

2015

Identification of the Causal Agent of Leaf and Crown Rot of Liriope in Louisiana

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IDENTIFICATION OF THE CAUSAL AGENT OF LEAF AND CROWN ROT OF *LIRIOPE*
IN LOUISIANA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Plant, Environmental and Soil Sciences

By

Carla Milena Proano Herrera
B.S., Pan American School of Agriculture Zamorano, 2010
August 2015

ACKNOWLEDGEMENTS

First of all I would like to thank God and my family for giving me the strength, support, love, and being the reason that encouraged me to keep pursuing and finish my degree.

I would like to give my special thanks to Dr. Kuehny for giving me the opportunity to work with him, for his time, knowledge, guidance, patience and help during this process. Also, I would like to thank to my committee members Dr. Raghuwinder Singh, Dr. Edward Bush and Dr. Don Labonte for providing me their knowledge, help and guidance.

I am also really thankful to the Botanic Gardens staff and student workers for being really helpful during my project and Timothy Burks for teaching me the laboratory methods needed for my research.

My special gratitude to the LSU AgCenter and Dr. William Richardson for providing financial support which allowed me to study at Louisiana State University.

Finally, I would like to thank my friends of ZAS (Zamorano Agricultural Society), for being my adopted family, for their unconditional support and friendship during all this time. My special gratitude to my friends that helped me any time I needed them: Fabián Díaz, Damir Torrico, José Brandao, to help me finish my project. This work would not have been possible without them. I am also thankful to Katherine Rubio and José Alonso for their unconditional support.

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ABSTRACT

Leaf and crown rot of *Liriope* is an increasing problem affecting *Liriope* in the nursery and landscape for the past eight years primarily in the southeastern United States. Symptoms start with water soaking of leaves at the crown area, followed by yellowing of the entire leaf starting at the base. Affected crowns rot and leaves turn brown leading to death of the plants.

Phytophthora palmivora and *Fusarium oxysporum* were isolated and identified from ‘Big Blue’ *Liriope* symptomatic plants taken from the nursery and landscape. The pathogens were positively identified by morphological features of the pathogens and then confirmed with polymerase chain reaction.

Pathogenicity tests were performed in the greenhouse using three *Liriope* cultivars including ‘Emerald Goddess’ and ‘Super Blue’, considered to be tolerant to this disease, and ‘Big Blue’ which was considered to be more susceptible. Four inoculation treatments were used: water control, *Phytophthora palmivora*, *Fusarium oxysporum* and *P. palmivora* + *F. oxysporum*. Results from this study confirm the pathogenicity of previously identified *P. palmivora* and *F. oxysporum* microorganisms causing leaf and crown rot. *P. palmivora* was the primary microorganism causing leaf and crown rot on ‘Emerald Goddess’ and *F. oxysporum* on ‘Big Blue’ leading to increased disease incidence and a decrease in fresh and dry weight of leaves. ‘Super Blue’ showed no significant differences in fresh and dry weight of leaves and roots, and in percentage of disease incidence between the inoculation treatments ($P > 0.05$).

A survey was conducted at 11 wholesale and 7 retail nurseries in Louisiana to confirm that leaf and crown rot was a problem in *Liriope* production. *P. palmivora* and *F. oxysporum* were recovered from plants exhibiting leaf and crown rot symptoms which indicated that the disease was present in all nurseries. Poor sanitation and cultural practices were found to be the primary factors leading to the development and spread of leaf and crown rot disease.

CHAPTER 1: INTRODUCTION

1.1 Ornamental industry in the United States and Louisiana

Ornamental horticulture became an important sector in the United States beginning in the 19th century (Wyman, 1973), and it has continued to increase in value across the country (Haynes et al., 2007). United States production and marketing of ornamental crops includes greenhouse, nursery, and ground cover crops which are primarily used in the landscapes (Hodges et al., 2010).

According to Hodges et al. (2011) the contribution of the Green Industry to the United States economy in 2007 was approximately \$175 billion. The two sectors of the Green Industry that had the greatest impact were of landscape services (\$50.28 billion) and greenhouse production (\$27.14 billion). Value added for all plant enterprises was \$3.19 billion in 2013 in the United States and total value to economy of Louisiana was \$7.34 billion (LSU AgCenter, 2013).

According to Bracy (2012), the economic contribution of Louisiana nursery and landscape industry ranked third in the agriculture industry at \$2.2 billion per year, and employed more than 56,000 workers.

1.2 Ground Covers

The total value of sales in nursery crops in 2006 was \$4.65 billion in the United States. Out of all the nursery crops, ground covers ranked fifth in 2006 with a gross sales estimated at \$359 million. The gross sales of ground covers in the United States increased by 31% in 2006 from 8% in 2003. Florida had the highest sales of ground covers at 39 % of sales, followed by California at 32% and Texas 6% (USDA, 2007). Ground covers and vines were the top commodities among other nursery crops with a 17.4% gross sale in Louisiana in 2005 (Brooker et al., 2005). The total sales of ground covers in 2008 decreased by 3% of total plant sales in the nursery industry in the United States (Hodges et al., 2010).

