermatum	PAS3	1.8 a	3.0 abc	1.3 a	2.0 A
P. aphanidermatum	PAR4	1.4 a	2.8 abc	1.5 a	1.9 A
P. aphanidermatum	PAR5	1.0 a	2.4 abc	1.3 a	1.5 A
P. aphanidermatum	PAR6	1.5 a	2.6 abc	1.1 a	1.8 A
***P. aphanidermatum* mean**		**1.4**	**3.0**	**1.4**	**2.0**
P. irregulare	PIS7	1.1 a	1.6 c	1.0 a	1.3 A
P. irregulare	PIS8	1.0 a	2.1 abc	1.4 a	1.5 A
P. irregulare	PIS9	1.0 a	1.8 bc	1.1 a	1.3 A
P. irregulare	PIR10	1.0 a	1.9 abc	1.3 a	1.4 A
P. irregulare	PIR11	1.1 a	1.6 c	1.3 a	1.3 A
P. irregulare	PIR12	1.0 a	2.1 abc	1.6 a	1.6 A
***P. irregulare* mean**		**1.0**	**1.9**	**1.3**	**1.4**
Non-inoculated Control	NEG	1.0 a	1.5 c	1.1 a	1.2 A
Mean[/e]		1.2 A	2.3 A	1.3 A	
Pr > F[/f]		*0.1115*	*0.0001*	*0.3462*	

[/a] Isolate designations PAS = *P. aphanidermatum* sensitive to mefenoxam; PAR = *P. aphanidermatum* resistant to mefenoxam; PIS = *P. irregulare* sensitive to mefenoxam; PIR = *P. irregulare* resistant to mefenoxam
[/b] Data from two trials was combined and analyzed using PROC GLIMMIX in SAS (Version 9.3); least squares means shown with Tukey-Kramer grouping (alpha = 0.05) within host type.
[/c] Rating scale 1-5 with 1= healthy plant and 5= dead plant. Higher plant health ratings correspond to more disease symptoms
[/d] Mean isolate effect across hosts. Isolate differences are indicated by upper case letters.
[/e] Mean host effect across isolates. Host differences are indicated by upper case letters.
[/f] Isolate effect within host. Differences within host are indicated by lower case letters.

Table 1.11. Top weights of petunia, Gerbera daisy, and poinsettia inoculated with mefenoxam sensitive and resistant isolates of *Pythium aphanidermatum* and *P. irregulare* in the greenhouse

Species	Isolate[/a]	Petunia[/b,c]	Poinsettia	Gerbera	Mean [/d]
P. aphanidermatum	PAS1	31.5 a	28.7 a	17.2 ab	25.8 A
P. aphanidermatum	PAS2	32.5 a	29.0 a	18.4 ab	26.6 A
P. aphanidermatum	PAS3	30.9 a	47.1 a	19.8 ab	32.6 A
P. aphanidermatum	PAR4	38.0 a	51.3 a	16.2 ab	35.2 A
P. aphanidermatum	PAR5	35.1 a	47.3 a	21.0 ab	34.5 A
P. aphanidermatum	PAR6	32.9 a	53.3 a	23.1 a	36.4 A
***P. aphanidermatum* mean**		**33.5**	**42.8**	**19.3**	**31.9**
P. irregulare	PIS7	37.5 a	53.4 a	18.8 ab	36.5 A
P. irregulare	PIS8	39.2 a	40.5 a	19.0 ab	32.9 A
P. irregulare	PIS9	40.4 a	49.9 a	19.4 ab	36.6 A
P. irregulare	PIR10	40.6 a	60.5 a	19.3 ab	40.1 A
P. irregulare	PIR11	37.6 a	57.9 a	16.3 ab	37.3 A
P. irregulare	PIR12	38.4 a	47.3 a	15.3 ab	33.7 A
***P. irregulare* mean**		**39.0**	**51.6**	**18.0**	**36.2**
Non-inoculated Control	NEG	35.1 a	59.0 a	20.1 b	38.0 A
Mean[/e]		36.1 A	48.1 A	18.8 A	
Pr > F[/f]		*0.3080*	*0.0501*	*0.0520*	

[/a] Isolate designations PAS = *P. aphanidermatum* sensitive to mefenoxam; PAR = *P. aphanidermatum* resistant to mefenoxam; PIS = *P. irregulare* sensitive to mefenoxam; PIR = *P. irregulare* resistant to mefenoxam

[/b] Data from two trials was combined and analyzed using PROC GLIMMIX in SAS (Version 9.3); least squares means shown with Tukey-Kramer grouping (alpha = 0.05) within host type.

