TEMPORAL AND HOST RELATED VARIATION OF *PYTHIUM* AND *GLOBISPORANGIUM* SPECIES IN FLORICULTURAL CROPS

By

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Abstract: Several species of *Pythium* and *Globisporangium* cause damping off of seedlings, seed rot, stem lesions, and root rot in greenhouse ornamental crops. Two hundred seven isolates from three greenhouses located in Long Island, New York, were collected from chrysanthemum plants in 2014 to identify the diversity of species present at these locations. Analysis of DNA sequences based on ITS region identified fifteen species of *Globisporangium* (n=4) and *Pythium* (n=11). *Globisporangium irregulare*, *G. cryptoirregulare* and *P. aphanidermatum* were the most common species found at the studied facilities. The *G. irregulare* s.l. complex (*G. irregulare*, *G. cryptoirregulare*) was the most prevalent species in 2014, which agrees with results of a previous study that analyzed samples collected over an eleven year period (2002 - 2013) from geranium and other spring season crops. The 2014 results showed the same predominant species in each greenhouse. Moderate to high genetic structure was found between greenhouse populations for each species of the *G. irregulare* complex. Allelic frequencies and distribution suggest local sources of inoculum as well as common sources of inoculum for Pythium diseases of chrysanthemum in the floricultural greenhouse operations studied. When the genetic composition of *G. irregulare* s.l. populations associated with geranium over different seasons and years (2009-2013) were compared, closely related genotypes occurred in the same location over different years, suggesting inoculum may have survived in greenhouse populations from year to year.

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CHAPTER I

INTRODUCTION

Pythium and *Globisporangium* are two sister genera of plant pathogenic oomycetes with ubiquitous distribution that are responsible for infecting diverse crops (Kirk et al. 2001; Moorman 2004). More than 140 host species have been described and around 40 more have been reported since 2000 (Kageyama 2014; Feng et al. 2015) . Soilborne pathogens in these two genera prob. Damping off and rot roots are the most common diseases in ornamental greenhouses, which limit production and cause crop losses (Agrios 2005; Daughtrey 2011).

Traditionally the identification of *Pythium* species has been based on morphological characteristics. However, due to the similarity and intraspecific variation between close related species, morphological identification inaccuracies, have emerged (Garzón et al. 2005a). Biochemical, molecular and phylogenetic criteria have been used for species identification to supplement the morphology based taxonomy (Martin 2000). Nevertheless, identification continues to be difficult because of cryptic morphology and the lack of sequence variation in genetic barcode loci between some species (Levesque and De Cock 2004; Garzón et al. 2007; Garrido 2014).

The ability to identify pathogens causing diseases is important to implement management strategies, particularly in greenhouse production where losses by *Pythium* are documented annually (Garzón et al. 2005b). Several studies have been performed to address this question, most of them on floriculture and ornamental crops (Daughtrey and Benson 2005; Lee et al. 2010; Garrido 2014; Castillo-Munera 2015). In these studies *G. irregulare* (formerly *Pythium irregulare*) along with *P. aphanidermatum* have been identified as the two most common and important pathogens in greenhouse production of ornamentals (Matsumoto et al. 2000; Daughtrey 2005; Garrido 2014).

Proper identification of pathogens to the species level using DNA technologies provide valuable information for the improvement of disease control strategies and have also helped to monitor their presence in greenhouses (Kageyama 2014). This research was initiated to determine the temporal and host related diversity of *Pythium* and *Globisporangium* species present in floricultural greenhouses because of their impact and assess the population structure of the most prevalent pathogenic species to obtain insights about their movement and potential sources of inoculum.

LITERATURE CITED

Agrios, G. N. 2005. Plant pathology. Elsevier Academic Press Amsterdam, The Netherlands:

Castillo-Munera, D. 2015. Characterization, population genetics and management of *Pythium* spp. from floriculture crops in Michigan. Michigan State University.

Daughtrey, M. 2005. Daughtrey Lab: Disease Management in Floral and Nursery Crops. Cornell University, Department of Plant Pathology and Plant-Microbe Biology.

Daughtrey, M. L., and Benson, D. M. 2005. Principles of plant health management for ornamental plants. Phytopatholy. 43:141-169.

Daughtrey, M. 2011. Disease Management in Floral and Nursery Crops. Cornell University, Riverhead, NY.

Feng, W., Ishiguro, Y., Hotta, K., Watanabe, H., Suga, H., and Kageyama, K. 2015. Simple detection of *Pythium irregulare* using loop-mediated isothermal amplification assay. FEMS Microbiology Letters 362(21).

Garrido, P. 2014. Molecular characterization of *Pythium* populations in ornamental greenhouse and nursery crops. Oklahoma State University, Stillwater, Oklahoma.

Garzón, C. D., Geiser, D. M., and Moorman, G. W. 2005a. Amplified fragment length polymorphism analysis and internal transcribed spacer and cox II sequences reveal a species boundary within *Pythium irregulare*. Phytopathology 95:1489-1498.

Garzón, C. D., Geiser, D. M., and Moorman, G. W. 2005b. Diagnosis and population analysis of *Pythium* species using AFLP fingerprinting. Plant Disease 89:81-89.

Garzón, C. D., Yánez, J. M., and Moorman, G. W. 2007. *Pythium cryptoirregulare*, a new species within the *P. irregulare* complex. Mycologia 99:291-301.

Kageyama, K. 2014. Molecular taxonomy and its application to ecological studies of *Pythium* species. Journal of General Plant Pathology 80:314-326.

Kirk, P., Cannon, P., David, J., and Stalpers, J. 2001. Ainsworth & Bisby's Dictionary of the Fungi, 2001. Wallingford UK: CAB International 9.

Lee, S., Garzón, C. D., and Moorman, G. W. 2010. Genetic structure and distribution of *Pythium aphanidermatum* populations in Pennsylvania greenhouses based on analysis of AFLP and SSR markers. Mycologia 102:774-784.

Levesque, C. A., and De Cock, A. W. 2004. Molecular phylogeny and taxonomy of the genus *Pythium.* Mycological Research 108:1363-1383.

Martin, F. N. 2000. Phylogenetic relationships among some *Pythium* species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. Mycologia:711-727.

Matsumoto, C., Kageyama, K., Suga, H., and Hyakumachi, M. 2000. Intraspecific DNA polymorphisms of *Pythium irregulare*. Mycological Research 104:1333-1341.

Moorman, G. 2004. Biology of Pythium root rot. The Pennsylvania State University.

CHAPTER II

LITERATURE REVIEW

Taxonomy

The genus *Pythium* was first described in 1858 by Pringsheim. Since then, several taxonomic descriptions have been reported, most of them based on the comparison of morphological characteristics among species (Van der Plaats-Niterink 1981; Paul and Masih 2000). The genus *Pythium* belongs to the Kingdom Chromista, phylum Oomycota, class Oomycetes, order Pythiales, and family Pythiaceae (Uzuhashi et al. 2010; Schroeder et al. 2013).

Several studies have been performed to understand the evolutionary organization of the genus and clarify its taxonomy (Kamoun et al. 2015). The genus is considered difficult for species identification, based on morphology, because some of the identification characteristics are similar among different species or are not formed in culture (Levesque and De Cock 2004). Biochemical and molecular analysis have been used for species identification to supplement the morphological taxonomy (Martin 2000). Studies using 28S rRNA (Briard et al. 1995), ITS region (Matsumoto et al. 1999), the mitochondrial gene *coxII* (Martin 2000) and nuclear gene β-tubulin (Villa et al. 2006) have found that *Pythium* is a polyphyletic group, in which monophyletic clades are

formed that correspond to the morphology of the sporangium while other features are polyphyletic (Martin 2000; Levesque and De Cock 2004; Garrido 2014).

Morphology complemented by phylogenetic analysis performed on two loci, LSU (large subunit) ribosomal DNA D1/D2 region and cytochrome oxidase *II* gene region, revealed the formation of five clades that reflects morphological variations in sporangia shape (Uzuhashi et al. 2010). Four new genera formerly included in *Pythium* sensu lato (s.l.) were described: *Ovatisporangium*, *Elongisporangium*, *Globisporangium* and *Pilasporangium,* and *Pythium* sensu stricto (s.s.). *Pythium* species were restricted to those with filamentous sporangium, while the genus *Globisporangium* includes species characterized by the production of globose sporangia (Uzuhashi et al. 2010). The recognition of the new four genera is still in flux, and some in the community prefer to follow the classical definition of *Pythium* (Ho et al. 2012; Schroeder et al. 2013). However, the validity of the name *Globisporangium* was accepted and are using it in recent scientific reports (Dr. Gloria Abad, pers, com.; Mycobank).

Biology

Pythium and G*lobisporangium* are two genera that include several soil-borne plant pathogenic species, with a wide host ranges. Several species are non-pathogenic and saprotrophs, some are mycoparasites; and at least one species is an animal pathogen (Daughtrey et al. 1995; Kammarnjesadakul et al. 2011; Schroeder et al. 2013). Reports have shown their potential of infecting seedlings and adult plants, usually through the

root of vegetables (Al-Sa'di et al. 2008a), field crops (Nzungize et al. 2012) , trees (Lazreg et al. 2013; Weiland et al. 2015), lawns, floriculture and ornamental crops, among others (Moorman 2002; Castillo-Munera 2015).

Both genera are the causal agents of a diversity of so-called Pythium diseases including pre and post emergence root rots, blackleg of cuttings, damping-off of seedlings, seed diseases, etc. (Agrios 2005). Most species are non-host selective and the diseases they cause are favored by wet and often cool conditions. Some species like *P. aphanidermatum* and *P. delicense*, are more severe in warm temperatures. Under field and greenhouse conditions *Pythium* spp. represent a problematic pathogenic group because some species have the potential to kill emerging or newly emerged seedlings and reduce crop yield and quality (Schroeder et al. 2013).

Reproduction

Species are routinely identified based on the morphology of asexual and sexual structures. From those the shape of the sporangia and the ornamentation of the oogonium are key characters. Heterothallic and homothallic sexual reproduction, has been used as one of the characteristics for identification along with the origin of antheridium, number of spores by oogonium, zoospores production, etc. (Dick 1991; Garrido 2014). Most of the species are homothallic, and just seven species have been reported as heterothallic (Van der Plaats-Niterink 1981).

Asexual reproduction occurs through sporangia. Sporangia germinates and produce hyphae or vesicles. Zoospores (asexual motile spores) are formed in the vesicles and once they are mature they are released into liquid environment, through which they can swim thanks to their flagella. Zoospores encyst on host surfaces then cyst germinates and a germinal tube infect host tissues (Allen et al. 2004). Zoospores are a way for disease spread in water since they are mobile swimming structures and have access to adjacent healthy plants. Mycelia harbored in soil and plant debris serve as a source of vegetative inoculum in species that produce a few zoospores (Agrios 2005; Yates 2016).

The sexual structures, oogonium and antheridium, may be formed from the same or from two different hyphae. Most species are homothallic and self-fertile. The antheridium attaches to the oogonium, forms a fertilization tube and penetrates the oogonium. The nucleus of the antheridium is transferred to the egg cell within the oogonium. Both sexual structures fuse to form the diploid zygote. Oospores have a dormant phase, after which they germinate, producing a germ tube. The thick-walled oospore is resilient and can survive under adverse conditions, such as during periods of drought, and it can remain viable for a few years. Oospores and sporangia serve as primary inoculum, and their germination is determined by environmental conditions and temperature (Allen et al. 2004; Agrios 2005; Nzungize et al. 2012).

Symptoms and management

Pythium diseases can be diagnosed based on symptoms and pathogen isolation. On mature plants diagnosis is difficult because plants often remain asymptomatic until symptoms start to manifest after a period of plant stress (Schroeder et al. 2013). Symptoms usually are similar from one plant to another and include wilting, stunting, chlorosis, decayed roots, poor seed germination and emergence, resulting in reduction of crop quality (Daughtrey and Miller 2009).

Examining crops regularly to look for signs and symptoms is a good practice but management strategies need to be more about prevention and eradication. Prevention starts with the use of disease free plant material or surface sterilized propagative materials. Sanitation should include discarding potential sources of inoculum (sterilizing pots and removing infected plant material). Eradication should include scouting and application of fungicide treatments and disposal of symptomatic plants (Agrios 2005; Al-Sa'di et al. 2008b; Garzón et al. 2011). Fungicides resistance to certain chemistries have been reported in some *Pythium* and *Globisporangium* species, particularly to mefenoxam and propamocarb (Moorman and Kim 2004). Fungicide resistance has been reported to have an effect on the genetic diversity of some species (Lee et al. 2010).

Pythium and *Globisporangium* in greenhouse floricultural crops

Within the agricultural sector, growing flowers and ornamental plants has been one of the fastest growing area in the United States and has generated great economic benefits. On last year's report, the floriculture production values showed an increase of four percent compared to 2014, with an estimated value of $4.37 billion in sales (USDA 2016).

It is common knowledge that the inoculum of the pathogen can be harbored in or on many potential sources including infected plant material, plant debris, soil, tools and equipment, potting mixtures, irrigation water, among others (Al-Sa'di et al. 2008b). Diseases usually appear in areas with poor drainage, high soluble salts or where conditions favorable for pathogen development and spread occur (Schroeder et al. 2013). With little knowledge about the movement of inoculum, epidemiological studies are challenging in ornamentals because of the diversity of crops and multiple potential sources of inoculum (Garrido 2014).

Increases in production of ornamental and floriculture crops have been accompanied with an increase in diseases caused by *Pythium* spp. and *Globisporangium* spp.; limiting factors for profitable production in greenhouses (Daughtrey 2011). Former studies show that the main species that affect floricultural crops are *G. irregulare, P. aphanidermatum* and members of Levesque et al. (2004) *Pythium* group F (Martin 2000; Levesque and De

Cock 2004). Of these, the predominant species are those belonging to the *Globisporangium irregulare* species complex (Moorman et al. 2002).

Molecular phylogeny

The identification and characterization of the species of *Pythium* and *Globisporangium* based on morphological characteristics is challenging due to the presence of cryptic species (Garzón et al. 2007). Internal transcript spacers (ITS) regions of the ribosomal DNA are widely used in phylogenetic studies thanks to the development of universal PCR primers (White et al. 1990) that amplify a highly variable region in all taxa (Levesque and De Cock 2004). The low level of sequence divergence among phylogenetically close species using the ITS region have boosted the use of other loci for phylogenetic analysis. Those include nuclear encoded genes, β-tubulin and LSU, as well as mitochondrial encoded genes as cytochrome oxidase I and II, including intergenic spacers. In oomycetes ITS and *coxII* gene are employed and recommended as DNA barcodes (Kageyama 2014).

Matsumoto et al. (1999) used ITS sequences for the differentiation of isolates at species level and found a relationship based on the sporangium morphology. Similar results were found by Levesque and Cock (2004). Levesque and Cock (2004) proposed the existence of 11 clades (A-K), using species identified by Van der Plaats-Niterink (1981), within the *Pythium* genus. Villa et al. (2006), in the analysis of *Pythium* and

Phytophthora species based on three genomic regions (ITS rDNA, cytochrome oxidase II and beta-tubulin gene), showed that members of the clade K were closer to *Phytophthora*. Finally was proposed the creation of a new genera called *Phytopythium* (Bala et al. 2010; Kageyama 2014; De Cock et al. 2015).

Globisporangium irregulare complex

Globisporangium irregulare is a species complex with worldwide distribution and broad host range. The complex includes *G. regulare, G. cylindrosporum, G. irregulare* s.s. and *G. cryptoirregulare* (Spies et al. 2011). This is a challenging group for identification because of the variability in morphology and the high levels of intraspecific genetic diversity (Garzón et al. 2005a). Matsumoto et al. (1999) performed RAPC-PCR (random amplified polymorphic DNA) analyses and phylogenetic analysis of the ITS region and found the formation of four groups (I, II, III, IV) within the complex. Groups III and IV were not significant as plant pathogens and different from groups I and II. Levesque and Cock (2004), based on ITS sequences, proposed the existence of 11 clades (A-K) in the genus *Pythium*. According to this study, *G. irregulare* s.l. belongs to clade F and includes four paraphyletic clusters. Garzon et al (2005a, 2007), using AFLP fingerprinting and sequencing of the ITS region and the *cox I-II* mitochondrial region, found that the complex has undergone speciation process and supported the separation of groups I and II sensu Matsumoto et al (1999). Those groups were defined as *G. irregulare* sensu stricto and *G. cryptoirregulare* respectively, while groups III and IV

clustered closer to *G. sylvaticum* than to *G. irregulare*, and remain still unresolved. Garrido (2014) performed a multigene phylogeny and her findings were congruent with Matsumoto and Garzon supporting the formation of four clades, that represents at least two sister species. Genetic differentiation within the species complex was found and one of the clades, clade II, was consistent with *G. cryptoirregulare* described by Garzon et al (2007). The analysis allowed to differentiate *G. irregulare* from *G. cryptoirregulare*. The existence of hybrids among *G. irregulare* s.s. and *G. cryptoirregulare* has been suggested (Garzón et al. 2007; Lee and Moorman 2008). Spies et al. (2011) after the molecular analysis with nuclear genes (ITS and β-tubulin), mitochondrial regions (coxI and coxII) and isozymes, found evidence of aneuploidy and putative hybridization, however, the authors suggested that the complex be maintained as one species *G. irregulare* (Spies et al. 2011).