Ground covers are low growing plants that grow less than 2 ft and are commonly used in the landscape as a cover crop. Ground covers provide many uses in the landscape such as, foot traffic control, reduction of soil erosion especially on pronounced slopes, weed control, reduction of glare, and to help reduce soil temperatures (Gill and Owings, 2007).

Although many different ground covers are available that exhibit different features such as, colorful flowers, fruits, growth habit, size, texture, and other characteristics, evergreen ground covers remained the most common type of ground cover used in the landscapes (Collier and Longenecker, n.d.). Most ground covers spread rapidly and grow easily in areas of the landscape with poor light conditions. They provide a good alternative to turfgrass because they are more drought tolerant compared to turfgrass. They thrive well under the drip line of trees and shrubs where most turf grasses cannot grow and maintenance is more difficult (MacKenzie, 1997).

Their fibrous root system prevents soil erosion and run-off especially on steep slopes, and their dense foliage prevents water droplets from hitting the ground directly and in turn reduce soil compaction. Ground covers also work well as a living mulch maintaining moisture and reducing weed growth (Relf, 2001).

1.3 *Liriope* in the United States and Louisiana

Liriope belongs to plant family *Rusaceae* (Nyffeler and Egli, 2010), and was first considered to be grown as a ground cover or a border grass in the southeastern United States beginning around the mid-20th century (Brooker et.al, 2005). In 1790, Loureiro described the genus *Liriope* originating from Cambodia, Laos and Vietnam, which is now known as *L. spicata*. *L. muscari* is one of the most widely used species as a perennial ground cover in landscapes (Leahy and Davison, 1999).

Bailey (1929) changed the name of *Ophiopogon muscari* to *Liriope muscari* and described two varieties known as var. *variegata* and var. *exiliflora*. *L. spicata* and *L. muscari* are the primary *Liriope* cultivated in the United States (Fantz, 1993).

Liriope is one of the most used ground cover or border grass in the southeastern United States including Louisiana (Winter, 2003; Harrison, 2005; Gill et al., 2012). The percentage of the total sales of ground covers in wholesale production was 18.4% and 13.5% in the southeast United States and Louisiana, respectively, in 2005 (Broussard, 2007).

1.4 Leaf and Crown Rot of *Liriope*

The primary purpose of landscape plants is to provide form and function and increase the aesthetic qualities of the landscape. It is important that all plant material grown for the landscape is free of diseases and insects so as to avoid plant damage that could affect the appearance of plants and to the landscape itself (MacKenzie, 1997).

Crown and rot root is one of the many diseases that infect numerous landscape plants. This disease is known to affect many other horticultural crops such as vegetable crops, fruit trees, shrubs, and woody ornamental (Perry, 2006). Some of the landscape plants that are most susceptible to crown rot disease are; *Hosta* spp. infected by *Fusarium oxysporum* (Wang and Jeffers, 2000); *Petunia* infected by *Phytophthora nicotianae* (Windham, n.d.); woody ornamentals such as *Rhododendron* spp. infected by *Phytophthora* spp. (Doubrava and Blake, 1999).

Ground covers have also been found to be infected with crown rot (Hartman and Witt, 1988). Leaf and crown rot is the most common disease infecting *Liriope muscari*, primarily in the southeastern states of the country both in nursery production and in the landscape. There is little information about the causal agents of leaf and crown rot of *Liriope*, but many pathogens have been attributed to be the cause of this disease. Some reports indicate that *Phytophthora*

spp. and *Pythium* spp. are the pathogens causing the disease (Smith and Cartwright, 2010), specifically *Pythium splendens*, in which symptoms start at the tip and then leaves becomes yellow and then brown, until the crown can be easily pulled off (Deputy, 1999c). According to Standberg (2002a), however, symptoms (yellow or brown) starting from the tip of the leaves is not related to crown rot and he proposed that the cause is related to other diseases or to nutritional or physiological problems present in the plant. *Phytophthora nicotinae* has been isolated from symptomatic plants; however, *Phytophthora palmivora* has also been indicated as a cause of *Liriope* leaf and crown rot (Leahy and Davison, 1999; Standberg, 2001; Standberg, 2002a; Ferrin, 2011). Other pathogens suspected to be the cause of crown rot in *Liriope* are *Fusarium oxysporum* and *Rhizoctonia solani*, and Standberg (2002a), proposed that *Fusarium* spp. is a secondary infection and that the primary cause of leaf and crown rot is *P. palmivora*. *Phytophthora* spp. was used to infect healthy and recently wounded plants causing a manifestation of crown rot symptoms and thus thought to be the primary pathogen (Erwin and Ribeiro, 1996). Inoculation with *P. palmivora* zoospores on *Liriope* plants caused symptoms on leaves, crown and roots, typical of leaf and crown rot disease (Standberg, 2002a). Insects, mites and nematodes that have been found on symptomatic plants but appear unrelated to the cause of the disease (Standberg, 2002a).