[/c] Top weights taken to the nearest gram

[/d] Mean isolate effect across hosts. Isolate differences are indicated by upper case letters.

[/e] Mean host effect across isolates. Host differences are indicated by upper case letters.

[/f] Isolate effect within host. Differences within host are indicated by lower case letters.

Table 1.12. Change in plant heights of poinsettia after inoculation with mefenoxam sensitive and resistant isolates of *Pythium aphanidermatum* and *P. irregulare* in the greenhouse

Species	Isolate[/a]	Run 1[/b,c]	Run 2
P. aphanidermatum	PAS1	13.5 a	-4.8 b
P. aphanidermatum	PAS2	13.6 a	-2.3 ab
P. aphanidermatum	PAS3	14.5 a	-5.9 b
P. aphanidermatum	PAR4	14.3 a	0.0 ab
P. aphanidermatum	PAR5	19.6 a	0.6 ab
P. aphanidermatum	PAR6	18.8 a	-0.4 ab
***P. aphanidermatum* mean**		**15.7**	**-2.1**
P. irregulare	PIS7	19.0 a	18.1 a
P. irregulare	PIS8	15.4 a	13.8 ab
P. irregulare	PIS9	19.0 a	12.5 ab
P. irregulare	PIR10	17.4 a	10.9 ab
P. irregulare	PIR11	22.6 a	13.5 ab
P. irregulare	PIR12	17.4 a	3.8 ab
***P. irregulare* mean**		**18.5**	**12.1**
Non-inoculated Control	NEG	18.5 a	9.5 ab
Pr > F[/d]		*0.1951*	*0.0026*

[/a] Isolate designations PAS = *P. aphanidermatum* sensitive to mefenoxam; PAR = *P. aphanidermatum* resistant to mefenoxam; PIS = *P. irregulare* sensitive to mefenoxam; PIR = *P. irregulare* resistant to mefenoxam

[/b] Data were analyzed using PROC Mixed in SAS (Version 9.3); least squares means shown with Tukey-Kramer grouping (alpha = 0.05) within host type.

[/c] Difference in initial and final heights to the nearest cm

[/d] Isolate effect within run

Table 1.13. Estimated cost of analyzing eight isolates (samples) of *Pythium* by the mefenoxam rapid screening assay

Item	Cost per Unit[a]	Unit	Total Units	Total Cost
Supplies				
Subdue Maxx 2EC	$0.1337	mL	0.0227	$0.0030
Mefenoxam 2 AQ	$0.1099	mL	0.0227	$0.0025
Microtiter Plates	$2.8100	plate	1	$2.8100
Petri Plates	$0.1562	plate	16	$2.4998
Bacto Agar	$0.3597	g	7.5	$2.6978
CMA	$0.1825	g	8.5	$1.5513
V8 Juice	$0.0029	mL	10	$0.0290
CaCO3	$0.1764	g	0.05	$0.0088
Pimaricin	$14.000	mL	0.2	$2.8000
Ampicillin	$0.0063	mg	125	$0.7875
Rifampicin	$0.1664	mg	5	$0.8320
PCNB	$0.0005	mg	50	$0.0250
Labor				
Hourly lab assistant	$10.805	hr	3.25	$35.116
Total Cost				**$49.16**

[a] Costs of materials as of May 15, 2013

Table 1.14. Estimated cost of applying fungicides labeled for control of Pythium root rot in commercial greenhouses in North Carolina