Microsatellite based population genetics

Single sequence repeats (SSRs) are a good markers to use for the analysis of intraspecific variation and genetic diversity (Schroeder et al. 2006). These neutral markers also called microsatellites, are short tandemly repeated nucleotide sequences used for genetic characterization studies. The main advantage of these markers are their high reproducibility and codominance (Abdel-Mawgood 2012).

Population genetic analyses are valuable for answering questions such as whether new organisms have been introduced to an area or have emerged from local populations and how populations are structured (Grünwald and Goss 2011; Hedrick 2011; Karlin 2012; Milgroom 2015). Several studies have developed SSR markers for *Pythium* genetic analysis specifically, for species such as *P. aphanidermatum*, *G. irregulare*, *G. cryptoirregulare* (Lee and Moorman 2008) and *P. helicoides* (Zhou et al. 2009). In the study of *P. aphanidermatum* isolates from different greenhouses located in Pennsylvania using SSR markers a population structure was determined by location and fungicide resistance rather than by host (Lee et al. 2010). Population genetics analysis of *Phytophthora ramorum*, the causal agent of sudden oak death, have shown evidence of their introduction to U.S. at least three times via nursery trade (Parke and Grünwald 2012). Examples like these provide evidence of the introduction of pathogens and the movement of inoculum highlighting the importance of this type of research.

LITERATURE CITED

Abdel-Mawgood, A. L. 2012. DNA based techniques for studying genetic diversity.

Agrios, G. N. 2005. Plant pathology. Elsevier Academic Press Amsterdam, The Netherlands.

Al-Sa'di, A., Drenth, A., Deadman, M., DeCock, A., Al-Said, F., and Aitken, E. 2008a. Genetic diversity, aggressiveness and metalaxyl sensitivity of *Pythium spinosum* infecting cucumber in Oman. Journal of Phytopathology 156:29-35.

Al-Sa'di, A., Drenth, A., Deadman, M., Al-Said, F., Khan, I., and Aitken, E. 2008b. Potential sources of *Pythium* inoculum into greenhouse soils with no previous history of cultivation. Journal of Phytopathology 156:502-505.

Allen, T. W., Martinez, A., and Burpee, L. L. 2004. Pythium blight of turfgrass. The Plant Health Instructor.

Bala, K., Robideau, G., and Lévesque, C. 2010. *Phytopythium* Abad, de Cock. Bala, Robideau, Lodhi & Levesque, gen. nov. and *Phytopythium sindhum* Lodhi, Shahzad & Levesque, sp. nov. Persoonia 24:136-137.

Briard, M., Dutertre, M., Rouxel, F., and Brygoo, Y. 1995. Ribosomal RNA sequence divergence within the Pythiaceae. Mycological Research 99:1119-1127.

Castillo-Munera, D. 2015. Characterization, population genetics and management of *Pythium* spp. from floriculture crops in Michigan. Michigan State University.

Daughtrey, M. L., Wick, R. L., and Peterson, J. L. 1995. Compendium of flowering potted plant diseases. American Phytopathological Society (APS Press).

Daughtrey, M., and Miller, W. 2009. Reducing root rot,bulb rot and vascular wil disease losses in floral crops by improvements in pathogen tracking and management.

Daughtrey, M. 2011. Disease Management in Floral and Nursery Crops. Cornell University, Riverhead, NY.

De Cock, A., Lodhi, A., Rintoul, T., Bala, K., Robideau, G., Abad, Z. G., Coffey, M., Shahzad, S., and Lévesque, C. 2015. *Phytopythium*: molecular phylogeny and systematics. Persoonia: Molecular Phylogeny and Evolution of Fungi 34:25.

Dick.M.W. 1991. Keys to *Pythium*. Mycologia 83:386-387.

Garrido, P. 2014. Molecular characterization of *Pythium* populations in ornamental greenhouse and nursery crops. Oklahoma State University, Stillwater, Oklahoma.

Garzón, C. D., Geiser, D. M., and Moorman, G. W. 2005a. Amplified fragment length polymorphism analysis and internal transcribed spacer and cox II sequences reveal a species boundary within *Pythium irregulare*. Phytopathology 95:1489-1498.

Garzón, C. D., Yánez, J. M., and Moorman, G. W. 2007. *Pythium cryptoirregulare*, a new species within the *P. irregulare* complex. Mycologia 99:291-301.

Garzón, C. D., Molineros, J. E., Yánez, J. M., Flores, F. J., del Mar Jiménez-Gasco, M., and Moorman, G. W. 2011. Sublethal doses of mefenoxam enhance Pythium damping-off of geranium. Plant Disease 95:1233-1238.

Grünwald, N. J., and Goss, E. M. 2011. Evolution and population genetics of exotic and re-emerging pathogens: novel tools and approaches. Annual Review of Phytopathology 49:249-267.

Hedrick, P. W. 2011. Genetics of populations. Jones & Bartlett Learning.

Ho, H.-H., Chen, X.-X., Zeng, H.-C., and Zheng, F.-C. 2012. The occurrence and distribution of *Pythium* species on Hainan Island of South China. Botany 53:525-534.

Kageyama, K. 2014. Molecular taxonomy and its application to ecological studies of *Pythium* species. Journal of General Plant Pathology 80:314-326.

Kammarnjesadakul, P., Palaga, T., Sritunyalucksana, K., Mendoza, L., Krajaejun, T., Vanittanakom, N., Tongchusak, S., Denduangboripant, J., and Chindamporn, A. 2011. Phylogenetic analysis of *Pythium insidiosum* Thai strains using cytochrome oxidase II (COX II) DNA coding sequences and internal transcribed spacer regions (ITS). Medical mycology 49:289-295.

Kamoun, S., Furzer, O., Jones, J. D., Judelson, H. S., Ali, G. S., Dalio, R. J., Roy, S. G., Schena, L., Zambounis, A., and Panabières, F. 2015. The Top 10 oomycete pathogens in molecular plant pathology. Molecular plant pathology 16:413-434.

Karlin, S. 2012. Population Genetics and Ecology. Elsevier.

Lazreg, F., Belabid, L., Sanchez, J., Gallego, E., and Garrido-Cardenas, J. A. 2013. First Report of *Globisporangium ultimum* Causing Pythium Damping-Off on Aleppo Pine in Algeria, Africa, and the Mediterranean Region. Plant Disease 97:1111-1113.

Lee, S., and Moorman, G. W. 2008. Identification and characterization of simple sequence repeat markers for *Pythium aphanidermatum*, *P. cryptoirregulare*, and *P. irregulare* and the potential use in *Pythium* population genetics. Current genetics 53:81-93.

Lee, S., Garzón, C. D., and Moorman, G. W. 2010. Genetic structure and distribution of *Pythium aphanidermatum* populations in Pennsylvania greenhouses based on analysis of AFLP and SSR markers. Mycologia 102:774-784.

Levesque, C. A., and De Cock, A. W. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. Mycological Research 108:1363-1383.

Martin, F. N. 2000. Phylogenetic relationships among some *Pythium* species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. Mycologia:711-727.

Matsumoto, C., Kageyama, K., Suga, H., and Hyakumachi, M. 1999. Phylogenetic relationships of *Pythium* species based on ITS and 5.8 S sequences of the ribosomal DNA. Mycoscience 40 321-331.

Milgroom, M. G. 2015. Population Biology of Plant Pathogens: Genetics, Ecology, and Evolution. APS Press.

Moorman, G., Kang, S., Geiser, D., and Kim, S. 2002. Identification and characterization of *Pythium* species associated with greenhouse floral crops in Pennsylvania. Plant Disease 86:1227-1231.

Moorman, G., and Kim, S. 2004. Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. Plant Disease 88:630-632.

Nzungize, J. R., Lyumugabe, F., Busogoro, J. P., and Baudoin, J. P. 2012. Pythium root rot of common bean: biology and control methods. A review. Biotechnologie, Agronomie, Société et Environnement 16:405.

Parke, J. L., and Grünwald, N. J. 2012. A systems approach for management of pests and pathogens of nursery crops. Plant Disease 96:1236-1244.

Paul, B., and Masih, I. 2000. ITS1 region of the nuclear ribosomal DNA of the mycoparasite *Pythium periplocum*, its taxonomy, and its comparison with related species. FEMS Microbiology Letters 189:61-65.

Schroeder, K., Okubara, P., Tambong, J., Lévesque, C., and Paulitz, T. 2006. Identification and quantification of pathogenic *Pythium* spp. from soils in eastern Washington using real-time polymerase chain reaction. Phytopathology 96:637-647.

Schroeder, K. L., Martin, F. N., de Cock, A. W., Lévesque, C. A., Spies, C. F., Okubara, P. A., and Paulitz, T. C. 2013. Molecular detection and quantification of *Pythium* species: evolving taxonomy, new tools, and challenges. Plant Disease 97:4-20.

Spies, C. F., Mazzola, M., Botha, W. J., Langenhoven, S. D., Mostert, L., and Mcleod, A. 2011. Molecular analyses of *Pythium irregulare* isolates from grapevines in South Africa suggest a single variable species. Fungal Biology 115:1210-1224.

USDA, N. A. S. S. 2016. Floriculture Crops 2015 Summary (April 2016). U. S. D. o. Agriculture, ed.

Uzuhashi, S., Tojo, M., and Kakishima, M. 2010. Phylogeny of the genus *Pythium* and description of new genera. Mycoscience 51:337-365.

Van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium.* Studies in Mycology.

Villa, N. O., Kageyama, K., Asano, T., and Suga, H. 2006. Phylogenetic relationships of *Pythium* and *Phytophthora* species based on ITS rDNA, cytochrome oxidase II and β-tubulin gene sequences. Mycologia 98:410-422.

Weiland, J. E., Garrido, P., Kamvar, Z. N., Espíndola, A. S., Marek, S. M., Grünwald, N. J., and Garzón, C. D. 2015. Population Structure of *Pythium irregulare*, *P. ultimum*, and *P. sylvaticum* in Forest Nursery Soils of Oregon and Washington. Phytopathology 105:684-694.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18:315-322.

Yates, R. 2016. How To Prevent *Pythium* In Fall Garden Mums. in: Crop Inputs, Greenhouse grower, ed.

Zhou, W., Motohashi, K., Suga, H., Fukui, H., and Kageyama, K. 2009. Development of microsatellite markers for *Pythium helicoide*s. FEMS Microbiology Letters 293:85-91.

CHAPTER III

SPECIES DIVERSITY OF *PYTHIUM* AND *GLOBISPORANGIUM* SPECIES PRESENT IN ORNAMENTAL CROPS FROM LONG ISLAND, NEW YORK

Introduction

Pythium and *Globisporangium* species (Straminopila, Oomycota) are genetically diverse with great variation in virulence, host range and distribution (Adhikari et al. 2013). Taxonomic descriptions in Pythiaceae family are based on morphological characteristics (Van der Plaats-Niterink 1981) and molecular criteria are now commonly used to supplement the taxonomy (Kamoun et al. 2015).

In ornamental greenhouses, damping off and rot roots are the most common Pythium diseases. These diseases can limit the production of seedlings and become problematic since reservoirs of the pathogen can remain in soils, sediments, water sources, tools and residues from previous crops (Agrios 2005; Daughtrey and Miller 2009). While the flower industry continue its growth, growers of ornamental crops cannot afford the presence of diseased plants that represent economic losses (Daughtrey 2011).

Research has been done to identify the diversity of species found in floricultural crops (Moorman et al. 2002; Garrido 2014; Castillo-Munera 2015). Garrido (2014) performed an analysis of species diversity from different greenhouses located at Long

Island N.Y. from various floricultural hosts. 22 *Pythium* species were identified including pathogenic and nonpathogenic species. The predominant species were *G. irregulare* complex, *P. aphanidermatum* and *G. ultimum*. A similar analysis was performed by Castillo-Munera (2015) to identify *Pythium spp*. associated with Michigan floriculture crops. *G. irregulare*, *P. aphanidermatum* and *G. ultimum* were again the most prevalent in a variety of crops. Both results are similar with what have been previously reported in Pennsylvania (Moorman et al. 2002).

The identification of the species associated with greenhouses using molecular techniques has helped to clarify phylogenetic relationships in diverse groups. One of the firsts steps is to identify pathogens since effective practices relies on the accurate species identification and knowledge of the pathogen biology (Weiland et al. 2013). The objective of this study was to identify the species of *Pythium* and *Globisporangium* affecting chrysanthemum in different greenhouses in Long Island, NY using DNA sequencing of ITS region and determine the most prevalent pathogenic group affecting the studied facilities.

Materials and Methods

Isolates

Two hundred seven isolates from *Chrysanthemum* plants were collected seasonally at three floricultural greenhouses located in Long Island, New York in 2014 (Supplementary Table 1). Isolates were identified morphologically (Van der Plaats-Niterink 1981) at Cornell University. Frozen mycelia for DNA purification were

provided by Margery Daughtrey (Cornell University's Long Island Horticultural Research and Extension Center).

DNA extraction

Mycelia were kept frozen upon arrival and lyophilized. Lyophilized mycelia were used to isolate DNA using DNeasy® Plant Mini kit (Qiagen, Hilden, Germany). DNA extractions were performed from 40 mg of mycelium according to manufacturer's instructions. DNA quantity and quality were measured using NanoDrop ND-1000 spectrophotometer (NanoDrop technologies, Wilmington, DE).

DNA amplification and sequencing

DNA sequence analysis of the internal transcribed spacer of the nuclear rDNA region (ITS) of all the isolates was performed. The ITS region was amplified using previously reported PCR conditions (Moorman et al. 2002; Garrido 2014) and primers ITS2 and ITS4, described in Table III-1. Amplification reactions were performed in 25 µL reactions containing 1.25 µL of each 5µM primer, 2 µL of 25 ng/µL DNA, and 12.5 µL of 2X GoTaq Green Master Mix (Promega, Madison, WI, USA). To check for the presence of PCR products, 5 uL of the PCR reaction mixture was loaded in 1% (Takara Bio) agarose gel, electrophoresed at 95 V for 1 hour, stained with ethidium bromide, visualized and photographed under UV illumination. PCR products were prepared for sequencing using the enzymatic purification kit ExoSAP-IT (USB Corporation, Cleveland), following the manufacturer's instructions. Sequencing was performed using the same primers in the initial PCR step. Sequencing reactions products were run on a

ABI 3100 DNA sequencer (Applied Biosystems) at the Recombinant DNA and Protein Core Facility of Oklahoma State University.

Table III-1. Primers used for PCR amplification and sequencing of the ITS region

Locus	Primer name	Primer sequence (5'-3')	Ta (˚C)	Reference	Fragment length
ITS	ITS 1 ITS 4	TCC GTA GGT GAA CCT GCG G TCC TCC GCT TAT TGA TAT GC	59	(White et al. 1990)	773-959 bp

Ta = annealing temperature

Identification

Isolates identities were verified at species level comparing ITS sequences with sequences available at the National Center for Biotechnology Information (NCBI) nucleotide database using the tool BLASTn**.** Sense and antisense sequences were aligned, edited and assembled using Geneious 6.0.6 (Kearse et al. 2012) to create consensus sequences for each isolate.

Results

ITS DNA sequence analysis of 207 isolates from three greenhouses located in Long Island NY, collected in 2014 identified fifteen plant pathogenic and non-pathogenic species of two genera, *Globisporangium* (n=4) and *Pythium* (n=11) (Fig. III-1). The most abundant species associated at the three greenhouses were *G. irregulare* sensu lato (s.l.) (59.90%), *P. aphanidermatum* (13.5%) and *G. ultimum* (11.6%). In each greenhouse the *Pythium* and *Globisporangium* species community compositions was different, but in the three greenhouses the most prevalent species was *G. irregulare* s.l.

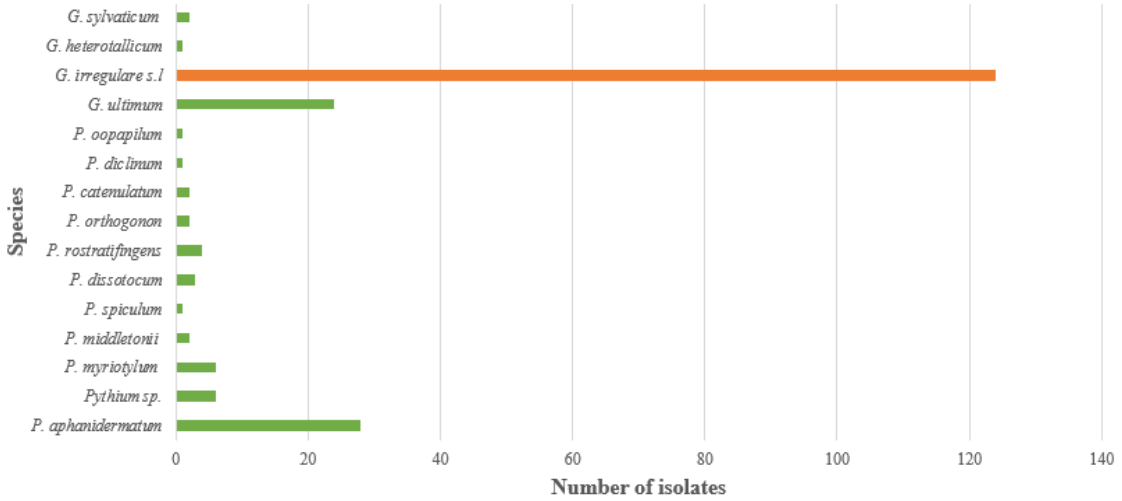

Figure III-1. Total number of *Pythium* and *Globisporangium* species identified with ITS region associated with *Chrysanthemum* greenhouses from Long Island, NY in 2014. *P = Pythium*; *G = Globisporangium*

An update of the species diversity found at 8 different greenhouses in Long Island NY from isolates collected from 2002 to 2015 were performed. 29 species of *Pythium* (n=21), *Globisporangium* (n=8) were identified. The species recovered from the different greenhouses were diverse, but *G. irregulare* complex (58.94%) continued to be the most prevalent followed by *P. aphanidermatum* (8.16%) (Fig. III-2).