Symptoms of *Liriope* leaf and crown rot commonly appear in late spring and early summer with increasing temperatures and high amounts of rainfall or overwatering with the use of overhead sprinkler irrigation. Disease symptoms decrease when temperatures are lower at the end of the fall and early spring (Ferrin, 2011). Standberg (2002a) stated that leaf and crown rot of *Liriope* is more related to the use of excess water rather than temperature. Symptoms can appear on mature and immature leaves starting with a brown coloration and rotted tissue at the base of the leaves near the crown. Infected leaves become yellow from the bottom to the tip after

one or two weeks of root infection. After two to four weeks, the leaves become necrotic and subside. Diseased leaves can be easily pulled from the crown (Standberg, 2002a; Ferrin, 2011).

Thus the disease can be easily reproduced and spread by splashing surfaces or standing water with the use of overhead sprinkler irrigation or rain water. The closer the spacing of plants the higher the incidence of infection of healthy plants (Ferrin, 2011). Phytotoxicity caused by excess fertilization (Leahy and Davison, 1999), and burying the crown during planting (MacKenzie, 1997), may increase the susceptibility of the plant to leaf and crown rot. One of the most important methods of disease spread is through propagation. The most common type of propagation used in *Liriope* is division (Broussard, 2007). This type of propagation results in high incidence of disease due to division of infected roots and movement of pathogen in contaminated soils (Leahy and Davison, 1999; Standberg, 2002a). Injury during division also makes the plants more susceptible to the disease (Popenoe, 2008).

The substrate used in transplanting can favor the development of the pathogen. Substrates with low porosity and low oxygen levels can enhance the incidence of disease (Ferrin, 2011). A substrate using 100% builders sand resulted in no disease on 'Evergreen Giant' when inoculated with *Phytophthora palmivora* (Standberg, 2002b).

The most effective method to prevent disease spread is to use disease free plants, but because of poor sanitation and cultural practices during production in nurseries, it is difficult to obtain plants free of leaf and crown rot. The fungicides mefenoxam (Subdue®) and fosetyl aluminum (Aliette®) and Phosphite, may help reduce the development of the disease when used preventatively as a preplant soil drench, but these chemicals are often used to cure the disease after infection has occurred. Standberg (2006) reported that there was less incidence of leaf and crown rot in *Liriope* when cyazofamid (Segway®), dimetromorph (Stature DM), potassium

phosphite (Vital), or clothianidin (Celero 16WSG) fungicides were applied as a preventative drench.

There are many cultural practices that can be used to reduce disease incidence, if appropriate protocols are followed (Standberg, 2002a), however once established, the disease is difficult to manage or eradicate.

1.5 Objectives

Previous studies have suggested that there is a complex of more than one causal agent involved in causing leaf and crown rot of *Liriope*. Studies have also reported that different pathogens alone may cause leaf and crown rot of *Liriope*. Evidence suggests that some species and cultivars of *Liriope* are more susceptible to leaf and crown rot such as ‘Evergreen Giant’ (Standberg, 2002a; Ferrin, 2011). ‘Emerald Goddess’ has been reported to be more tolerant to leaf and crown rot and other fungal pathogens (Pategas and Pategas, 2011a; Owings, 2012b). ‘Big Blue’ *Liriope muscari*, is the most popular commercial variety, but it also appears to be susceptible to leaf and crown root rot (Allen Owings, Personal Communications).

The three objectives of this thesis were: 1) Identification of the micro-organism responsible for causing leaf and crown rot of *Liriope* in Louisiana.; 2) Determine the susceptibility of commercially available *Liriope* cultivars to leaf and crown rot; 3) Survey of the incidence of leaf and crown rot of *Liriope* in wholesale and retail nurseries in Louisiana.

CHAPTER 2: LITERATURE REVIEW

2.1 *Liriope*

Liriope and *Ophiopogon* are two genera first known as liriomondos (Bailey, 1929), and today are commonly referred to as liriopogons (Skinner, 1971). Species of both genera are named under this group due to problems in taxonomy (taxonomic confusion). Common names for *Liriope* species are “lilyturf”, “monkeygrass”, and “aztecgrass”, the last two names are also used for *Ophiopogon* spp. (Fantz, 1993). Liriopogons have become an important commodity in the landscape trade (Fantz, 1993). They are primarily grown in the southeast and central United States and they are widely used in landscaping as a perennial ground cover, in mass or as edging plants (Nesom, 2010).