Chemical trade name	Application Rate [a,b]	Cost / 1 oz [c]	Cost / 100 gal	Cost + Labor / 1000 pots [d]	Cost + labor / 10000 pots
Subdue Maxx 2EC	1 oz / 100 gal	3.95	3.95	7.23	72.37
Mefenoxam 2 AQ[e]	1 oz / 100 gal	3.25	3.25	7.02	70.19
Terrazole	3.5 oz / 100 gal	2.36	8.26	8.58	85.83
Terrazole	10 oz / 100 gal	2.36	23.60	13.37	133.73
Truban	3 oz / 100 gal	1.67	5.01	7.57	75.72
Truban	10 oz / 100 gal	1.67	16.70	11.23	112.33

[a] Higher rates used for peat and other high organic potting media
[b] Applied as a soil drench; 4 oz / 6-in pot
[c] Cost in dollars as of May 15, 2013
[d] Labor rates calculated based on $10.81 / hr wages
[e] Generic form of mefenoxam

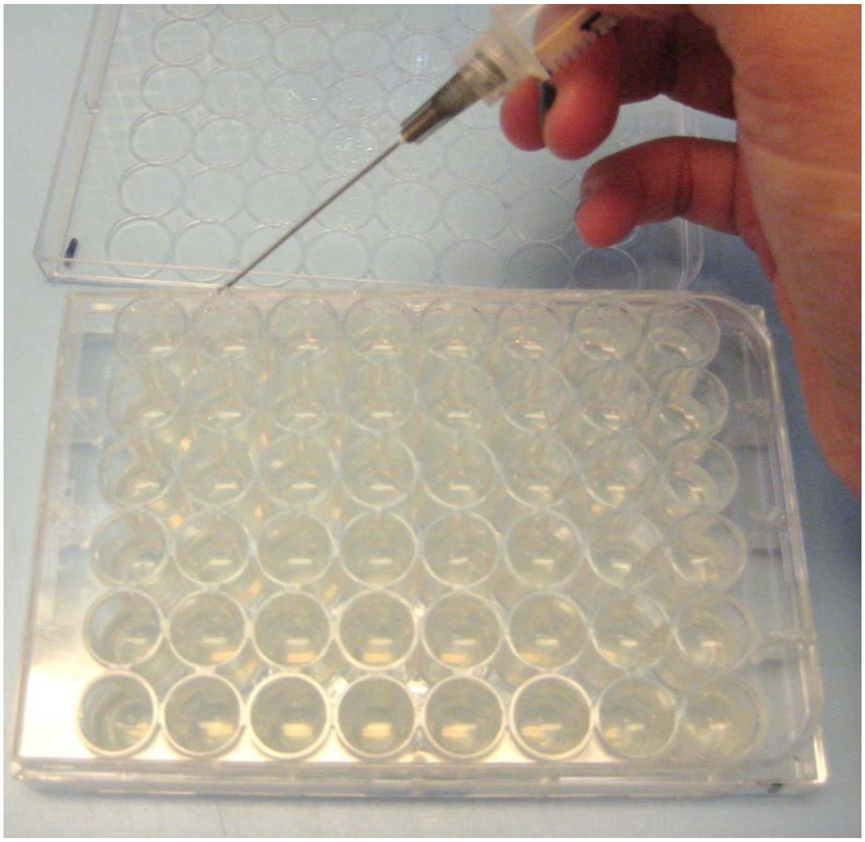

Figure 1.1. Rapid mefenoxam screening assay using a 48-well microtiter plate. Top three rows have media amended with 100 ug a.i./ml and bottom three rows have non-amended media. *Pythium* agar plugs are transferred with a hypodermic needle into each well.