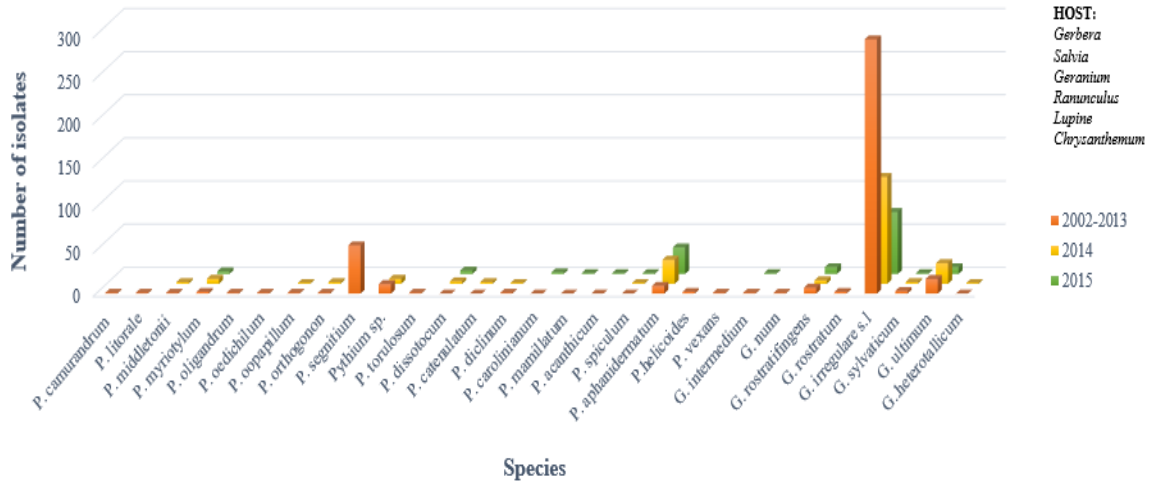

Figure III-2. Total number of species of *Pythium*, *Globisporangium* species identified with ITS region associated with floricultural greenhouses from Long Island, NY collected by year.

Discussion

Pythium and *Globisporangium* species are among the main diseases causal agents in ornamental crops, affecting seed germination, seedling development, and established plants' aesthetics, causing important economic losses. In addition to pathogenic species, diverse non-pathogenic species can also be found in ornamental greenhouses, which do not require management. Since the application of disease management strategies is often decided based only on the presence of oospores in plant roots, unnecessary and costly treatments may be applied. Therefore, effective identification of each species has become a necessity to improve epidemiological studies and the implementation of better management strategies to control Pythium diseases (Daughtrey & Benson, 2005).

The diversity of *Pythium* and *Globisporangium* species affecting chrysanthemum in three ornamental greenhouses located in Long Island New York, was conducted using a collection of isolates sampled in 2014. ITS region sequences identified *G. irregulare* s.l (59.9%) and *P. aphanidermatum* (13.5%) as the most common plant pathogenic species at those facilities. This two species were also found by Garrido (2014) in samples collected from 2002 to 2013 in Long Island NY. *G. irregulare* s.l continue to be the most predominant species causing disease to floricultural greenhouse crops, as has been reported in previous studies (Moorman et al. 2002; Garzón et al. 2005a; Daughtrey and Miller 2009). *G. irregulare* is characterized by their globose sporangia, their virulence at cooler temperatures and their high morphological and genetic diversity (Martin and Loper 1999; Adhikari et al. 2013).

The second most common species found, *P. aphanidermatum*, has been reported as a causal agent of root and basal-stem rots in garden mums (Yates 2016). This species is more aggressive as a pathogen compared to *G. irregulare*, and little is known about its population biology (Lee et al. 2010). The pathogen spread is favored by warm temperatures, making it an important issue in greenhouses (Parker 2001).

The isolates used in this study came from a single host, *Chrysanthemum.* Moorman et al. (2002) found that *Begonia*, *Chrysanthemum* and *Antirrhinum* are commonly infected by *G. irregulare* and *G. ultimum*. The potential sources of inoculum for diseases of floricultural greenhouse crops are many: plant debris, soil, irrigation tools, water, etc. Additionally, the movement of workers and of equipment between greenhouses can facilitate the movement of the pathogen from one location to another (Al-Sa'di et al. 2008b). In general, disease management of *Pythium* diseases have been accomplished by practices like exclusion: discarding potential sources of inoculum, the use of disease free plant material, sterilized soil and prevention via sanitation (Agrios 2005; Garzón et al. 2011). The presence of isolates of the same species in all three greenhouses suggest the possibility of common sources of inoculum. However, *G. irregulare* s.l. is a common soil inhabitant, therefore, further analysis are needed to determine if the inoculum for chrysanthemum infections at the studied facilities were local or introduced through infected propagative materials or infested potting materials.

Sequences used for species identification purposes are known as DNA barcodes. ITS region is one of the recommended barcodes for identification of oomycetes (Robideau et al. 2011). It has been widely used because of the high sequence variability in the

intergenic spacers, which are flanked by highly conserved rDNA regions in oomycetes which allows the use of the same primers in a wide range of eukaryotic species (Tambong et al. 2006). Although ITS region can be useful to differentiate most *Pythium* and *Globisporangium* species, this barcode alone cannot resolve the closely related and cryptic species within the *G. irregulare* complex. No evident morphological differences between *Gl. irregulare* and *Gl. cryptoirregulare* have been reported. In order to discriminate closely related species a multilocus sequence typing (MLST) approach is required (Robideau et al. 2011). The discrimination among isolates of the *G. irregulare* complex and their population biology will be addressed in the next chapter.

LITERATURE CITED

Adhikari, B. N., Hamilton, J. P., Zerillo, M. M., Tisserat, N., Lévesque, C. A., and Buell, C. R. 2013. Comparative genomics reveals insight into virulence strategies of plant pathogenic oomycetes. PLoS One 8:e75072.

Agrios, G. N. 2005. Plant pathology. Elsevier Academic Press Amsterdam, The Netherlands.

Al-Sa'di, A., Drenth, A., Deadman, M., Al-Said, F., Khan, I., and Aitken, E. 2008b. Potential sources of *Pythium* inoculum into greenhouse soils with no previous history of cultivation. Journal of Phytopathology 156:502-505.

Castillo-Munera, D. 2015. Characterization, population genetics and management of Pythium spp. from floriculture crops in Michigan. Michigan State University.

Daughtrey, M. L., and Benson, D. M. 2005. Principles of plant health management for ornamental plants. Phytopatholy 43:141-169.

Daughtrey, M., and Miller, W. 2009. Reducing root rot,bulb rot and vascular wil disease losses in floral crops by improvements in pathogen tracking and management. Department of Plant Pathology, Cornell University, Riverhead, NY.

Daughtrey, M. 2011. Disease Management in Floral and Nursery Crops. Cornell University, Riverhead, NY.

Garrido, P. 2014. Molecular characterization of *Pythium* populations in ornamental greenhouse and nursery crops. Oklahoma State University, Stillwater, Oklahoma.

Garzón, C. D., Geiser, D. M., and Moorman, G. W. 2005a. Amplified fragment length polymorphism analysis and internal transcribed spacer and cox II sequences reveal a species boundary within *Pythium irregulare*. Phytopathology 95:1489-1498.

Garzón, C. D., Molineros, J. E., Yánez, J. M., Flores, F. J., del Mar Jiménez-Gasco, M., and Moorman, G. W. 2011. Sublethal doses of mefenoxam enhance Pythium damping-off of geranium. Plant Disease 95:1233-1238.

Kamoun, S., Furzer, O., Jones, J. D., Judelson, H. S., Ali, G. S., Dalio, R. J., Roy, S. G., Schena, L., Zambounis, A., and Panabières, F. 2015. The Top 10 oomycete pathogens in molecular plant pathology. Molecular Plant Pathology 16:413-434.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., and Duran, C. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649.

Lee, S., Garzón, C. D., and Moorman, G. W. 2010. Genetic structure and distribution of *Pythium aphanidermatum* populations in Pennsylvania greenhouses based on analysis of AFLP and SSR markers. Mycologia 102:774-784.

Martin, F. N., and Loper, J. E. 1999. Soilborne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. Critical Reviews in Plant Sciences 18:111-181.

Moorman, G., Kang, S., Geiser, D., and Kim, S. 2002. Identification and characterization of *Pythium* species associated with greenhouse floral crops in Pennsylvania. Plant Disease 86:1227-1231.

Parker, K. C. 2001. *Pythium aphanidermatum*. N. S. University, ed.

Robideau, G. P., De, c., Arthur, W., Coffey, M. D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D. W., Désaulniers, N., and Eggertson, Q. a. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Molecular Ecology Resources 11:1002-1011.

Tambong, J., De Cock, A., Tinker, N., and Lévesque, C. 2006. Oligonucleotide array for identification and detection of *Pythium* species. Applied and Environmental Microbiology 72:2691-2706.

Van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium.* Studies in Mycology.

Weiland, J., Littke, W., and Haase, D. 2013. Forest nurseries face critical choices with the loss of methyl bromide fumigation. California Agriculture 67:153-161.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A guide to Methods and Applications 18:315-322.

Yates, R. 2016. How To Prevent *Pythium* In Fall Garden Mums. in: Crop Inputs, Greenhouse grower, ed.

CHAPTER IV

PHYLOGENETIC ANALYSIS AND POPULATION STRUCTURE OF *Globisporangium irregulare* s.l FROM LONG ISLAND, NY GREENHOUSES IN 2014

Introduction:

Globisporangium irregulare s.l is a species complex that includes two cryptic species, *G. cryptoirregulare* and *G. irregulare* s.s (Garzón et al. 2005a; Garzón et al. 2007). *G. irregulare* s.l have a worldwide distribution, a wide host range (Van der Plaats-Niterink 1981; Matsumoto et al. 2000) and have been reported as one of the most common species in floricultural and ornamental crops (Moorman and Kim 2004; Garrido 2014; Castillo-Munera 2015; Lookabaugh et al. 2015).

SSR markers have been used to determine the population structure and distribution of *Pythium* species: *G, irregulare* s.s, *G. cryptoirregulare, P. aphanidermatum* and *P. helicoides* (Lee and Moorman 2008; Zhou et al. 2009); some of them transferable to additional species and in need of studies to address their informativeness. Mostly, the genetic structure of the genus have been based on culture collections with few isolates and isolates from different hosts and locations. However, little is known about the genetic population structure of *Pythium* species (Weiland et al. 2015).

In previous years *Gl. irregulare* s.l was found to be the most prevalent species in different greenhouses at Long Island NY, where diverse hosts were sampled, with predominance of geranium isolates. Phylogenetic analysis of the complex revealed the formation of four clades within the complex, supporting the separation of *G. irregulare* s.s. and *G. cryptoirregulare*. The isolates under study were grouped in four groups including *G. irregulare* s.s. and three clusters in *G. cryptoirregulare*. Garrido (2014) suggested that the pathogens had a local and remote origins, possibly introduced on infected plant material. Also, the existence of putative hybrids between *G. cryptoirregulare* and *G. irregulare* was detected, providing evidence of possible genetic exchange between the two species (Garrido 2014). In another study, Weiland et al. (2015) characterized three *Pythium* species, including *G. irregulare* s.s., from forest nurseries in the U.S, and found an intrinsic population structure explained by the prevalence of two lineages within the species and evidence of the movement of isolates between nurseries.

Molecular sequencing has helped to clarify phylogenetic relationships of organisms. In the need to achieve adequate management and control of diseases caused by *Pythium* species, the first step is to make an accurate identification of the pathogen (Weiland et al. 2013), a task that is challenging when closely related species are also morphologically cryptic. The two objectives of this study were to: i) perform a phylogenetic analysis of the *G. irregulare* s.l. isolates from three different *Chrysanthemum* greenhouses at NY collected in 2014 and ii) asses their population structure using SSRs markers.

Materials and Methods

Isolates

Isolates were identified by means of morphological criteria and sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA and cytochrome oxidase subunit II, including the cox I-II spacer. For the phylogenetic analysis, 58 isolates identified as *G. irregulare* s.l., based on DNA sequences of the ITS region, that represented the genetic diversity of the 2014 populations under study on were used.

coxII gene sequencing

The mitochondrial *coxII* gene, including the intergenic spacer and a partial *coxI* sequence, was amplified. Amplification was performed using the following PCR conditions: 2 min denaturation at 95ºC, 35 cycles of denaturation at 94ºC (1 min), annealing at 56.4ºC (1 min), and extension at 72ºC (1 min), 10 min final extension at 72ºC (Martin 2000; Garrido 2014), and primers described in Table IV-1. Amplification reactions were prepared as previously described for ITS region. PCR products were purified using ExoSap-IT, sequenced and compared with reference sequences at NCBI using BLAST as described in chapter III.

Table IV-1. Primers used for PCR amplification and sequencing of *coxII* gene

Locus	Primer name	Primer sequence (5'-3')	Ta (˚C)	Reference	Fragment length
coxII	FM 35 FM 52	CAG AAC CTT GGC AAT TAG G GTT GTG CTA ATT CCA TTC TAA	56.4	(Martin 2000)	563 bp

Phylogenetic analyses

Analyses were performed on DNA sequences of the ITS region and the *cox*II gene. Sequences were aligned and manually edited with MEGA 6.0. Only high quality sequences were included in posterior analyses. Reference sequences for *G. irregulare* and *G. cryptoirregulare* obtained from NCBI were included in the analysis, and the sequence of a *Globisporangium sylvaticum* reference isolate (OM121) was used as the outgroup species (Table IV-2). Poorly aligned positions of multiple sequence alignment datasets were excluded with Gblocks server (Castresana 2000). Phylogenetic trees were constructed by maximum likelihood (ML) methods in R v. 3.3.1 using the packages *phangorn* 2.0.4 and *ape* 3.5 (Paradis et al. 2004; Schliep 2011). Bootstrapping was performed with 1000 replicates for ML trees with the two loci. Trees were visualized using FigTree version 1.4.3. Isolates were determined to be *G. irregulare* or *G. cryptoirregulare* based on their phylogenetic clustering with the reference isolates. Additionally, a SSR-based UPGMA tree was generated using Nei's genetic distances, and a minimum spanning network (MSN) using Bruvo's distances were used to compare clusters supported by SSR analysis with the nuclear and mitochondrial sequence based phylogenies.

Table IV-2. Reference isolates and GenBank accession numbers for the ITS and coxII region used for the phylogenetic analysis

Collection id.	Species	Gen Bank accession numbers		Reference
		ITS	*cox*II	
P50	*G. cryptoirregulare*	AY907893.1	AY907918.1	(Garzón et al. 2005a)
MAFF305572	*G. irregulare* s.s.	AB107999.1	DQ071384	(Matsumoto et al. 1999, Villa et al. 2006)

Collection id.	Species	Gen Bank accession numbers		Reference
		ITS	*cox*II	
325393	*G. cryptoirregulare*	AY907901.1	AY907926.1	(Garzón et al. 2007)
OM121	*G. sylvaticum*	AJ233459	DQ071397	(Matsumoto et al. 2000, Villa et al. 2006)

SSR genotyping and SSR allele scoring

Eleven polymorphic *G. irregulare* and *G. cryptoirregulare* SSR markers developed by Lee and Moorman (2008) and standardized by Garrido (2014) were utilized to genotype isolates (Table IV-3). For all primer combinations the reaction conditions were the same, 20 µL reactions: 1 µL of each 5µM primer, 2 µL of 35 ng/µL DNA, and 10 µL of 2X GoTaq Colorless Master Mix. Forward primers were labeled with either fluorescent dyes 6-Fam, Ned or Pet (Applied Biosystems®, Thermo Fisher Scientific, Waltham, MA, USA). The PCR amplification program consisted of an initial denaturing step of 94ºC for 2 min, followed by 35 cycles of 94ºC for 30 s, annealing temperature 55 - 60ºC (depending on the specific primer set) for 25 s, 72ºC for 1 min, and a final extension of 72ºC for 10 min. PCR products were diluted and pooled into a multiplex set of 3 SSRs according to their expected amplicon size and dye, to optimize genotyping cost and avoid overlapping fragments. PCR products were resolved by multiplexed capillary electrophoresis on an ABI 3730 DNA Analyser (Applied Biosystems) by loading 1 µL of the diluted PCR product, 9 µL Hi-Di™ formamide, and 0.4 µL GeneScan_500 LIZ® Size standard (Applied Biosystems). Electropherograms were inspected using Peak scanner v 1.0 (Thermo Fisher Scientific) software and alleles were called using GeneMapper v.4.0 (Thermo Fisher Scientific) software. A genotypic matrix was constructed and analyzed using GenALex 6.501 (Peakall and Smouse 2012).