They are preferred over other types of ground covers because they are known to be more resistant to or adapt better to adverse environmental conditions in the landscape, and for their resistance to pests and diseases (Fantz, 1993).

2.1.1 Morphology or Phytography of Liriopogons

Liriopogons are acaulescent herbaceous perennial plants and they are similar in appearance to turfgrass (Fantz, 2008a). Liriopogons are monocot plants that have been assigned to many families, but are recently considered belonging to the plant family *Ruscaceae* (Nyffeler and Egli, 2010). Foliage of liriopogons is generally green, but cultivars with variegated margins or stripes in cream, yellow, white or silver color are also available. Colorful clustered flowers grow among the leaves with colors like lilac to violet or white (Fantz, 1993). It was found that liriopogons number of chromosomes are $x = 18$ (Kim et al., 2010; Lattier et al., 2014). *Liriope muscari* was determined to be tetraploid and *Liriope spicata* hexaploid (Kim et al., 2010)

Liriope is considered to be a native from Japan, Vietnam, Taiwan and the east of Asia primarily from China (Bailey, 1929; Valder, 1999; Winter, 2003). In China, *Liriope*

(Convallariaceae) plants are known to be used in gardens as a ground cover or as edging plants (border grass), but they are also grown in pots for indoor and outdoor uses (Valder, 1999). It was introduced in the United States specifically in the southeastern part of the country approximately 150 years ago (Deputy, 1999a). Hume (1961) described five species of *Liriope* including two new species known as *L. graminifolia* and *L. gigantea* and eleven cultivars of *L. muscari*.

Liriope spp. are commonly known to have spreading or clumping growth habit. The spreading type also referred as invasive plants, are rhizomatous plants that have a caudex or subterranean stem from which rhizomes grow from the mother plant and become a daughter plant or as stoloniferous (Deputy, 1999a; Fantz, 2008b; Nesom, 2010). These plants are commonly used in shaded and large areas that need to be filled with plant material. *L. spicata* and *L. graminifolia* are considered to have spreading type of growth (Nesom, 2010). Most cultivars of *L. muscari* have clumping growth habit, but some can grow as spreading types (Deputy, 1999a).

Flowers of liriopogons are grouped in fascicles that grow among the leaves (Fantz, 1993). The flower is composed of six sepals and petals (perianth) and six stamens, and floral whorls found in multiples of three (Fantz, 2008a; Lattier et al., 2014). Inflorescences are panicles, spikes or racemes (Cutler, 1992).

The three most common species of *Liriope* grown in the southeastern United States are *L. muscari*, *L. gigantea* and *L. spicata* (Owings, 2012b).

Liriope muscari is distributed in the United States mostly in Alabama, Georgia, Kansas, Louisiana, Maryland, Mississippi, South Carolina, and the most common names used for this species are big blue liriopogon, blue lilyturf, blue-flowered snakes beard. It is described to have a clumping growth with clumps of approximately 15 to 30 cm tall, and it is commonly used as a border or a massing plant. *Liriope muscari* is described to have short stolons (Nesom, 2010) or

non-stolons (Chen and Tamura, 2000). Leaves are green and measure 20 to 30 cm long with stiff veins (Chen and Tamura, 2000; Fantz, 2008b). Flowers are erect and grouped in clusters of three to eight, and consist of 3-6 mm long setiform bracts and a hypogynous ovary. Seeds are globose and appear purplish-black at maturity (Chen and Tamura, 2000; Broussard, 2007; Nesom, 2010).

The three most widely used cultivars in the landscape today are ‘Big Blue’ which is considered a cultivar of *L. muscari*, and ‘Emerald Goddess’ and ‘Evergreen Giant’, cultivars of *L. gigantea* (Nesom, 2010).

2.1.2 Culture

Liriope is described to adapt better in USDA hardiness zones 6 to 10 (Winter, 2003) or 7 to 11 (Harrison, 2005). It is known to adapt to adverse environmental conditions and can be grown in shaded areas (Harrison, 2005). *Liriope* can be substituted for turfgrass because of its shade tolerance. Tolerance to sun depends on the species or cultivar used. For example, *L. muscari* is more tolerant to sun than *L. spicata* (Harrison, 2005), but Odenwald and Turner (2000) reported that leaves sunscorch when exposed to direct sunlight. *Liriope muscari* can be planted in any season, but planting in fall and early winter is best for its establishment in spring (James, 2014). All cultivars of *L. muscari* have a tendency for the foliage to flatten in winter (Broussard, 2007)

Liriope is claimed to be drought tolerant (Deputy, 1999c), and can tolerate a wide variety of soil types (Harrison, 2005). However, *Liriope* prefers acidic soils (MacKenzie, 1997) with an approximate pH 6.0 (Deputy, 1999b).