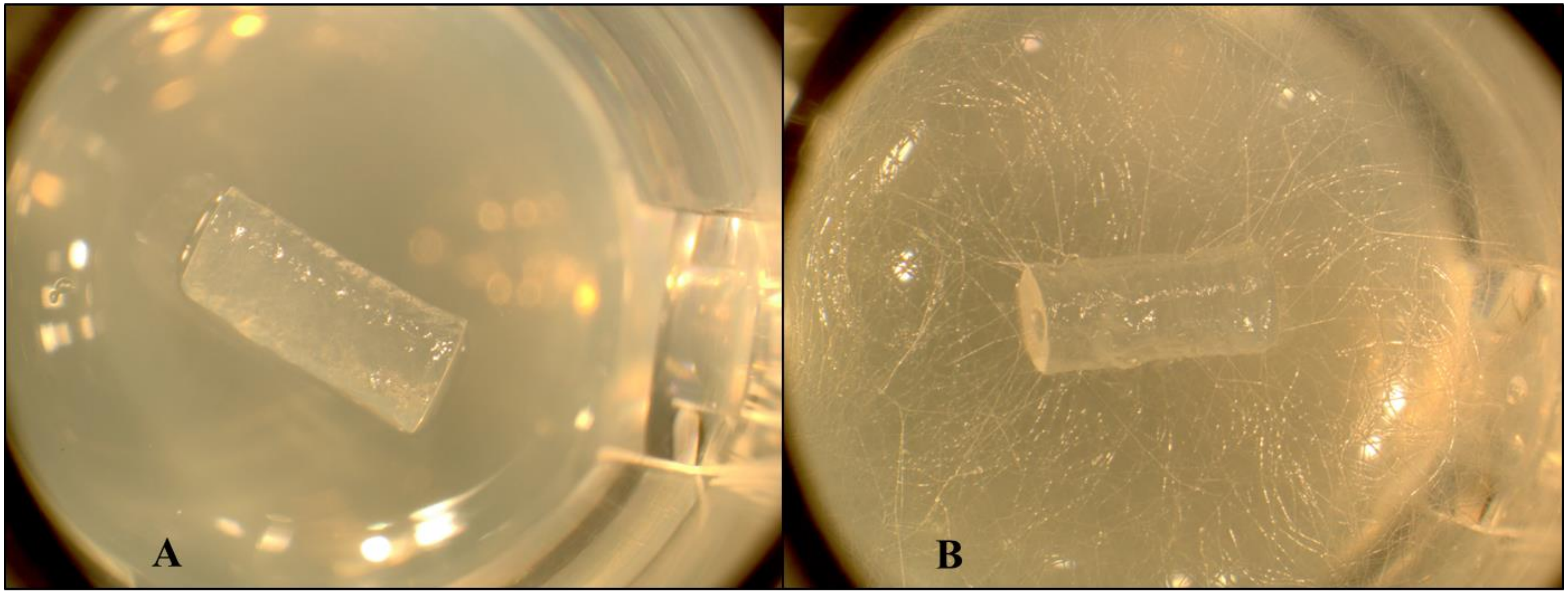

Figure 1.2. Rapid mefenoxam sensitivity assay rating scale. Colonization was scored on a rating scale were 0=no growth, 1= a few hyphae growing from plug but only visible microscopically, 2= hyphae growing uniformly around plug but visible only microscopically, 3= hyphae growing uniformly around plug and just visible macroscopically, 4= hyphae visible macroscopically but not completely covering agar surface, 5= agar surface completely covered by mycelium and growth equal to that in non-amended wells. Isolates with mean sensitivity scores of 4 were considered resistant to mefenoxam. A represents a rating of 0 (sensitive) and B represents a rating of 5 (resistant).

Figure 1.3. Plant health rating scale. At the end of the experiment, the health of each plant was evaluated on a visual scale of 1= healthy, 2= slightly stunted, 3= chlorosis, moderate stunting and/or defoliation, 4= wilting and/or severe stunting, 5= dead.