Table IV-3. Loci, primer sequences, repeat motifs, dye and fragment sizes of polymorphic loci for *G. irregulare* and *G. cryptoirregulare* (Lee and Moorman 2008)

Locus	Target species	Motif	Dye	Expected Size
63108ACA1-67	*G. irregulare*	$(ACA)_7$	FAM	130
63108CAA2-41	*G. irregulare*	$(CAA)_{10}$	NED	189
63108AG3-90	*G. irregulare*	$(AG)_{28}$	FAM	115
63108AG2-33	*G. irregulare*	$(AG)_{15}$	NED	214
P50TC2-23	*G. cryptoirregulare*	$(TC)_{20}$	PET	121
P50CT1-58	*G. cryptoirregulare*	$(CT)_{15}$	NED	177
P50AC3-33	*G. cryptoirregulare*	$(AC)_{19}$	PET	225
P50AG3-30	*G. cryptoirregulare*	$(AG)_{18}$	FAM	117
P50GA3-20	*G. cryptoirregulare*	$(GA)_{13}(GT)_{11}$	FAM	179
P50GAA3-42	*G. cryptoirregulare*	$(GAA)_{10}$	PET	322
P50TG2-93	*G. cryptoirregulare*	$(TG)_{18}$	PET	113

Data analysis

SSR data formatting and population genetic analyses were conducted using GenAlEx 6.5 (Peakall and Smouse 2012) and R 3.3.1 (R Core Team 2013). SSR multilocus genotypes from a total of 120 *G. irregulare* s.l isolates were included in this study. The formatted genotypes were imported to R for use with *Poppr* 2.2.1 (Kamvar et al. 2014) and *Adegenet* 2.0.1 (Jombart 2008) packages. A clonal correction was done to include just one individual representative for each multilocus genotype observed to assess the genotypic diversity. Multilocus genotype data were stratified in population hierarchies (Greenhouse, Species and Mefenoxam test). The information about fungicide sensitivities were provided by Margery Daughtrey. Based on their power and utility (Grünwald et al. 2003), the following genotypic diversity parameters were chosen: Shannon-Weiner diversity (*H*), the Stoddart and Taylor's index (*G*), (Shannon 1949; Stoddart and Taylor

1988), and the genotypic richness was calculated as E_5 (Pielou 1975; Ludwig and Reynolds 1988; Grünwald et al. 2003).

For assessing population structure two methods were used: bootstrap analysis of Nei's genetic distance with the unweighted pair group method with arithmetic means (UPGMA) algorithm, and minimum spanning networks (MSN) using Bruvo's genetic distance for microsatellite loci (Bruvo et al. 2004; Kamvar et al. 2014). Statistical support for the branches was obtained using 1000 bootstrapped samples.

Analysis of molecular variance (AMOVA) was performed to detect and compare population differentiation within and between the population hierarchical levels. Statistical significance of the F-statistics was assessed by randomization test with 9999 permutations.

Results

Phylogenetic analysis

ML trees based on DNA sequences of ITS region and *cox*II gene revealed the formation of two well supported branches. One branch comprises isolates from *G. irregulare* and other from *G. cryptoirregulare*, both with strong bootstrap support (Fig. IV-1A and IV-1B). Comparison of the groupings formed with both trees were topologically similar.

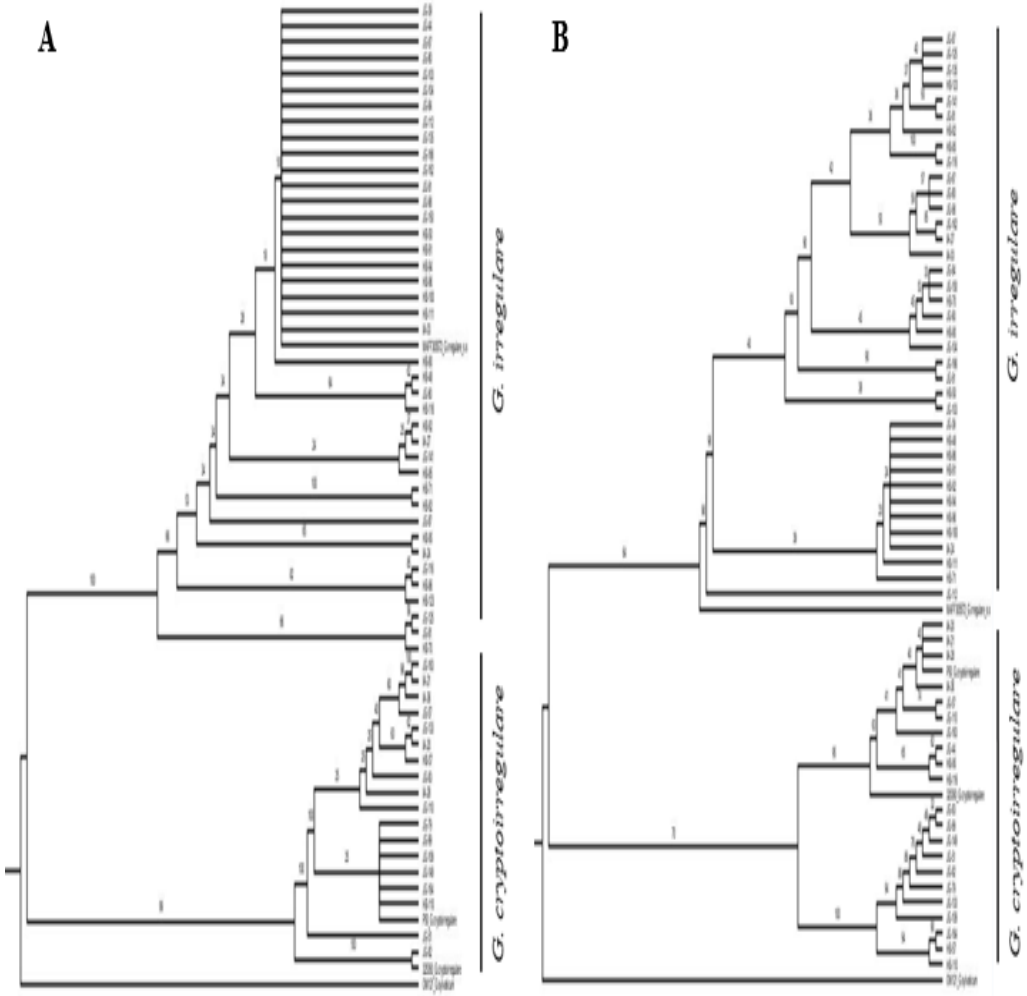

Figure IV-1. ML trees showing the relationship of *G. irregulare* complex obtained by maximum likelihood analysis of DNA sequences of **A**. ITS region and **B.** *coxII* gene.

The SSR based UPGMA tree revealed similar results. Two branches formed, one comprising *G. irregulare* isolates and the other comprising *G. cryptoirregulare* isolates (Fig IV-2). In the UPGMA tree, isolates (JG-44, HB-95 and HB-116) grouped with *G. irregulare.* Different results were found with the nuclear and mitochondrial sequence based phylogenies.

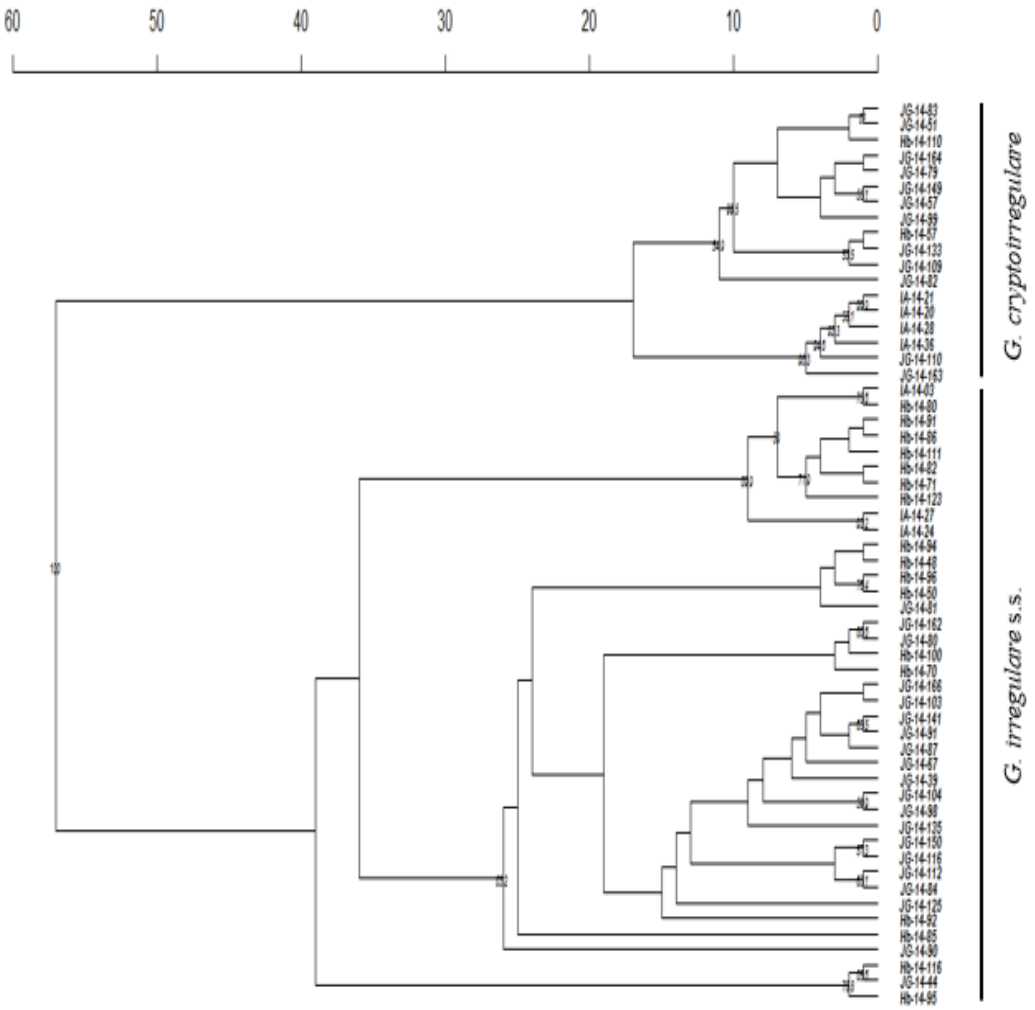

Figure IV-2. UPGMA tree of *G. irregulare* complex, based on genetic distance obtained with SSRs markers.

In order to explore in detail this isolates a MSN was constructed. The MSN showed that the three isolates, JG-44, Hb-95, Hb-116, are in the middle of the *G. cryptoirregulare* and *G. irregulare*, sharing alleles with both species suggesting potential hybrids (Fig IV-3).

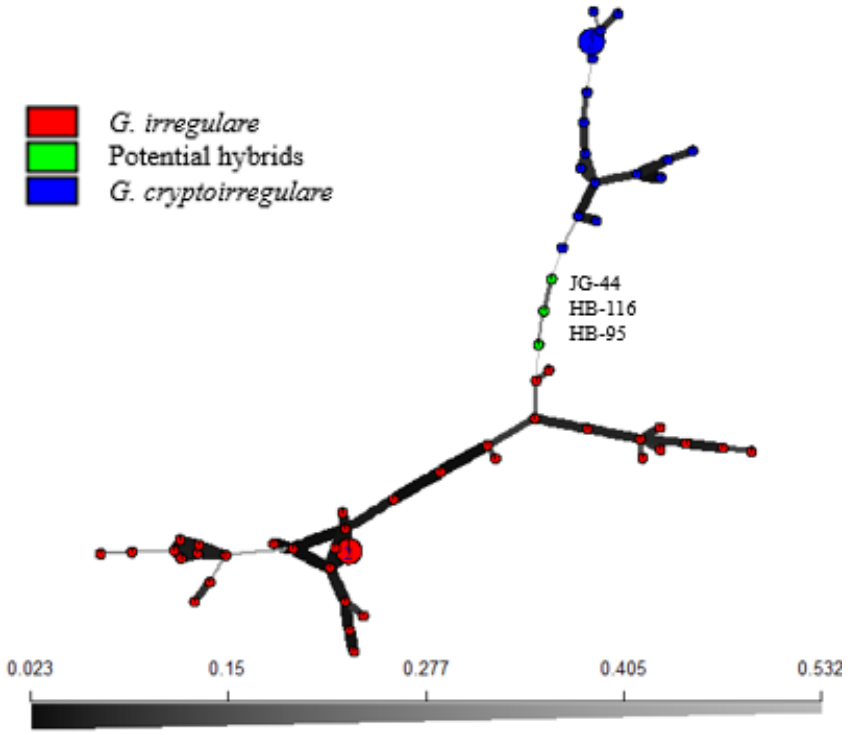

Fig IV-3. Minimum spanning network of isolates used for phylogenetic analysis. Nodes (circles) represent individual multilocus genotypes. The size of the circle is relative to the number of individuals represented in the data. Nodes more closely related have darker and thicker lines whereas nodes more distantly related have lighter and thinner lines.

The groupings found with the phylogenetic trees, the UPGMA and MSN were used as reference to identify isolates of the rest of the sample for posterior analyses.

Population genetic analysis of isolates of the *G.irregulare* complex from *Chrysanthemum* grown in floricultural greenhouses in Long Island, NY in 2014

Genetic diversity

In total, 111 multilocus genotypes (MLG) were detected among de 120 isolates. Most of MLG were unique and only 7 clonal lineages were found, with up to three

isolates each. Allelic and genotype diversity within loci revealed that the number of alleles varied from 5 to 27 microsatellite alleles per locus. P50AC3-33 had the highest number of alleles (n=27) with the highest Simpson diversity (0.92). Locus 63108CAA2-41 and P50GAA3-42 has the most evenly distributed alleles (0.89) (Table IV-4).

Table IV-4. Diversity statistics for all SSR loci investigated in the study (Lee and Moorman, 2008)

Locus	allele	1-D	Hexp	Evenness
P50AG3-30	26	0.89	0.90	0.70
P50TG2-93	20	0.90	0.91	0.79
P50CT1-58	9	0.83	0.84	0.87
63108CAA2-41	5	0.68	0.68	0.89
P50TC2-23	17	0.88	0.89	0.79
P50GA3-20	13	0.76	0.77	0.68
63108AG2-33	19	0.88	0.89	0.75
63108ACA1-67	10	0.80	0.80	0.78
63108AG3-90	15	0.75	0.75	0.59
P50GAA3-42	6	0.73	0.74	0.89
P50AC3-33	27	0.92	0.92	0.73
mean	15.18	0.82	0.82	0.77

Allele = Number of observed alleles; 1-D = Simpson index; Hexp = Nei's 1978 gene diversity

Genotypic diversity (either *H* or *G*) were higher in greenhouse A and in *G. cryptoirregulare* when data was analyzed by species. If all genotypes were equally abundant, G value would be the number of MLGs. Evenness (E5 = 0.945) and richness (*eMLG* = 10.9) were similar in all populations (by greenhouse and species). There were few genotypes that occurred more than two times, as expected from the high *E5* (Table IV-5).

Table IV-5. Diversity statistics of *G. irregulare* s.l. populations in greenhouses A,B or C. Diversity was measured for each greenhouse, and by clade (each representing a species or lineage).

Population	**N**	**MLG**	**eMLG**	**H**	**G**	**E_5**
A	80	73	10.8	4.25	65.31	0.933
B	29	28	10.9	3.32	27.13	0.981
C	11	10	10	2.27	9.31	0.955
Total	120	111	10.9	4.67	101.41	0.945
Clade	**N**	**MLGs**	**eMLGs**	**H**	**G**	**E_5**
G. cryptoirregulare	64	57	9.80	3.99	50.0	0.922
G. irregulare	46	44	9.91	3.77	42.3	0.977
Potential hybrids	10	10	10	2.30	10.0	1.000
Total	120	111	9.93	4.67	101.4	0.945

N = number of individuals observed; MLG = Number of multilocus genotypes observed; eMLG = the number of expected MLG at the smallest sample size ≥ 10 based on rarefaction; H = Shannon-Wiener Index of MLG diversity; G = Stoddart and Taylor's Index of MLG diversity; E5 = Evenness.

Population structure and differentiation

When all isolates were displayed on the UPGMA the basal branches split the 120 isolates into two subpopulation groups that corresponded *G. cryptoirregulare* and *G. irregulare* (Fig IV-4). Most of the isolates from greenhouse A were grouped in the *G. cryptoirregulare* branch. Most of the isolates from greenhouse B were grouped in the *G. irregulare* branch. Isolates from greenhouse C were grouped in both branches without a clear predominance of either of them. Looking in detail, there were some clades where the clear predominance of isolates from one lineage per greenhouse suggested local sources of inoculum, while closely related genotypes shared by isolates from different greenhouses suggested common sources of inoculum.

MSN (Fig IV-5) revealed isolates clustering together according to phylogenetic relationships. Structure by location (greenhouse) was masked by phylogenetic relationships. MSN (Fig IV-5A) revealed similar results as the UPGMA. *G. irregulare* isolates were grouped together and separated from isolates identified as *G.*

cryptoirregulare, also the potential hybrids were located between the two species, showing that they are sharing alleles with both (Fig IV-B). MSN based on mefenoxam sensitivity revealed that most of the isolates grouped in *G. irregulare* were sensitive to mefenoxam while *G. cryptoirregulare* isolates were sensitive, resistant or intermediate. Some clades within this group had predominance of resistant isolates. No clonal genotypes were shared among greenhouses.