Plants perform best when the soil is kept moist (Deputy, 1999a), but avoid saturated soils (Harrison, 2005). Overwatering plants can create an environment conducive for the development of root rot diseases (MacCubbin, 2002), thus it is recommended to plant in soils with good porosity and it is preferable plants be watered early in the morning (Harrison, 2005).

After establishment, *Liriope* does not require constant fertilization. Application of light fertilizer from February to September may improve aesthetic characteristics with a slow release fertilizer with high nitrogen (Deputy, 1999a). At planting time it is recommended to use “2 pounds of a slow release 12-6-6 fertilizer/ 100 sqft) (Winter, 2003). *Liriope* should be fertilized once per year, preferably in spring, (MacCubbin, 2002; Gill et al., 2012) or it can be top dressed with “1/2 inch of compost”, used as a nitrogen supplier (Deputy, 1999a) or fertilized twice a year in early spring and the middle of the summer (Midcap and Clay, 2014).

When planting *Liriope*, the crowns should not be planted below the soil line (Deputy, 1999a). Recommended spacing is 20 to 25 cm as ground cover or as a border plant (MacCubbin, 2002), plant spacing of 31- 41 cm is recommended with pint/quart sized plants (MacKenzie, 1997).

Liriope can be propagated both asexually and sexually, but asexual propagation by division is the most common method used (Harrison, 2006). Clumps of crowns termed “daughter plants” are divided from the larger clump termed the “mother plant” (Ingram, 1987). It is recommended that division occur prior to new growth (Broussard, 2007). Tissue culture can be used as a propagation method (Frett and Dirr, 1983). Pruning or mowing is a recommended cultural practice prior to spring growth, primarily for aesthetic purposes and to reduce diseases (Deputy, 1999a; Broussard, 2007). Harrison (2005) recommends mowing in early spring before new growth of clumps begins just above the crown of the plant. Mowing has also been suggested to reduce infestation primarily of scale insects and anthracnose (Killebrew, 1999).

2.1.3 Insects and Diseases

Scales are one of the most common insects known to affect *Liriope*. They primarily affect the aesthetics of the plant in late summer by producing yellow spots on the lower surface of the

leaves and near the crown. Heavy scale infestation can cause leaves to drop and affect the plant growth (Deputy, 1999a; Popenoe, 2008; Midcap and Clay, 2014).

Liriope is susceptible to a common fungal disease called Anthracnose. The disease is caused by *Collectrichum* sp., and it produces red to brown lesions on the tips or margins of the leaves. It appears primarily in late summer and fall when there is overwatering caused by rainfall or overhead irrigation, but symptoms can remain in winter (Owings, 2012b; Russ, 2014). Free water on foliage is required for the fungal spores to germinate and start infection (Popenoe, 2008). Root knot nematodes can also infect *Liriope*. Root knot (*Meloidogyne* spp.) and reniform (*Rotylenchus* spp.) nematodes have been reported to cause major damage to *Liriope* roots (Hagan, 2005). Symptoms include yellowing of the leaves, and stunting of plants.

Leaf and crown rot has become a major disease of *L. muscari* in the landscapes of the southeastern United States (Standberg, 2002a). Symptoms start with root rot and the pathogen causes small bright red lesions on the affected roots. As the disease develops, crowns of the plants are infected and the leaves start to rot at the base with a water soaked brownish appearance. The rest of the leaf turns yellow from base up. Later on, the affected leaves wilt and turn brown and sections of the plant die. Infected leaves can be easily pulled from the crowns (Popenoe, 2008). Roots may become sloughed and look discolored (Russ, 2014). Leaf and crown rot primarily appears in late spring and early summer during high humidity and high temperature conditions (Ferrin, 2011). Many pathogens have been attributed to cause these symptoms, but the primary cause has still yet to be determined. Deputy (1999a) indicated that *Pythium splendens* is the pathogen causing this disease, however, numerous studies suggested that the pathogen causing the disease is *Phytophthora palmivora* (Standberg, 2002a; Armitage, 2008; Popenoe, 2008; Ferrin, 2011; Owings, 2012b; Russ, 2014). Smith and Cartwright (2010) attributed the symptoms to be caused by *Pythium* sp. and *P. palmivora*, and Russ (2014) proposed *P.*

palmivora being the primary pathogen and *Fusarium oxysporum* and *Rhizoctonia solani* as the secondary pathogens.

Once leaf and crown is established, there are no control measures to eradicate it (Owings, 2012b), but proper cultural practices may help reduce the development of the disease. For container production adequate drainage should be used to avoid standing water and wide spacing between plants reduces spread (Ferrin, 2011). Ferrin (2011) also recommended to avoid overwatering and overfertilizing the plants. Fungicides can be used to manage disease, but not to eliminate it (Russ, 2014). It was found that fungicides such as mefenoxam (Subdue®), fosetyl aluminum (Aliette®) and Phosphite have been found to help to control the disease. Lower disease incidence occurred with the drench application of cyazofamid (Segway®), demetromorph (Stature® DM), potassium phosphite (Vital®), and Fluoxastrobin (TM-473®) (Standberg, 2006). However, sporangia in soil may not be affected by these fungicides, and the disease can appear again (Popenoe, 2008). The best control of this disease is to plant healthy plants and practice cultural techniques to help avoid the development of the disease (Ferrin, 2011).