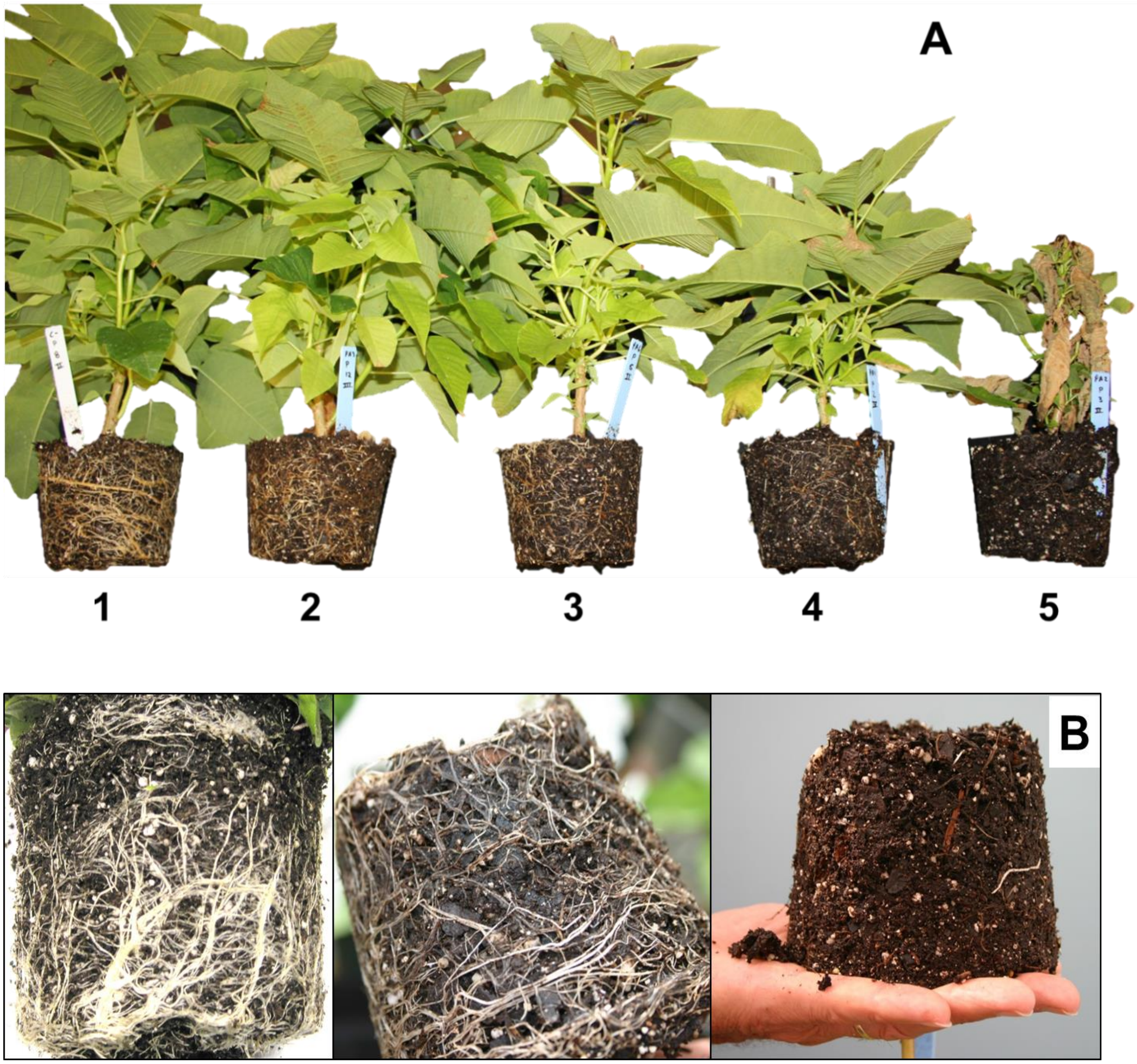

Figure 1.4. Root Rot rating scale. Plants were carefully inverted, the pot was removed, and the roots were observed with the growth media intact. **(A)**Visual root rot ratings were assessed based on the size and integrity of the root ball and root color. The rating scale used was 1= healthy white roots with root ball completely intact, 2= 25% root rot, some root discoloration present, 3= 50% root rot, brown discoloration evident throughout root system, root ball integrity fairly weak, root cortex sloughs off easily, 4= 75% root rot, brown dead roots evident throughout root system, root ball integrity severely compromised, very few white roots, 5= 100% brown dead roots, root ball lost all integrity. **(B)** Close up view of root systems.