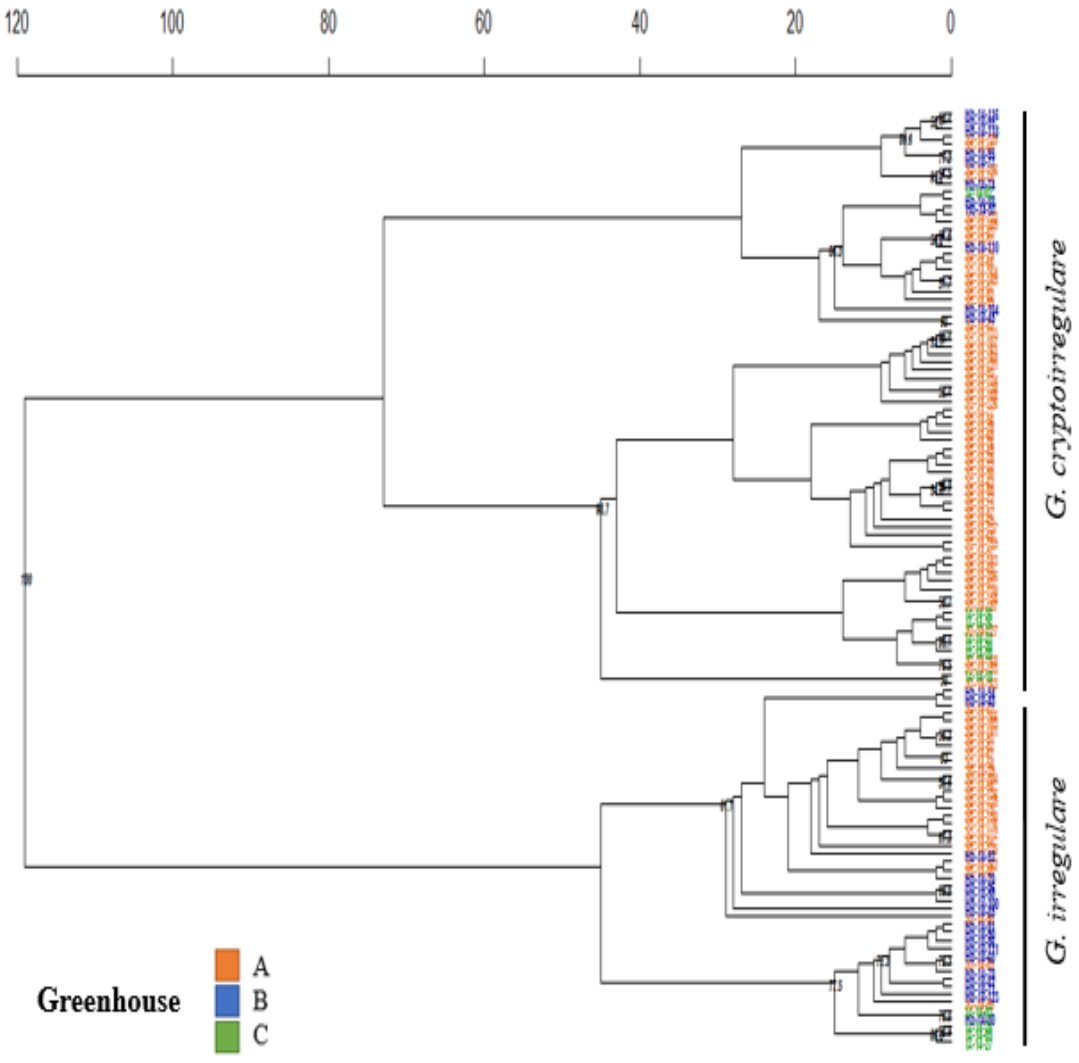

Figure IV-4. UPGMA tree of *G. irregulare* complex, based on genetic distance obtained with SSRs markers.

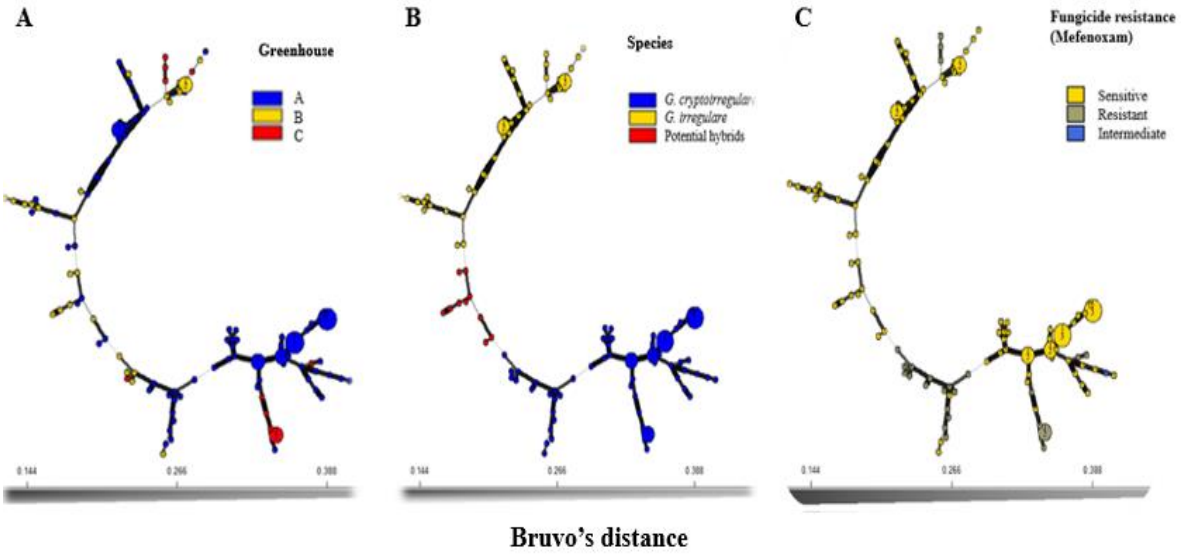

Fig IV-5. Minimum spanning network based on Bruvo's genetic distance. **A**. by greenhouses; **B**. by Species; **C**. by Fungicide resistance to mefenoxam. Nodes (circles) represent individual multilocus genotypes. The size of the circle is relative to the number of individuals represented in the data. Nodes more closely related have darker and thicker lines whereas nodes more distantly related have lighter and thinner lines.

AMOVA revealed significant moderate genetic differentiation among greenhouse populations (F_{ST} = 0.101) without discrimination of phylogenetic clades, and significant high genetic differentiation when analysis were performed per location on each individual phylogenetic clade (*G. irregulare* s.s. and *G. cryptoirregulare*) (Table IV-6). This results shows the importance of perform the analysis separating the species within the complex to do not lose information.

Table IV-6. Analysis of molecular variance (AMOVA) comparing *G. irregulare* s.l. isolates for two different hierarchies: by location (greenhouses) and by species.

Level	F_{ST}	*p*
G. irregulare s.l. **(by location)**	0.101	0.001*
By species	0.289	0.001*
G. irregulare (by location)	0.256	0.001*
G. cryptoirregulare (by location)	0.138	0.002*
Potential hybrids	0.038	0.170

F_{ST} = fixation index. F_{ST} < 0.05 = low genetic differentiation; 0.05 to 0.15 = moderate; 0.15 to 0.25 = great; and, >0.25 = very great). *p* values were based on 999 permutations; * indicates significant differences.

Discussion

The most common species previously reported in floricultural crops in Long Island NY. were *G. irregulare* s.l and *P. aphanidermatum* (Garrido 2014). The same pathogenic species have been found in other states (Moorman et al. 2002; Castillo-Munera 2015). In *Chrysanthemum*, *G. irregulare* have also been reported to cause disease (Yates 2016). In Pennsylvania, no spatial and temporal patterns were found over time in greenhouses, suggesting that the pathogen was likely introduced with planting material or growing media (Moorman et al. 2002; Daughtrey and Miller 2009). Garrido (2014) performed SSR analysis of *G. irregulare* s.l isolates from multiple hosts, with predominance of isolates from *Pelargonium* (geranium), and concluded that plant pathogenic isolates in the studied greenhouses were likely introduced by infected plant material and moved between greenhouses (Garrido 2014).

In this study, the analysis of *G. irregulare* s.l, isolates support the genetic differentiation of *G. cryptoirregulare* and *G. irregulare* as previously reported (Garzón et al. 2007; Garrido 2014). This results were revealed by ITS and COXII sequences, and supported by SSR analysis, showing that this method is an alternative for the characterization of species of the *G. irregulare* complex. Potential hybrids found between *G. irregulare* and *G. cryptoirregulare* suggested that occasional genetic exchange was possible between species (Garrido 2014), which was confirmed by the findings of the current study. Interestingly, putative hybrids clustered in a separate clade. Further comparisons with Garrido 2014 data will be discussed in chapter V.

Analysis of genotypic diversity is an important part of genetic structure analysis of populations. Genotypic diversity is calculated from genotype frequencies (Grünwald et al. 2003). In this study higher diversity values were observed, which could be caused in part by samples size differences between greenhouses. When data was analyzed by clades, *G. cryptoirregulare* was overall more diverse compared to *G. irregulare* s.s. Similar results were previously reported by Garzón et al. (2007). Genetic diversity is a source of variability of pathogenic strains in crop hosts and could be used to identify alleles controlling key pathogenicity traits (Bennett and Stone 2016).

SSR based cluster analyses, UPGMA and MSN, performed for inference of population structure and lineage membership allowed to determine how isolates grouped together and helped to detect isolates that were misidentified based on morphology. An intrinsic genetic population structure was found due to phylogenetic discrimination of *G. irregulare* and *G. cryptoirregulare*. Significant genetic population structure was found by location and fungicide resistance (mefenoxam). Some lineages were shared among greenhouses. Those findings suggest potential common sources of inoculum, while lineages present in a single greenhouse suggest local sources of inoculum. In the case of the mefenoxam sensitivity, a higher number of resistant isolates were found in *G. cryptoirregulare* from the three different greenhouses. Studies, have shown that isolates of *Pythium* species with similar genetic backgrounds can show variation in their sensitivity to fungicides and that genetic variation can help to explain fungicide resistance (Al-Sa'di et al. 2008; Al-Sa'di et al. 2008a; Garrido 2014).

All of the isolates in this study came from the same host (chrysanthemums) making just possible to analyze population differentiation by location, by fungicide sensitivity (MSN only), and by the species in the complex. Based on that, clades with potential local sources of inoculum were identified as well as clades showing potential common sources. The results showed that isolates may have been moved between the greenhouses; although no clonal genotypes were shared among greenhouses. Molecular characterization is a way to quantify variation, while population structure can provide insights regarding factors that contribute to the movement and distribution of pathogens (Schroeder et al. 2013). Studies analyzing the diversity of *Pythium* and *Globisporangium* populations can help to understand the epidemiology of the diseases they cause on plants. When a pathogen population is highly diversified, the management may be harder than when a population consist of clones, since high diversity may reflect multiple sources of inoculum which could be difficult to identify.

Little is known about the movement and distribution of inoculum in greenhouses but it can be harbored in many potential sources including infected plant material, plant debris, soil, tools, equipment, potting mixtures, irrigation water, and also can be splashed around potting mixes and other substrates in greenhouses (Al-Sa'di et al. 2008b). In the future growers should consider better management practices to reduce the risk of introduction of infected materials. In depth analysis of the diverse sources of inoculum should be examined in future studies. Additionally, since *G. irregulare* s.l. lineages were shared among the three greenhouses, further analysis is required to examine each group individually to further understand the relevant differences and similarities among these plant pathogens.

LITERATURE CITED

Al-Sa'di, A., Drenth, A., Deadman, M., and Aitken, E. 2008. Genetic diversity, aggressiveness and metalaxyl sensitivity of Pythium aphanidermatum populations infecting cucumber in Oman. Plant Pathology 57:45-56.

Al-Sa'di, A., Drenth, A., Deadman, M., DeCock, A., Al-Said, F., and Aitken, E. 2008a. Genetic diversity, aggressiveness and metalaxyl sensitivity of Pythium spinosum infecting cucumber in Oman. Journal of Phytopathology 156:29-35.

Al-Sa'di, A., Drenth, A., Deadman, M., Al-Said, F., Khan, I., and Aitken, E. 2008b. Potential sources of Pythium inoculum into greenhouse soils with no previous history of cultivation. Journal of Phytopathology 156:502-505.

Bennett, P., and Stone, J. 2016. Assessments of population structure, diversity, and phylogeography of the Swiss needle cast fungus (*Phaeocryptopus gaeumannii*) in the US Pacific Northwest. Forests 7:14.

Bruvo, R., Michiels, N. K., D'SOUZA, T. G., and Schulenburg, H. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. Molecular Ecology 13:2101-2106.

Castillo-Munera, D. 2015. Characterization, population genetics and management of *Pythium* spp. from floriculture crops in Michigan. Michigan State University.

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17:540-552.

Garrido, P. 2014. Molecular Characterization of *Pythium* populations in ornamental greenhouse and nursery crops. Oklahoma State University, Stillwater, Oklahoma.

Garzón, C. D., Geiser, D. M., and Moorman, G. W. 2005a. Amplified fragment length polymorphism analysis and internal transcribed spacer and cox II sequences reveal a species boundary within *Pythium irregulare*. Phytopathology 95:1489-1498.

Garzón, C. D., Yánez, J. M., and Moorman, G. W. 2007. *Pythium cryptoirregulare*, a new species within the P. irregulare complex. Mycologia 99:291-301.

Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., and Fry, W. E. 2003. Analysis of genotypic diversity data for populations of microorganisms. Phytopathology 93:738-746.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405.

Kamvar, Z. N., Tabima, J. F., and Grünwald, N. J. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Lee, S., and Moorman, G. W. 2008. Identification and characterization of simple sequence repeat markers for *Pythium aphanidermatum*, *P. cryptoirregulare*, and *P. irregulare* and the potential use in *Pythium* population genetics. Current Genetics 53:81-93.

Lookabaugh, E., Ivors, K., and Shew, B. 2015. Mefenoxam Sensitivity, Aggressiveness, and Identification of *Pythium* Species Causing Root Rot on Floriculture Crops in North Carolina. Plant Disease 99:1550-1558.

Ludwig, J. A., and Reynolds, J. F. 1988. Statistical ecology: a primer in methods and computing. John Wiley & Sons.

Martin, F. N. 2000. Phylogenetic relationships among some *Pythium* species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. Mycologia:711-727.

Matsumoto, C., Kageyama, K., Suga, H., and Hyakumachi, M. 2000. Intraspecific DNA polymorphisms of *Pythium irregulare*. Mycological Research 104:1333-1341.

Moorman, G., and Kim, S. 2004. Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. Plant Disease 88:630-632.

Paradis, E., Claude, J., and Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289-290.

Peakall, R., and Smouse, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537-2539.

Pielou, E. 1975. Ecological diversity–John Wiley & Sons. New York.

Schliep, K. P. 2011. phangorn: phylogenetic analysis in R. Bioinformatics 27:592-593.

Schroeder, K. L., Martin, F. N., de Cock, A. W., Lévesque, C. A., Spies, C. F., Okubara, P. A., and Paulitz, T. C. 2013. Molecular detection and quantification of *Pythium* species: evolving taxonomy, new tools, and challenges. Plant Disease 97:4-20.

Shannon, C. E. 1949. WEAVER; W.(1949): The Mathematical Theory of Communication. Urbana.

Stoddart, J. A., and Taylor, J. F. 1988. Genotypic diversity: estimation and prediction in samples. Genetics 118:705-711.

R Core Team. 2013. R: A language and environment for statistical computing.

Van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium*. Studies in Mycology.

Villa, N. O., Kageyama, K., Asano, T., and Suga, H. 2006. Phylogenetic relationships of *Pythium* and *Phytophthora* species based on ITS rDNA, cytochrome oxidase II and β-tubulin gene sequences. Mycologia 98:410-422.

Weiland, J., Littke, W., and Haase, D. 2013. Forest nurseries face critical choices with the loss of methyl bromide fumigation. California Agriculture 67:153-161.

Weiland, J. E., Garrido, P., Kamvar, Z. N., Espíndola, A. S., Marek, S. M., Grünwald, N. J., and Garzón, C. D. 2015. Population Structure of *Pythium irregulare*, *P. ultimum*, and *P. sylvaticum* in Forest Nursery Soils of Oregon and Washington. Phytopathology 105:684-694.

Yates, R. 2016. How To Prevent Pythium In Fall Garden Mums. in: Crop Inputs, Greenhousegrower, ed.

Zhou, W., Motohashi, K., Suga, H., Fukui, H., and Kageyama, K. 2009. Development of microsatellite markers for *Pythium helicoides*. FEMS Microbiology Letters 293:85-91.

CHAPTER V

POPULATION STRUCTURE OF *Globisporangium irregulare* s.l. FROM LONG ISLAND, NY GREENHOUSES: HOST COMPARISON *Chrysanthemum* vs. *Pelargonium*

Introduction:

Globisporangium irregulare s.l has been reported as one of the most common species in floricultural and ornamental crops (Moorman and Kim 2004; Garrido 2014; Castillo-Munera 2015; Lookabaugh et al. 2015). Phylogenetic analysis of the complex revealed the formation of four clades within the complex, supporting the separation of *G. irregulare* s.s. and *G. cryptoirregulare*.(Garzón et al. 2005a; Garrido 2014). Analysis of the population structure in Long Island NY with samples collected from 2009 to 2013, grouped the complex in four groups including *G. irregulare* s.s. and three clusters in *G. cryptoirregulare* (Garrido 2014).

The population genetic analysis of samples from different locations and different years offers a reference frame for population analysis (Garzón et al. 2005b). Understanding the dynamics over time can help to find epidemiological patterns. The objectives of this study were to compare the clusters observed on *Chrysanthemum* in 2014 to the clades identified by Garrido (2014), and compare the genetic composition of

G. irregulare s.l. populations associated to geranium over different seasons and years (2009-2013) on the same floricultural greenhouses studied by Garrido.