According to Standberg (2002a), all *Liriope* plants are susceptible to this disease, but the most susceptible seems to be ‘Evergreen Giant’ (Leahy and Davison, 1999; Armitage, 2008; Russ, 2014). ‘Emerald Goddess’ seems to be the most resistant (Pategas and Pategas, 2011b).

2.2 *Phytophthora* spp.

Phytophthora spp. is commonly known as one of the most severe and destructive pathogens worldwide as exemplified by potato late blight disease caused by *Phytophthora infestans* in Ireland in 1845 (Clement, 1993). *Phytophthora* was first recorded in northeastern United States in 1843 (Erwin and Ribeiro, 1996). In 1876, Anton de Bary was the first to describe *P. infestans* as the cause of potato late blight (Erwin and Ribeiro, 1996). The name

Phytophthora comes from the greek phyto (plant) pthora (destructive), and there are at least 8000 species recognized to cause disease (Clement, 1993). Most *Phytophthora* spp. are known as soilborne pathogens, and can survive a certain period of time without a host (Erwin and Ribeiro, 1996). It is considered a facultative saprophyte (Ellis et al., 2008). *Phytophthora* is diploid at the reproductive phase unlike other fungi which are haploid at this stage (Sansome, 1965).

Phytophthora cell walls do not contain chitin as do many other fungi (Erwin and Ribeiro, 1996). *Phytophthora* and *Pythium* are known to belong to the *Pythiaceae* family (Erwin and Ribeiro, 1996) to the Oomycetes genus, and Chromalveolata kingdom (Adl et al., 2005). *Phytophthora* was first named to the Chromista kingdom in 1986 by Cavalier Smith (Erwin and Ribeiro, 1996), considered as “not true fungi”, being part of the water mold group, meaning that they need wet conditions to reproduce and develop (Rytönen, 2011). Oomycetes are known to be sexually reproduced by thick wall oospores, and can withstand adverse environmental conditions, and can survive in soil without a host for long periods of time (Gallegly, 1970). Antheridium and oogonium are the sexual structures of *Phytophthora* that produce oospores. There are two ways in which antheridium can be attached to the oogonium; if the antheridium surrounds the oogonium it is called amphigynous, but if it is attached to the side of the oogonium it is called paragynous (Fry and Grünwald, 2010).

Production of oospores in species of *Phytophthora* can be homothallic, heterothallic or both. Homothallic produce oospores by self-reproduction whereas heterothallic production of oospores depends on two mating types commonly known as A1 and A2 (Savage et al., 1968).

Survival of *Phytophthora* for long periods of time depends on the oospores (sexual structure) and chlamydopores (asexual structure) (Gallegly, 1970). Temperature seems to have an effect on oospores survival which seems to be susceptible to higher temperatures (> 40 °C) (Fry and Grünwald, 2010). Sporangia are formed by the germination of oospores or

chlamydospores. Sporangia is the asexual spore, and it has different morphological features that can change within species (Erwin and Ribeiro, 1996). There are two methods of sporangia dispersal; one that can be dispersed by wind to long distances called caducous, and the other that can be dispersed by the presence of water known to be non caducous in which zoospores are released by cold temperatures and free water (Goodwin, 1997). Zoospores have flagella (biflagellate) which give them the ability to swim, however they can cyst which means that they develop a cell wall caused by some shaking or any impact (Erwin and Ribeiro, 1996). They are known to have chemostatic and electrical qualities that can predispose them to be attracted to the host (Goodwin, 1997). Dispersal of zoospores are related to soils with high moisture and conversely drought environments (Rytönen, 2011).

Most of the *Phytophthora* spp. are commonly known to be soilborne pathogens (Goodwin, 1997), and the cause of disease in many important crops (Erwin and Ribeiro, 1996; Drenth and Guest, 2004). Since 1980's it has been found that more than 30 *Phytophthora* spp, cause disease in ornamental nurseries around the world (Lamour, 2013). Common names have been given to this disease by the symptoms on ornamental crops such as leaf blight, crown and root rot (Lamour, 2013; Schreier, 2013; Williams-Woodward and DeMott, 2014). Symptoms will depend on the host and the species of *Phytophthora* that is causing the infection, however, symptoms on most of host plants related to *Phytophthora* infections are when root and stems, specifically the lower portion, become rotted (Agrios, 2004). *Phytophthora* root rot is known to infect many fruit trees (Wilcox, 1992; Strand, 2002; Schnabel and Miller, n.d.), herbaceous plants and woody ornamental plants such as azalea (*Rhododendron*), japanese holly (*Ilex crenata*), boxwood (*Buxus sempervirens*), hemlock (*Tsuga*), dogwood (*Cornus Virginiana*), camellia (*Camellia japonica*), and others (Smiley et al., 1999 ; Hagan, 2000; Perry, 2006; Lamour, 2013) and many important agricultural crops (Kendrick, 1922; Tsao, 1990). This