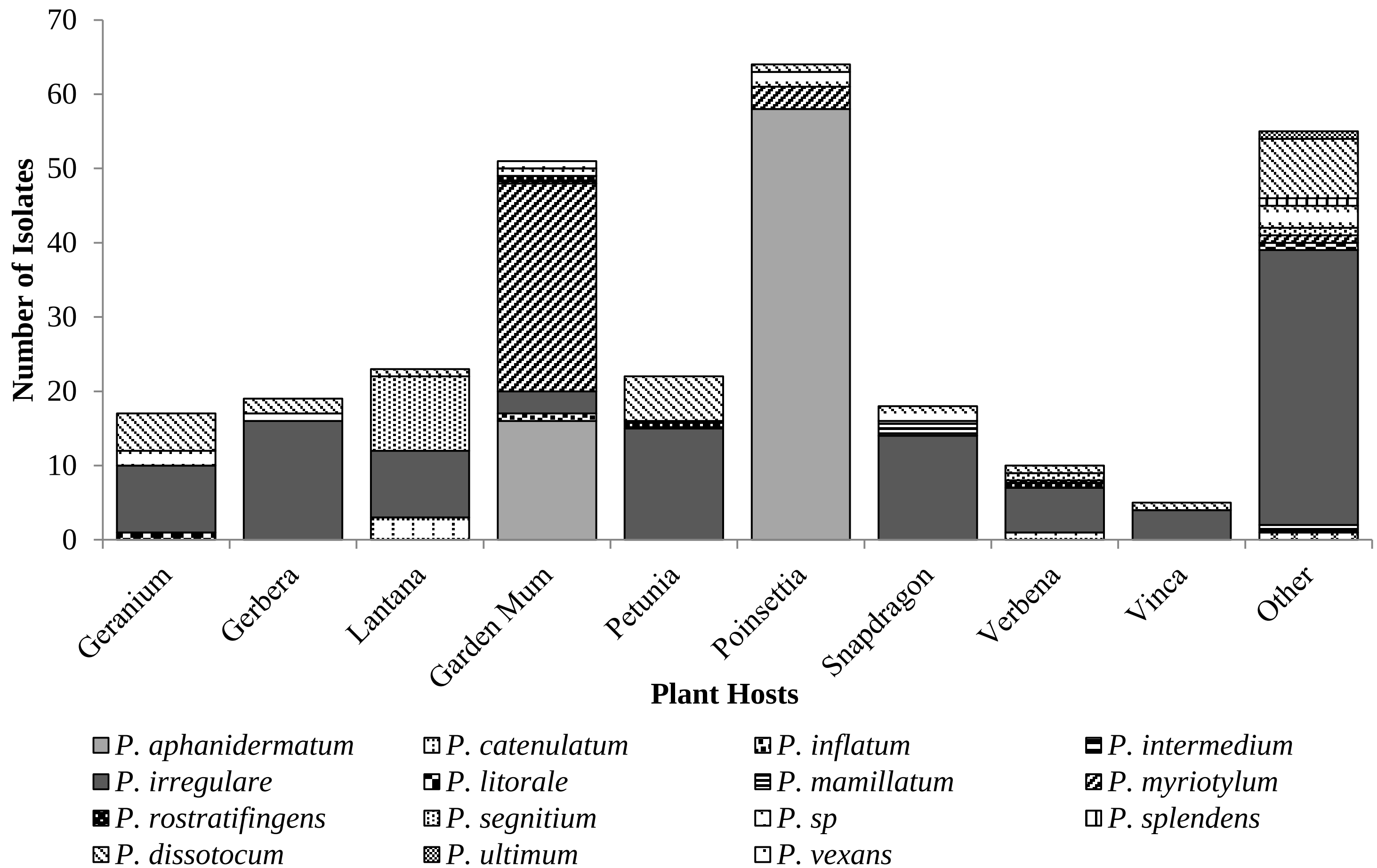

Figure 1.5. Diversity of *Pythium* species isolated from hosts sampled from North Carolina greenhouses. Shading patterns represent different *Pythium* species.