Materials and Methods

Identification of clusters

Data formatting and population genetic analyses were conducted using R 3.3.1 (R Core Team 2013). Garrido (2014) genotypic matrix were used (Supplementary table 2) and imported to R for use with *Poppr* 2.2.1(Kamvar et al. 2014) and *Adegenet* 2.0.1 (Jombart 2008) packages. Minimum spanning networks using Bruvo's genetic distance for microsatellite loci (Bruvo et al. 2004; Kamvar et al. 2014) were used to find grouping of 120 isolates from chrysanthemums using *G. irregulare* and *G. cryptoirregulare* (I, II and II) using eight isolates previously characterized by Garrido (2014) as reference isolates.

Dynamics over time with focus on Geranium

The multilocus genotype data set of isolates from *Pelargonium* spp. (geranium) from Garrido (2014) was stratified in population hierarchies (greenhouse, species and year). The genotypic diversity parameters used were: Shannon-Weiner diversity (*H*), the Stoddart and Taylor's index (*G*) (Shannon 1949; Stoddart and Taylor 1988), and the genotypic richness E_5 (Pielou 1975; Ludwig and Reynolds 1988; Grünwald et al. 2003). Discriminant analysis of principal components (DAPC) were used to show temporal dynamics of isolates collected from 2009 to 2013. DAPC is a multivariate approach to clustering based on prior population information (Jombart et al. 2010). Minimum

spanning networks using Bruvo's genetic distance for microsatellite loci (Bruvo et al. 2004; Kamvar et al. 2014) were also used.

Results

Identification of clusters

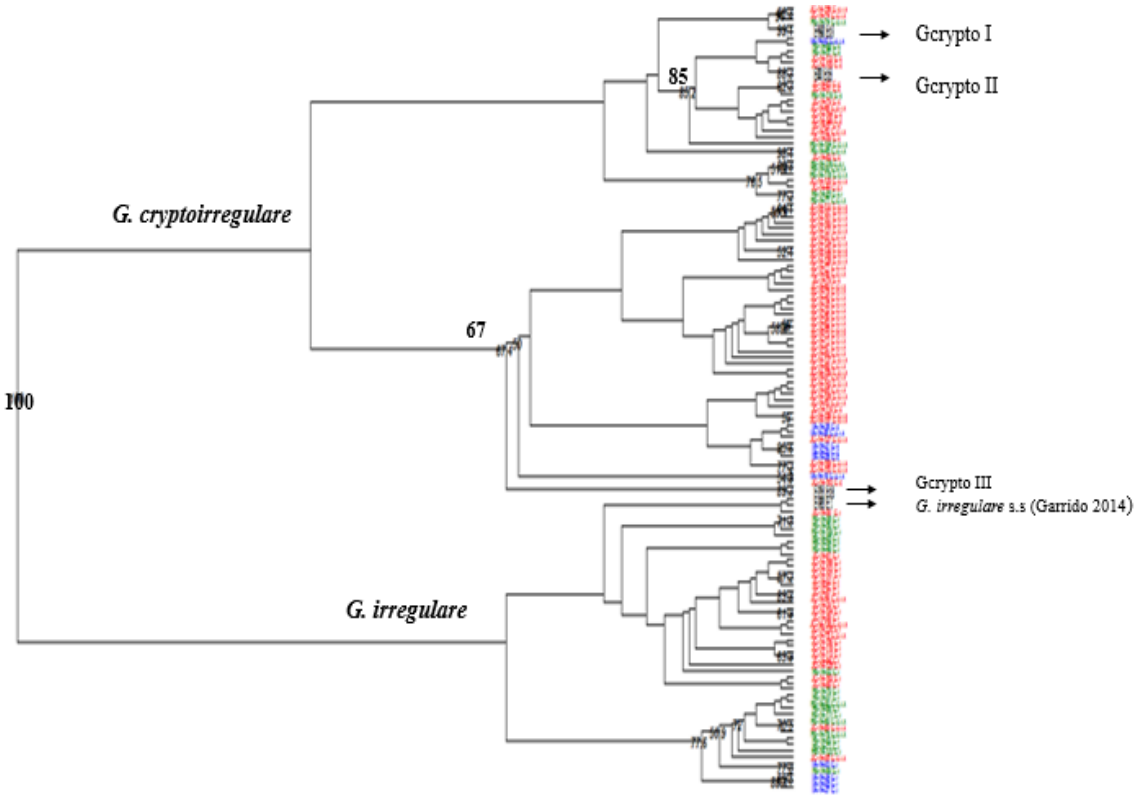

Figure V-1. UPGMA tree of *G. irregulare* complex, based on genetic distance obtained with SSRf markers and their clustering with Garrido (2014) groups. Color corresponds to greenhouses; Red = A; Green = B; Blue = C.

When all isolates from chrysanthemums were displayed on the UPGMA the basal branches split the 120 isolates into two subpopulation groups that corresponded to *G. cryptoirregulare* and *G. irregulare.* Within *G. cryptoirregulare*, Garrido (2014) groups Gcrypto I and Gcrypto II were found. Garrido (2014) group *G. irregulare* s.s. grouped with *G. irregulare* isolates (Fig V-1). Gcrypto III reference isolates did not group within

any isolate cluster from chrysanthemum, but several *G. cryptoirregulare* isolates grouped close to the two Gcrypto III than to either of the other clades, with statistical support, therefore they are refered as GcryptoIII.

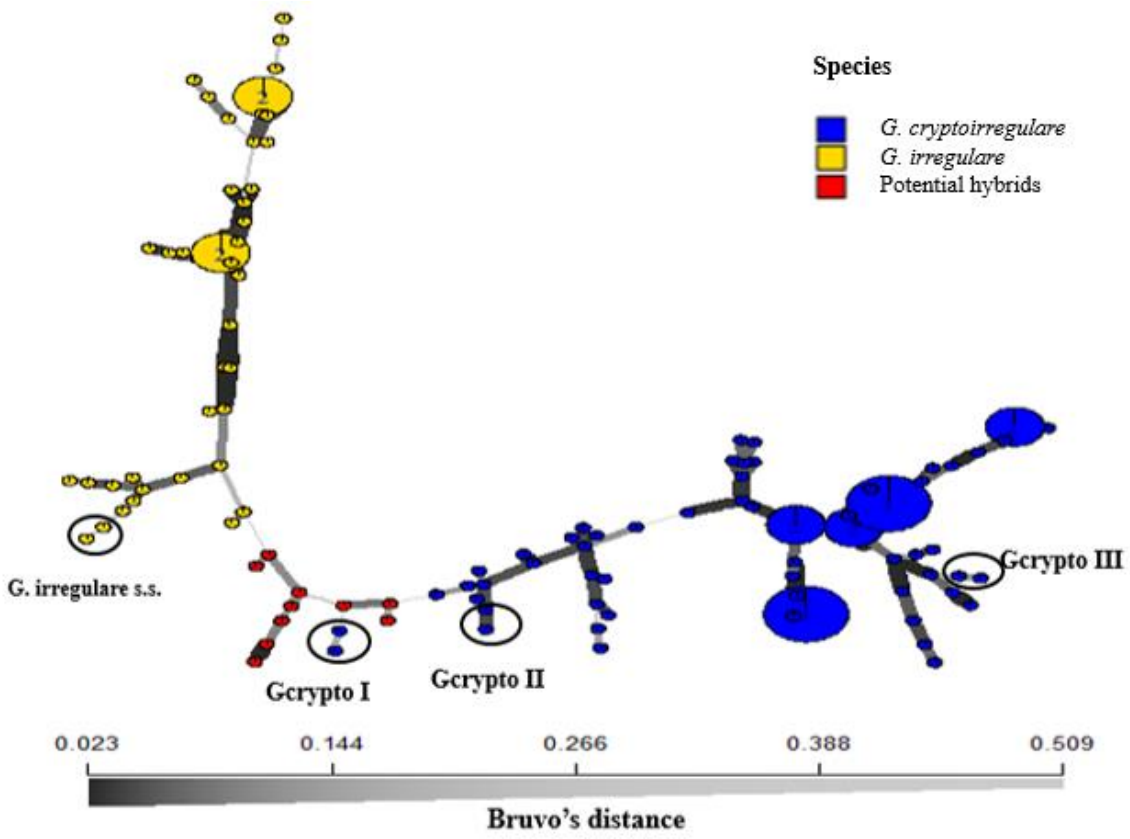

Fig V-2. Minimum spanning network of isolates from chrysanthemums based on Bruvo's genetic distance by species and intraspecific clades. Nodes (circles) represent individual multilocus genotypes. The size of the circle is relative to the number of individuals represented in the data. Nodes more closely related have darker and thicker lines whereas nodes more distantly related have lighter and thinner lines

MSN (Fig V-2 and 3) revealed that the isolates from 2014 clustered with reference isolates of the groups found by Garrido. MSN (Fig V-2) revealed that from the four groups, isolates within two of them (*G. irregulare* s.s and Gcrypto II) seem to be closely related among them, which can be interpreted based on the thickness of the branches connecting the genotypes within each cluster, while the other two seems to have more distant

relationships. An interesting finding was that Gcrypto I reference isolates were grouped with the potential hybrids previously identified. The reference Gcrypto III isolates were close to a large *G. cryptoirregulare* clade, but their connection was not as close as that observed in the other clades.

MSN (Fig V-3) based on the fungicide resistance to mefenoxam revealed that most of the isolates grouped in *G. irregulare* were sensitive to mefenoxam while *G. cryptoirregulare* included both sensitive and resistant isolates. All the isolates within *G. crypto* II were mefenoxam resistant. While the other three groups included isolates that were mefenoxam sensitive and intermediate resistant.

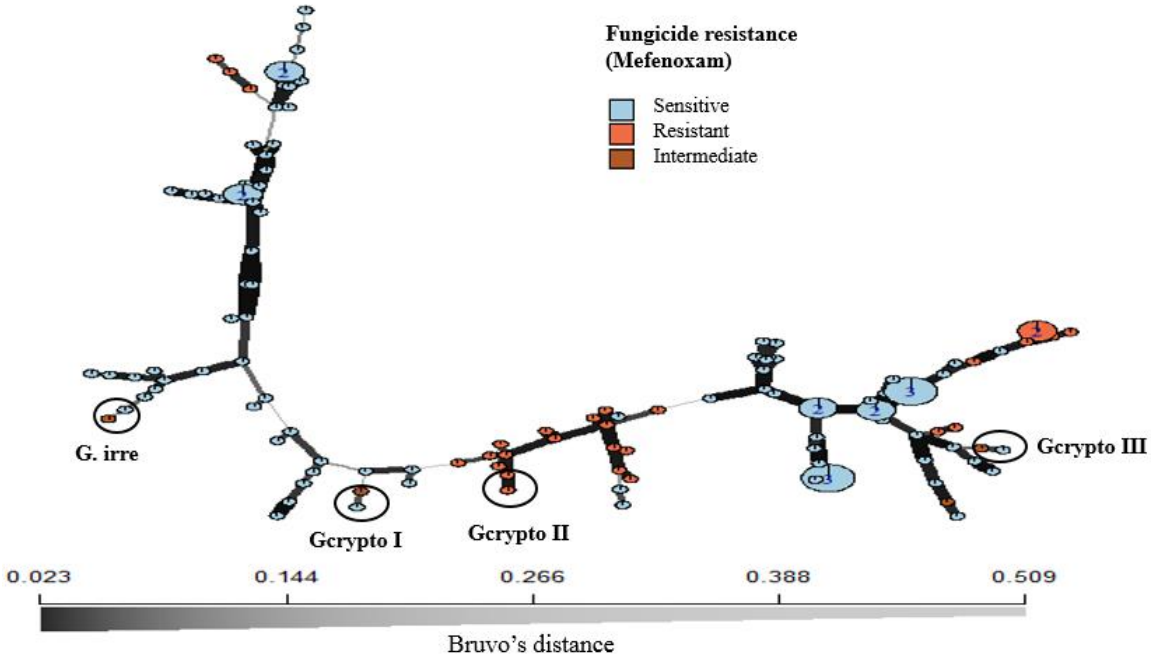

Fig V-3. Minimum spanning network of isolates from chrysanthemums based on Bruvo's genetic distance by species and interspecies clades, depicting in color differences in mefenoxam sensitivity. Nodes (circles) represent individual multilocus genotypes. The size of the circle is relative to the number of individuals represented in the data. Nodes more closely related have darker and thicker lines whereas nodes more distantly related have lighter and thinner lines.

Dynamics over time with focus on Geranium

Genetic diversity was assessed by year. Unique multilocus genotypes (MLG) were found in datasets from *Geranium* plants with clonal lineages found with up to six isolates each. Genotypic diversity (either *H* or *G*) were higher in 2011 and 2012 probably due to big sample size. A lower E5 (0.770) value in 2011 was expected due to clones were found just in this year (Table V-1).

Table V-1. Diversity statistics of *G. irregulare* s.l populations investigated in Garrido (2014) study

Year	N	MLG	eMLG	H	G	E_5
2009	2	2	2	0.693	2	1
2011	66	51	9.4	3.767	33.5	0.770
2012	38	38	10	3.638	38	1
2013	10	10	10	2.302	10	1
Total	116	101	9.8	4.513	74.8	0.818

N = number of individuals observed; MLG = Number of multilocus genotypes observed; eMLG = the number of expected MLG at the smallest sample size ≥ 10 based on rarefaction; H = Shannon-Wiener Index of MLG diversity; G = Stoddart and Taylor's Index of MLG diversity; E5 = Evenness.

DAPC clustering allowed to establish the proximities between populations inside the entire space (Fig V-4). Populations were defined by year. The results showed that the first discriminant component almost separated samples from 2009 from the 2011 population, but not enough samples were included from 2009. The second principal component showed that populations from 2012 and 2013 accumulated genetic changes that distanced them from the 2009 and 2011 populations. While the 2011 population was distinct from the 2012 and 2013 populations, the 2012 and 2013 populations shared several alleles and where closely related. The scatterplot shows the temporal changes in the population genetic composition.

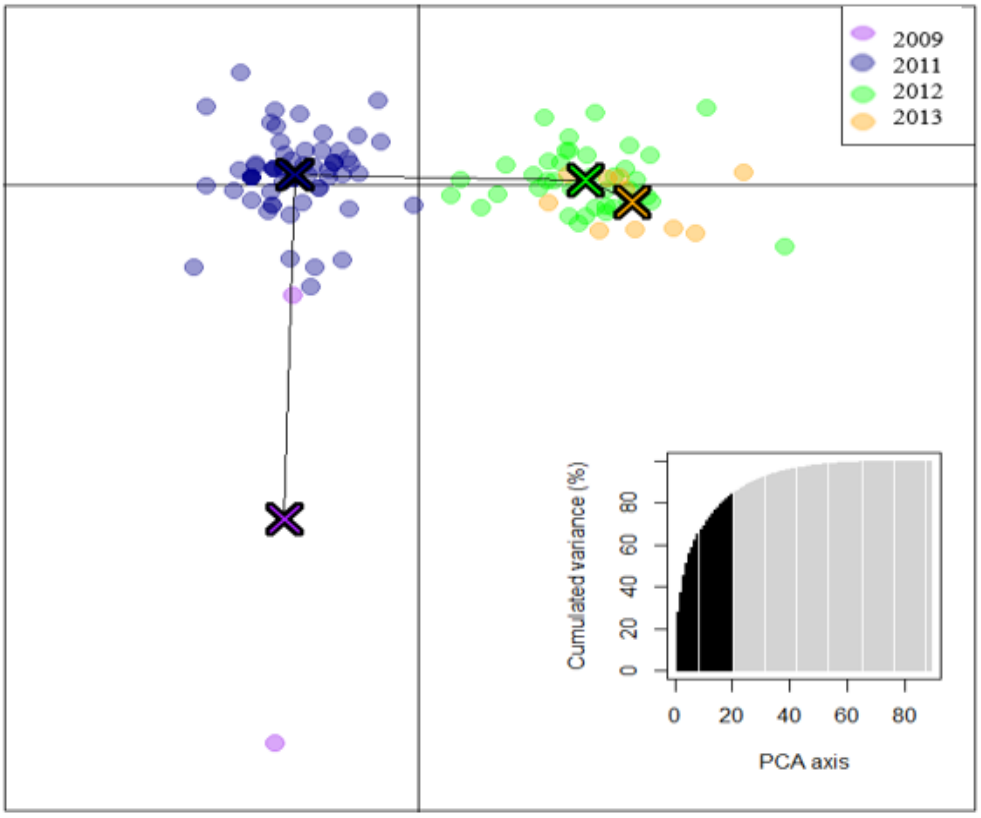

Fig V-4. Scatterplot from discriminant analysis of principal components (DAPC) discriminating *G. irregulare* sl. isolates collected from diferent years in *Geranium*. Individuals are represented as dots. The center of each groups is indicated with crosses.

MSN (Fig V-5) revealed that most of the genotypes were related between populations from 2012 and 2013 as was showed in the DAPC. Among isolates from geranium, clonal lineages were found in 2011, but not in the other years. In general terms some genotypic lineages were maintained through the years in the populations studied.