particular disease has been the cause of damage of many landscape and ornamental plants produced in nurseries in the Southeast part of the United States and also to all states in the country (Ferguson and Jeffers, 1999; Olson et al., 2013). *Phytophthora* crown rot appears primarily when conditions are appropriate for the development of the pathogen such as excessive water and cool temperatures (15-30 °C) (Strand, 2002). Some of the production practices that can promote the development of the disease are attributed to the reuse of water for irrigation, reusing pots and not having the adequate drainage (Yakabe et al., 2009; Lamour, 2013). The disease can be easily spread by water, contaminated soil, infected plants, and tools that haven't been disinfected previously, and insects could be a possible vector (Adlam, 2014).

Morphological characteristics have been used for the identification of *Phytophthora* spp., but difficult because of similarities between species (Erwin and Ribeiro, 1996). Waterhouse (1963) was the first to divided species into six groups according to morphological features, and then described by Stamps (1990) and Erwin and Ribeiro (1996). Some of the characteristics that are used to differentiate between species are morphology of sexual and asexual structures such as position of the anteridium, morphology of sporangia (size, shape) if a papilla is present (papillate, semipapillate, non papillate), if it is heterothallic or homothallic, temperature during growth, if chlamydospores are present. Molecular identification is another tool used for accurate identification, mainly between species that have similar morphology. Identification is difficult by physiological characteristics (Erwin and Ribeiro, 1996). ELISA (enzyme linked immunosorbent assay) is used as a tool for identification of numerous fungi such as *Phytophthora*, *Pythium* and *Rhizoctonia*. There are kits available which can help to identify *Phytophthora* presence in a short period of time (Kabashima et al., 1997). These kits are commonly used in nurseries, to detect presence in irrigation water (Ali-Shtayeh et al., 1991). One of the molecular tools for DNA identification (Lamour, 2013) of *Phytophthora* spp. is PCR-SSCP (single strand conformation

polymorphism) (Gallegly and Hong, 2008). Restriction fragment length polymorphism (PCR-RFPL) is also used to determine *Phytophthora* spp. by the ITS region (Cooke et al., 2000).

2.2.1 *Phytophthora palmivora*: Biology, Ecology, Taxonomy

P. palmivora was described by Butler in 1919 (Lamour, 2013), it is commonly known to cause disease primarily in the subtropical and tropical plants (Erwin and Ribeiro, 1996).

According to Mchau and Coffey (1994), *P. palmivora* originated in Asia, or Central and South America, but because of the high number of tropical and subtropical plants affected by this pathogen it seems that Central and South America are the primary areas where the pathogen originated (Erwin et al., 1983). This pathogen is known to cause several diseases in the same plant, and to have more than 150 plant hosts (Zentmyer, 1973; Chliyeh et al., 2014c).

P. palmivora is primarily known to cause a severe disease called black pod in cocoa (*Theobroma cacao* L.), that caused a worldwide crop loss of approximately 20 to 30 %, but can also cause stem canker and chupon wilt (Erwin and Ribeiro, 1996). There are many other important crops that are known to be affected by this pathogen causing diseases such as foot rot in rubber (*Hevea brasiliensis*) and black pepper (*Piper nigrum* L), patch canker in durian (*Durio zibethinus*), bud rot and fall of premature coconut (*Cocos nucifera*), fruit rot of breadfruit (*Artocarpus altilis*), root and fruit rot of papaya (*Carica papaya*) (Erwin and Ribeiro, 1996; Chliyeh et al., 2014c), root rot and leaf blight in citrus (Zitko et al., 1991; Drenth and Sendall, 2001), and bud rot in African oil palm (*Elaeis guineensis*) (Torres et al., 2010).

P. palmivora is classified in Group II (Waterhouse, 1963) and considered to be a heterothallic species, with caducous ellipsoid and ovoid, pyriform sporangia with a papillate, short pedicels (5 μ), formed in groups in the sporangiospore (20 sporangias in one sympodium) (Erwin and Ribeiro, 1996; Drenth and Sendall, 2001). *P. palmivora* is mainly recognized when

compared with others by different papillate present in the sporangia and also by the production of chlamydospores (Erwin and Ribeiro, 1996; Drenth and Sendall, 2001).