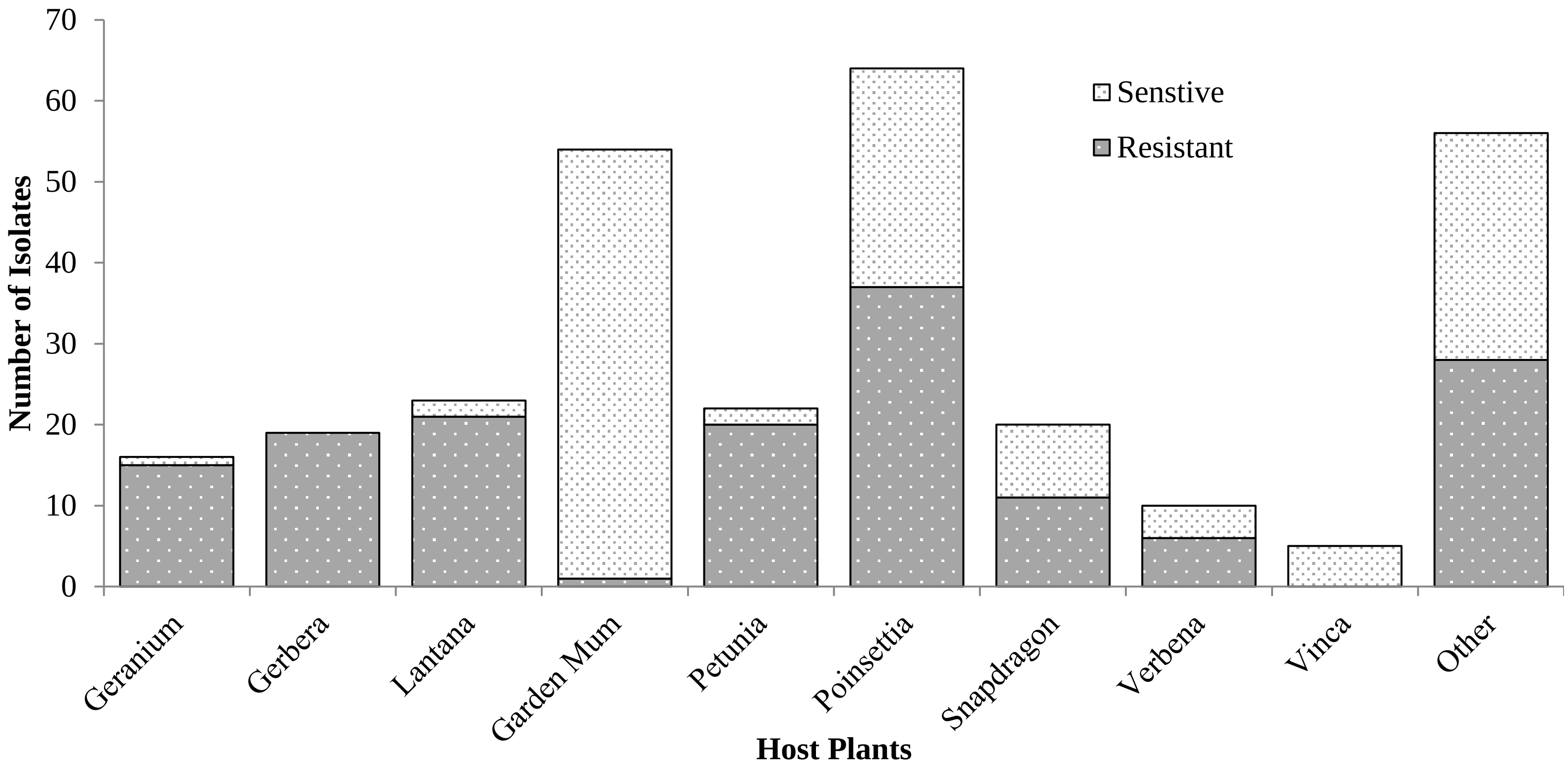

Figure 1.6. Mefenoxam resistance of *Pythium* species grouped by host species of isolation. Shaded dots represent isolates resistant to mefenoxam at 100 µg a.i./ml and white dots represent sensitive isolates.