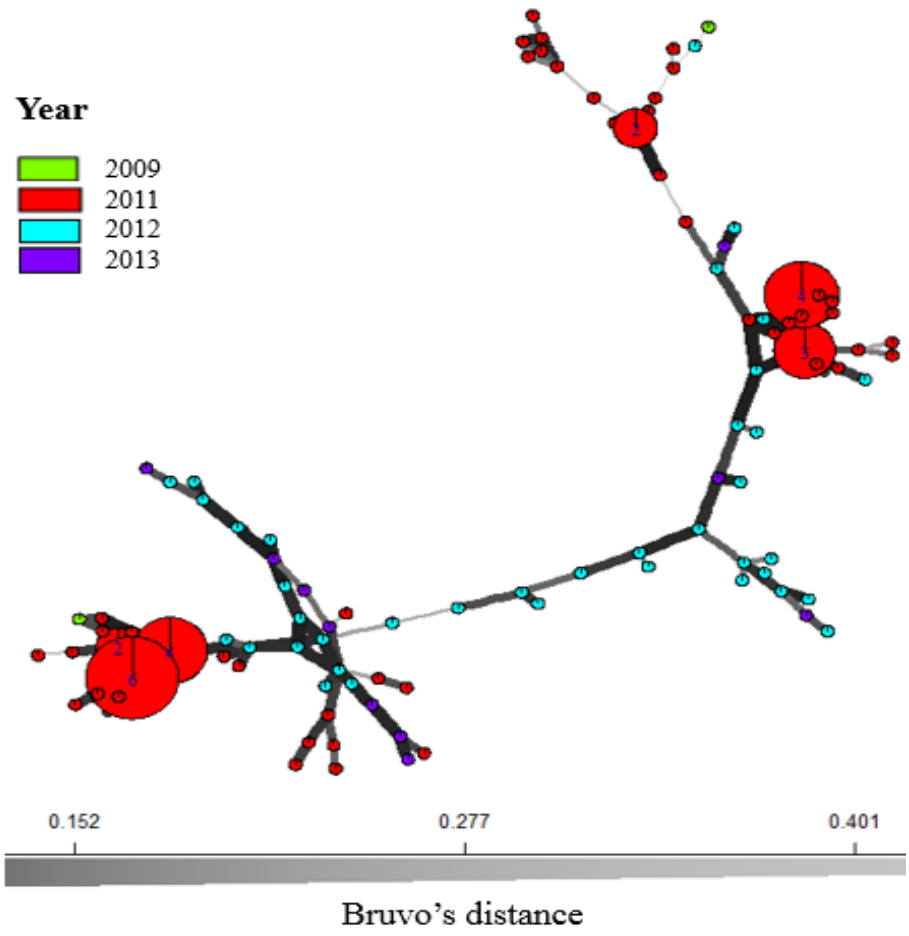

Fig V-5. Minimum spanning network based on Bruvo's genetic distance by years. Nodes (circles) represent individual multilocus genotypes. The size of the circle is relative to the number of individuals represented in the data. Nodes more closely related have darker and thicker lines whereas nodes more distantly related have lighter and thinner lines

MSN (Fig V-6) revealed that some clonal genotypes were shared between greenhouses but no particular lineages were associated to specific greenhouses probably they were randomly associated to geraniums. Also, was possible to found close related genotypes on the same location over different years, suggesting that inoculum may have survived in greenhouses populations from year to year.

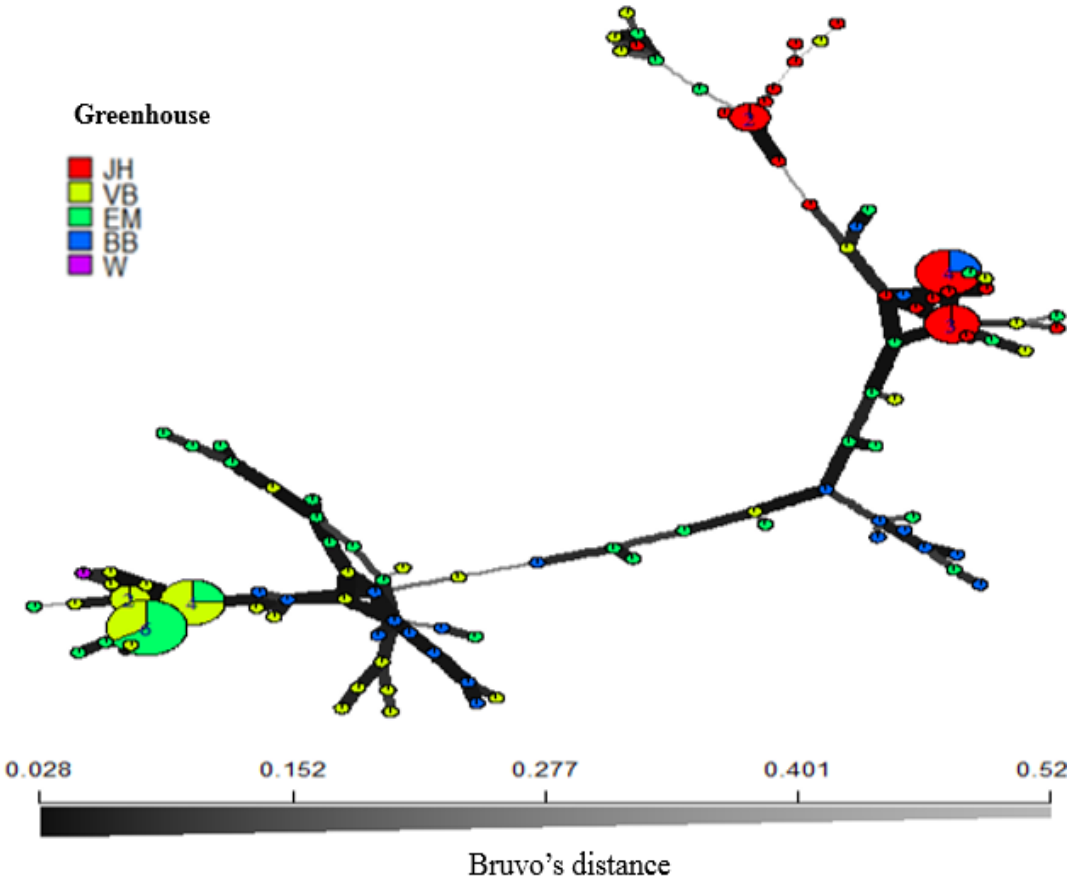

Fig V-6. Minimum spanning network based on Bruvo's genetic distance by greenhouses. Nodes (circles) represent individual multilocus genotypes. The size of the circle is relative to the number of individuals represented in the data. Nodes more closely related have darker and thicker edges lines whereas nodes more distantly related have lighter and thinner lines.

MSN (Fig V-7) revealed that lineages in *G. irregulare* s.l. are shared among different hosts. An intrinsic genetic population structure was better explained by the phylogenetic relationships of the species in the complex and masked structure by location (greenhouses) (Fig V-7). Mostly unique MLG were found, with some clonal lineages in populations of isolates collected from geranium, same results were found in chrysanthemums. No closely related clusters were formed from geranium and chrysanthemum isolates as was expected.

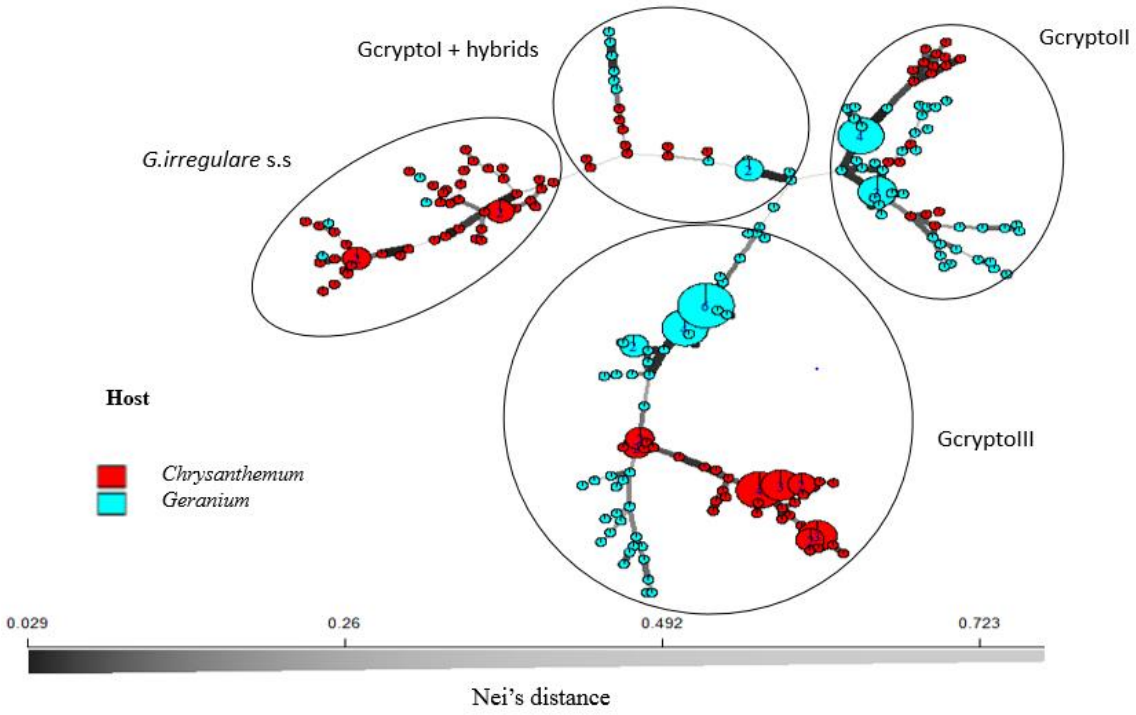

Fig V-7. Minimum spanning network based on Nei's genetic distance by Host. Nodes (circles) represent individual multilocus genotypes. The size of the circle is relative to the number of individuals represented in the data. Nodes more closely related have darker and thicker lines whereas nodes more distantly related have lighter and thinner lines.

Discussion

Garrido (2014) in her analysis of *G. irregulare* s.l isolates using SSR markers concluded that diseases caused by *Pythium* species in the greenhouses, she studied, were likely to be introduced by infected plant material and potentially by movement of materials between greenhouses or from common sources. It was also suggested that analysis of the population dynamics in greenhouses overtime could help to elucidate probable sources of inoculum.

In this study, was analyzed the population structure of *G. irregulare* s.l samples collected in 2014 from three *Chrysanthemum* greenhouses, and independently analyzed data sets generated for isolates from *Pelargonium* by Garrido et al. (unpublished) from

2009 to 2013. Garrido (2014) in her analysis used isolates collected in different years and from different greenhouses at Long Island New York. None of the greenhouses were the same, neither the host analyzed. Thus, a correlation between her results with ours were difficult to address.

Previous results have grouped *G. irregulare* complex in four groups including *G. irregulare* s.s. and three clusters in *G. cryptoirregulare* (Gcrypto I, II and III). Using isolates previously characterized as reference for this study, the four clades identified by Garrido (2014) among isolates from geranium were also found among isolates from chrysanthemums collected in 2014. Gcrypto II included mefenoxam resistant isolates in this study and also the one performed by Garrido. The 2014 Gcrypto I isolates are putative hybrids of *G. irregulare* s.s. and *G. cryptoirregulare*.

In this part of the study was interesting to find that even when analysis were performed on populations from different host, geranium and chrysanthemum, and locations (greenhouses), over five years, similar results were found regarding representation of four clades of the *G. irregulare* s.l. species complex. Unique MLG were found, with some clonal lineages in populations of isolates collected from both hosts. Clades with potential local sources of inoculum were identified as well as clades showing potential common sources. The results shown that isolates have been moved among the greenhouses. Little is known about the movement and distribution of inoculum in greenhouses but it can be harbored in many potential sources (Al-Sa'di et al. 2008b). Better practices to reduce the risk of introduction of infected material should be used.

Lineages in *G. irregulare* s.l. are shared among different greenhouses. An intrinsic genetic population structure was found by the phylogenetic relationships of *G. irregulare* and *G. cryptoirregulare* and masked structure by location. This results suggest that further analysis should study each phylogenetic group individually.

Closely related genotypes on the same location (geranium) over different years were found, suggesting inoculum may have survived in local greenhouse populations from year to year. Investigate the genetics of populations at many loci can provide insights of the genetic variability and possible movement of genes among populations (Lee et al. 2010). This part of the study, with focus on geranium, showed that analyze the population dynamics of *Globisporangium irregulare* complex over time is important to determine if the pathogen are from a particular greenhouse, are moving through contaminated material or are surviving from year to year, later the information generated can help to design better management strategies.

LITERATURE CITED

Al-Sa'di, A., Drenth, A., Deadman, M., Al-Said, F., Khan, I., and Aitken, E. 2008b. Potential sources of *Pythium* inoculum into greenhouse soils with no previous history of cultivation. Journal of Phytopathology 156:502-505.

Bruvo, R., Michiels, N. K., D'Souza, T. G., and Schulenburg, H. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. Molecular Ecology 13:2101-2106.

Castillo-Munera, D. 2015. Characterization, population genetics and management of Pythium spp. from floriculture crops in Michigan. Michigan State University.

Garrido, P. 2014. Molecular characterization of *Pythium* populations in ornamental greenhouse and nursery crops. Oklahoma State University, Stillwater, Oklahoma.

Garzón, C. D., Geiser, D. M., and Moorman, G. W. 2005a. Amplified fragment length polymorphism analysis and internal transcribed spacer and cox II sequences reveal a species boundary within *Pythium irregulare*. Phytopathology 95:1489-1498.

Garzón, C. D., Geiser, D. M., and Moorman, G. W. 2005b. Diagnosis and population analysis of *Pythium* species using AFLP fingerprinting. Plant Disease 89:81-89.

Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., and Fry, W. E. 2003. Analysis of genotypic diversity data for populations of microorganisms. Phytopathology 93:738-746.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405.

Jombart, T., Devillard, S., and Balloux, F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:1.

Kamvar, Z. N., Tabima, J. F., and Grünwald, N. J. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Lee, S., Garzón, C. D., and Moorman, G. W. 2010. Genetic structure and distribution of *Pythium aphanidermatum* populations in Pennsylvania greenhouses based on analysis of AFLP and SSR markers. Mycologia 102:774-784.

Lookabaugh, E., Ivors, K., and Shew, B. 2015. Mefenoxam Sensitivity, Aggressiveness, and Identification of Pythium Species Causing Root Rot on Floriculture Crops in North Carolina. Plant Disease 99:1550-1558.

Ludwig, J. A., and Reynolds, J. F. 1988. Statistical ecology: a primer in methods and computing. John Wiley & Sons.

Moorman, G., and Kim, S. 2004. Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. Plant Disease 88:630-632.

Pielou, E. 1975. Ecological diversity–John Wiley & Sons. New York.

Shannon, C. E. 1949. WEAVER; W.(1949): The Mathematical Theory of Communication. Urbana.

Stoddart, J. A., and Taylor, J. F. 1988. Genotypic diversity: estimation and prediction in samples. Genetics 118:705-711.

R Core Team. 2013. R: A language and environment for statistical computing.

APPENDICES

Supplementary table 1. *Pythium* and *Globisporangium* isolates used in the identification and characterization of species present in floricultural crops from Long Island, New York in 2014.

Isolate No.	Morphology ID	DNA ID (ITS)	Mtest[1]
JG-14-32	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-35	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-39	*P. irregulare*	*P. irregulare*	S
JG-14-44	*P. irregulare*	*P. irregulare*	S
JG-14-47	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-49	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-37	*P. aphanidermatum*	*P. aphanidermatum*	S
JG-14-38	*P. aphanidermatum*	*P. aphanidermatum*	S
JG-14-42	*P. aphanidermatum*	*P. aphanidermatum*	S
JG-14-50	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-51	*P. irregulare*	*P. cryptoirregulare*	R
JG-14-53	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-54	*P. irregulare*	*P. irregulare*	S
JG-14-57	*P. irregulare*	*P. cryptoirregulare*	R
JG-14-52	*P. irregulare*	*P. cryptoirregulare*	R
JG-14-55	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-56	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-60	*P. irregulare*	*P. irregulare*	S
JG-14-62	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-63	*P.rost/seg/no Pyth*	*P. ultimum*	S
JG-14-65	*P.spinosum?*	*P. ultimum*	S
JG-14-66	*P. irregulare*	*P. cryptoirregulare*	R
JG-14-67	*P. irregulare*	*P. irregulare*	S
JG-14-70	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-60	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-68	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-69	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-71	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-72	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-73	*P.acanthicum*	*Pythium sp.*	S
JG-14-74	*P.ultimum*	*Pythium sp.*	S
JG-14-75	*P. irregulare*	*P. irregulare*	S
JG-14-76	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-77	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-78	*P.myriotylum/ P.irregulare?*	*P. myriotylum*	S
JG-14-79	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-80	*P. irregulare*	*P. irregulare*	S
JG-14-93	*P. irregulare*	*P. cryptoirregulare*	S

Isolate No.	Morphology ID	DNA ID (ITS)	Mtest[1]
JG-14-95	*P. irregulare*	*P. irregulare*	S
JG-14-85	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-97	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-83	*P. irregulare*	*P. cryptoirregulare*	R
JG-14-70	*P.irregulare*	*P. irregulare*	S
JG-14-82	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-87	*P.irregulare*	*P. irregulare*	S
JG-14-91	*P.irregulare*	*P. irregulare*	S
JG-14-98	*P.irregulare*	*P. irregulare*	S
JG-14-81	*P. irregulare*	*P. irregulare*	S
JG-14-99	*P. irregulare*	*P. cryptoirregulare*	R
JG-14-74	*P.ultimum*	*P. irregulare*	S
JG-14-103	*P.irregulare*	*P. irregulare*	S
JG-14-104	*P.irregulare*	*P. irregulare*	S
JG-14-102	*P.myriotylum*	*P. miryotilum*	S
JG-14-114	*P.myriotylum*	*P. miryotilum*	S
JG-14-90	*P. irregulare*	*P. irregulare*	S
JG-14-84	*P. irregulare*	*P. irregulare*	S
JG-14-89	*P. irregulare*	*P. irregulare*	S
JG-14-106	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-110	*P. irregulare*	*P. cryptoirregulare*	R
JG-14-112	*P. irregulare*	*P. irregulare*	S
JG-14-116	*P. irregulare*	*P. irregulare*	S
JG-14-121	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-122	*P.myriotylum*	*P.myriotylum*	S
JG-14-124	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-109	*P.irregulare*	*P. cryptoirregulare*	R
JG-14-107	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-108	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-115	*P.myriotylum*	*P. aphanidermatum*	S
JG-14-130	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-131	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-133	*P.irregulare*	*P. cryptoirregulare*	R
JG-14-127	*P. irregulare*	*P. cryptoirregulare*	R
JG-14-129	*P. irregulare*	*P. irregulare*	S
JG-14-135	*P. irregulare*	*P. irregulare*	S
JG-14-136	*P. irregulare*	*P. irregulare*	S
JG-14-137	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-138	*P. irregulare*	*P. spiculum*	S
JG-14-141	*P. irregulare*	*P. irregulare*	S
JG-14-143	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-142	*?*	*Pythium rostratifingens*	S
JG-14-149	*P.irregulare*	*P. cryptoirregulare*	R
JG-14-151	*?*	*Pytium sp.*	S
JG-14-125	*P. irregulare*	*P. irrregulare*	S
JG-14-126	*P. irregulare*	*P. cryptoirregulare*	S

Isolate No.	Morphology ID	DNA ID (ITS)	Mtest[1]
JG-14-150	*P.?*	*P. irrregulare*	S
JG-14-157	*P. irregulare*	*P. cryptoirregulare*	I
JG-14-158	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-160	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-161	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-166	*P. irregulare*	*P. irrregulare*	S
JG-14-168	*P. irregulare*	*P. cryptoirregulare*	S
JG-14-86	*P.rost./seg*	*P. rostratifingens*	S
JG-14-88	*P.rost./seg*	*P. rostratifingens*	S
JG-14-139	*P.rost./seg*	*Pythium sp.*	S
JG-14-150	*P.irregulare*	*P. irrregulare*	S
JG-14-153	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-156	*P.irregulare*	*P. irrregulare*	S
JG-14-159	*P.irregulare*	*P. cryptoirregulare*	S
JG-14-162	*P.irregulare*	*P. irrregulare*	S
JG-14-163	*P.irregulare*	*P. cryptoirregulare*	R
JG-14-164	*P.irregulare*	*P. cryptoirregulare*	R
JG-14-168	*?*	*P. cryptoirregulare*	S
JG-14-46	*P. aphanidermatum*	*P. aphanidermatum*	S
JG-14-125		*P. ultimum*	S
Hb-14-43	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-45	*P. irregulare*	*P. cryptoirregulare*	S
Hb-14-46	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-48	*P. irregulare*	*P. irregulare*	S
Hb-14-49	*P. irregulare*	*P. ultimum*	S
Hb-14-51	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-52	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-53	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-54	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-55	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-56	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-41	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-42	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-44	*P. irregulare*	*P. ultimum*	S
Hb-14-46	*P. aphanidermatum*	*P. aphanidermatum*	S
Hb-14-50	*P. irregulare*	*P. irregulare*	S
HB-14-57	*P. irregulare*	*P. cryptoirregulare*	R
HB-14-58	*P. irregulare*	*P. ultimum*	S
Hb-14-62	*P. irregulare*	*P. ultimum*	S
Hb-14-64	*P. irregulare*	*P. ultimum*	S
Hb-14-68	*P. irregulare*	*P. ultimum*	S
Hb-14-69	*P. irregulare*	*P. sylvaticum*	S
Hb-14-71	*P. irregulare*	*P. irregulare*	S
Hb-14-72	*P. irregulare*	*P. ultimum*	S
Hb-14-75	*P. irregulare*	*P. ultimum*	S
Hb-14-77	*P. irregulare*	*P. cryptoirregulare*	S

Isolate No.	Morphology ID	DNA ID (ITS)	Mtest[1]
Hb-14-74	*P. irregulare*	*P. cryptoirregulare*	S
Hb-14-80	*P. irregulare*	*P. irregulare*	S
Hb-14-85	*P. irregulare*	*P. irregulare*	S
Hb-14-86	*P.irregulare*	*P. irregulare*	S
HB-14-88	*P. irregulare*	*P. cryptoirregulare*	R
Hb-14-78	*P. irregulare*	*P. irregulare*	S
Hb-14-81	*P.irregulare*	*P. ultimum*	S
Hb-14-82	*P.irregulare*	*P. irregulare*	S
Hb-14-87	*P.irregulare*	*P. ultimum*	S
Hb-14-90	*P.irregulare*	*P. irregulare*	S
Hb-14-91	*P.irregulare*	*P. irregulare*	S
Hb-14-92	*P.irregulare*	*P. irregulare*	S
Hb-14-95	*P.irregulare*	*P. irregulare*	S
Hb-14-97	*P.aphanidermatum*	*P. aphanidermatum*	S
Hb-14-89	*P.irregulare*	*P. ultimum*	S
Hb-14-94	*P.irregulare*	*P. irregulare*	S
Hb-14-96	*P.irregulare*	*P. irregulare*	S
Hb-14-99	*P. irregulare*	*P. irregulare*	S
Hb-14-100	*P. irregulare*	*P. irregulare*	S
Hb-14-102	*P. irregulare*	*P. sylvaticum*	S
Hb-14-110	*P. irregulare*	*P. cryptoirregulare*	R
Hb-14-111	*P. irregulare*	*P. irregulare*	S
Hb-14-101	*?*	*P. dissotocum*	S
Hb-14-104	*P.irregulare*	*P. cryptoirregulare*	R
Hb-14-105	*?*	*P. dissotocum*	S
Hb-14-109	*P.irregulare*	*P. ultimum*	S
Hb-14-114	*?*	*P. ultimum*	S
Hb-14-115	*?*	*P. ultimum*	S
Hb-14-116	*P. irregulare*	*P. irrregulare*	S
Hb-14-118	*P. irregulare*	*P. irrregulare*	S
Hb-14-120	*?*	*P. ultimum*	S
Hb-14-123	*P. irregulare*	*P. irrregulare*	S
Hb-14-113	*?*	*P. rostratifingens*	S
Hb-14-103	*P. rosr./ sygn*	*Pythium sp.*	S
Hb-14-107	*P. rosr./ sygn*	*Pythium sp.*	I
Hb-14-117		*P. diclinum*	S
Hb-14-119		*P. oopapillum*	S
Hb-14-122	*P. irregulare*	*P. ultimum*	S
Hb-14-124		*P. ultimum*	S
Hb-14-60	*P. irregulare*	*P. irregulare*	S
Hb-14-63	*P. irregulare*	*P. ultimum*	S
Hb-14-70	*P. irregulare*	*P. irregulare*	S
Hb-14-98/A	*P. ultimum*	*Pythium sp.*	S
Hb-14-98/B		*P. ultimum*	S
HB-14-52		*P. aphanidermatum*	S
IA-14-01	*P.myriotylum*	*P. aphanidermatum*	S

Isolate No.	Morphology ID	DNA ID (ITS)	Mtest[1]
IA-14-06	*P.myriotylum*	*P. aphanidermatum*	S
IA-14-02	*P. irregulare*	*P. cryptoirregulare*	R
IA-14-07	*P.myriotylum*	*P. aphanidermatum*	S
IA-14-05	*P. irregulare*	*P. irregulare*	R
IA-14-08	*P.myriotylum*	*P. aphanidermatum*	S
IA-14-03	*P.irregulare*	*P. irregulare*	S
IA-14-11	*P.myriotylum*	*P. miryotilum*	S
IA-14-14	*P.myriotylum*	*P. aphanidermatum*	S
IA-14-16	*P.myriotylum*	*P. aphanidermatum*	S
IA-14-10	*P.myriotylum*	*P. aphanidermatum*	S
IA-14-17	*P.myriotylum*	*P. aphanidermatum*	S
IA-14-18	*P.irregulare*	*P. cryptoirregulare*	R
IA-14-20	*P.irregulare*	*P. cryptoirregulare*	R
IA-14-21	*P.irregulare*	*P. cryptoirregulare*	R
IA-14-22		*P. ultimum*	S
IA-14-23	*P.lutarium*	*P. orthogonon*	S
IA-14-24	*P. irregulare*	*P. irregulare*	R
IA-14-25	*P. irregulare*	*P. middletonii*	R
IA-14-26	*P.?*	*P. cryptoirregulare*	S
IA-14-28	*P. irregulare*	*P. cryptoirregulare*	R
IA-14-32	*P.aphanidermatum*	*P. aphanidermatum*	S
IA-14-34	*P.cryptoirregulare*	*P. ultimum*	S
IA-14-35	*P.myriotylum*	*P. myriotylum*	S
IA-14-12	*P.lutarium*	*P. catenulatum*	S
IA-14-13	*P.intermedium*	*P. catenulatum*	S
IA-14-27	*P.irregulare*	*P. irregulare*	R
IA-14-29	*P.*	*P. middletonii*	S
IA-14-36	*P.irregulare*	*P. cryptoirregulare*	R
IA-14-23	*P.lutarium*	*P. orthogonon*	S
IA-14-30	*?*	*P. dissotocum*	I
IA-14-19	*P. aphanidermatum*	*P. aphanidermatum*	S

[1]Mtest: Fungicide resistant test to mefenoxam. S = Sensitive; R = Resistant; I = Intermediate

Supplementary table 2. *Globisporangium irregulare* s.l. isolates used in Garrido (2014) study.

Code	Strain name	*Molecular ID*	Year
C20	JH-09-244	*G. irregulare s.l.*	2009
C32	W-171	*G. irregulare s.l.*	2009
C104	BB-11-128	*G. irregulare s.l.*	2011
C60	BB-11-130	*G. irregulare s.l.*	2011
C220	BB-11-134	*G. irregulare s.l.*	2011
C259	EM-11- 36	*G. irregulare s.l.*	2011
C231	EM-11- 37	*G. irregulare s.l.*	2011
C230	EM-11- 38	*G. irregulare s.l.*	2011
C240	EM-11- 39	*G. irregulare s.l.*	2011
C244	EM-11- 40	*G. irregulare s.l.*	2011
C248	EM-11- 43	*G. irregulare s.l.*	2011
C256	EM-11- 44	*G. irregulare s.l.*	2011
C258	EM-11- 45	*G. irregulare s.l.*	2011
C257	EM-11- 46	*G. irregulare s.l.*	2011
C260	EM-11- 47	*G. irregulare s.l.*	2011
C261	EM-11- 48	*G. irregulare s.l.*	2011
C44	JH-11-254	*G. cryptoII*	2011
C54	JH-11-255	*G. irregulare s.l.*	2011
C55	JH-11-256	*G. cryptoII*	2011
C109	JH-11-270	*G. irregulare s.l.*	2011
C117	JH-11-277	*G. irregulare s.l.*	2011
C160	JH-11-281	*G. irregulare s.l.*	2011
C152	JH-11-289	*G. irregulare* s.s.	2011
C147	JH-11-290	*G. irregulare s.l.*	2011
C149	JH-11-291	*G. irregulare s.l.*	2011
C153	JH-11-292	*G. irregulare s.l.*	2011
C156	JH-11-293	*G. cryptoI*	2011
C186	JH-11-295	*G. irregulare* s.s.	2011
C191	JH-11-311	*G. irregulare s.l.*	2011
C192	JH-11-312	*G. irregulare s.l.*	2011
C214	JH-11-315	*G. irregulare s.l.*	2011
C201	JH-11-316	*G. irregulare s.l.*	2011
C202	JH-11-317	*G. irregulare s.l.*	2011
C203	JH-11-319	*G. irregulare s.l.*	2011
C216	JH-11-321	*G. irregulare s.l.*	2011
C205	JH-11-323	*G. cryptoI*	2011
C206	JH-11-324	*G. irregulare s.l.*	2011
C243	JH-11-328	*G. irregulare s.l.*	2011
C241	JH-11-329	*G. irregulare s.l.*	2011
C242	JH-11-331	*G. irregulare s.l.*	2011
C232	JH-11-335	*G. irregulare s.l.*	2011
C80	VB-11-104	*G. irregulare s.l.*	2011
C82	VB-11-105	*G. irregulare s.l.*	2011

Code	Strain name	*Molecular ID*	Year
C83	VB-11-107	*G. irregulare s.l.*	2011
C88	VB-11-108	*G. irregulare s.l.*	2011
C84	VB-11-109	*G. irregulare s.l.*	2011
C90	VB-11-111	*G. irregulare s.l.*	2011
C105	VB-11-115	*G. irregulare s.l.*	2011
C33	VB-11-116	*G. irregulare s.l.*	2011
C34	VB-11-117	*G. irregulare s.l.*	2011
C35	VB-11-118	*G. irregulare s.l.*	2011
C121	VB-11-120	*G. irregulare s.l.*	2011
C123	VB-11-121	*G. irregulare s.l.*	2011
C64	VB-11-122	*G. irregulare s.l.*	2011
C124	VB-11-123	*G. irregulare s.l.*	2011
C65	VB-11-126	*G. irregulare s.l.*	2011
C132	VB-11-129	*G. irregulare s.l.*	2011
C140	VB-11-131	*G. irregulare s.l.*	2011
C136	VB-11-133	*G. irregulare s.l.*	2011
C135	VB-11-134	*G. irregulare s.l.*	2011
C131	VB-11-137	*G. cryptoIII*	2011
C162	VB-11-138	*G. cryptoIII*	2011
C171	VB-11-140	*G. irregulare s.l.*	2011
C264	VB-11-143	*G. irregulare s.l.*	2011
C223	VB-11-144	*G. irregulare s.l.*	2011
C247	VB-11-146	*G. irregulare s.l.*	2011
C236	VB-11-147	*G. irregulare s.l.*	2011
C254	VB-11-149	*G. irregulare s.l.*	2011
C255	VB-11-150	*G. irregulare s.l.*	2011
C267	BB-12-149	*G. irregulare s.l.*	2012
C268	BB-12-150	*G. irregulare s.l.*	2012
C269	BB-12-151	*G. irregulare s.l.*	2012
C270	BB-12-152	*G. irregulare s.l.*	2012
C272	BB-12-154	*G. irregulare s.l.*	2012
C274	BB-12-156	*G. irregulare s.l.*	2012
C275	BB-12-157	*G. irregulare s.l.*	2012
C276	BB-12-158	*G. irregulare s.l.*	2012
C277	BB-12-159	*G. irregulare s.l.*	2012
C278	BB-12-160	*G. irregulare s.l.*	2012
C279	BB-12-161	*G. irregulare s.l.*	2012
C281	BB-12-163	*G. irregulare s.l.*	2012
C283	BB-12-165	*G. irregulare s.l.*	2012
C285	BB-12-167	*G. irregulare s.l.*	2012
C286	BB-12-168	*G. irregulare s.l.*	2012
C290	EM-12-52	*G. irregulare s.l.*	2012
C293	EM-12-55	*G. irregulare s.l.*	2012
C294	EM-12-56	*G. irregulare s.l.*	2012
C300	EM-12-62	*G. irregulare s.l.*	2012
C302	EM-12-64	*G. irregulare s.l.*	2012

Code	Strain name	*Molecular ID*	Year
C303	EM-12-65	*G. irregulare s.l.*	2012
C304	EM-12-66	*G. irregulare s.l.*	2012
C305	EM-12-67	*G. irregulare s.l.*	2012
C306	EM-12-68	*G. irregulare s.l.*	2012
C307	EM-12-69	*G. irregulare s.l.*	2012
C308	EM-12-70	*G. irregulare s.l.*	2012
C309	EM-12-71	*G. irregulare s.l.*	2012
C310	EM-12-72	*G. irregulare s.l.*	2012
C311	EM-12-73	*G. irregulare s.l.*	2012
C325	VB-12-151	*G. irregulare s.l.*	2012
C327	VB-12-153	*G. irregulare s.l.*	2012
C328	VB-12-154	*G. irregulare s.l.*	2012
C329	VB-12-155	*G. irregulare s.l.*	2012
C331	VB-12-157	*G. irregulare s.l.*	2012
C332	VB-12-158	*G. irregulare s.l.*	2012
C333	VB-12-159	*G. irregulare s.l.*	2012
C334	VB-12-160	*G. irregulare s.l.*	2012
C336	VB-12-162	*G. irregulare s.l.*	2012
C399	BB-13-184	*G. irregulare s.l.*	2013
C400	BB-13-185	*G. irregulare s.l.*	2013
C409	BB-13-186	*G. irregulare s.l.*	2013
C401	BB-13-187	*G. irregulare s.l.*	2013
C418	EM-13-100	*G. irregulare s.l.*	2013
C414	EM-13-102	*G. irregulare s.l.*	2013
C419	EM-13-103	*G. irregulare s.l.*	2013
C415	EM-13-104	*G. irregulare s.l.*	2013
C420	EM-13-105	*G. irregulare s.l.*	2013
C422	EM-13-107	*G. irregulare s.l.*	2013

VITA

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