Phytophthora spp. have been attributed to root rot and collar rot in woody ornamentals and landscape plants (Smiley et al., 1999; Hagan, 2000; Vincelli and Hershman, 2005; Gillman, 2011). Root rot symptoms in ornamentals is associated with *P. palmivora* (Pérez Sierra and Jung, 2013). *P. palmivora* can cause disease to more than 200 ornamental plant species (Chliyah et al., 2014c). It has been recovered in greenhouses, nurseries and landscapes (Leonberger et al., 2012). Ground covers commonly used worldwide such as English ivy (*Hedera helix*) have being affected by stem and root rot caused by *P. palmivora*, found in North Carolina greenhouses (Hwang and Benson, 2005). In Italy *P. palmivora* was reported to cause root rot disease on *Grevillea* spp. (Cacciola et al., 2003), and in lavender (Davino et al., 2002). In Taiwan *P. palmivora* was recovered from maiden fern (Ann, 2000b), also causing blight in flowers of ornamental ginger (*Curcuma alsinifolia*) and wilt in *Petunia* plants (Ann, 2000a).

P. palmivora is the most known species to infect many palms, approximately 25 species, around the world primarily in tropical and subtropical regions (Elliott et al., 2004). *P. palmivora* also infects to other important ornamentals such as hibiscus (Balakrishnan, 1947), and bougainvillea (Erwin and Ribeiro, 1996).

2.2.2 Disease Cycle of *Phytophthora* Root Rot and Crown Rot

Phytophthora spp. is commonly related to be the cause of crown and root rot and damping off primarily on seedlings of some plants. Symptoms depend on the *Phytophthora* species that is attacking the plant, susceptibility of the host, and environmental factors such as soil moisture and temperature but symptoms mostly appear on the foliage and roots. Seedlings wilt and become chlorotic, and the pathogen may affect the normal development of the plant.

Roots become brown and sloughed, and development of new roots is affected (Drenth and Sendall, 2001; Perry, 2006)

Phytophthora spp. are mostly known as soilborne pathogens, but *Phytophthora palmivora* is known to also infect plants above ground (Drenth and Guest, 2004). Survival in soil without the presence of a host and when environmental conditions are not favorable for the development of the disease depends on oospores and chlamydospores sexual structures that can survive for several years (Erwin and Ribeiro, 1996; Rytönen, 2011). Mycelia is produced by the germination of oospores producing sporangia (asexual structure) in presence of water in the soil. This structure contains zoospores that are released by water and they can be spread by standing water, run-off water, splashing water, wind, free water on aerial plant parts and other vectors that makes them travel short and long distances and cause the plant infection mostly by roots or other infection sites (Drenth and Guest, 2004; Rytönen, 2011). Disease dispersal of *Phytophthora* spp. in the nursery can be also attributed to cultural practices that create favorable conditions for the development and dispersal of the disease (Lucas et al., 1991; Hagan, 2000; Daughtrey and Benson, 2005; Stewart-Wade, 2011). *Phytophthora* spp. have been found in irrigation water, especially recycled water (Hagan, 2000), and also in container mixes (Ferguson and Jeffers, 1999; Lamour, 2013). *P. palmivora* was found in recycled water used in Virginia nurseries (Bush et al., 2007).

2.2.3 Spread and Control

There are many factors or conditions that can promote the development or spread of the disease, but it can be controlled by proper management to prevent the outbreak of disease. Preventative methods should be practiced to avoid development or introduction of disease, because once established there are no curative methods that can eliminate the pathogen

completely. It is important to combine good cultural practices along with the use of fungicides as a preventative not as a curative practice (Lucas et al., 1991).

Sanitation practices, like those used by disease-free certified nurseries (Drenth and Guest, 2004; Ferrin, 2011) include sterile substrate (Koike, 2010), plants situated in places with proper drainage, spacing, and removing plant debris that could serve as a source of inoculum (Erwin and Ribeiro, 1996; Daughtrey and Benson, 2005; Ferrin, 2011).

Composted pine bark when used as at least 20% of the substrate may help reduce root rot disease caused by *Phytophthora* and *Pythium* (Koike, 2010). Substrate with high amounts of peat moss appear to increase disease development of these two pathogens (Hoitink et al., 1975), and also was related to increase disease incidence on leaf and crown rot on *Liriope* (Standberg, 2002b).

Water and temperature are the most important factors creating a favorable environment for the production of sporangia and zoospores (Erwin and Ribeiro, 1996). pH is another factor that can influence the development of disease, it has been found that a lower pH can suppress *Phytophthora* spp. growth (Erwin and Ribeiro, 1996; Daughtrey and Benson, 2005). According to (Leahy and Davison, 1999) over fertilization could make the plant more susceptible to *Phytophthora*.

2.2.3.1 Biological Control

Biological control using beneficial organisms (Pal and Gardener, 2006) can reduce disease development, and reduce the use of fungicides (Khetan, 2000). Some of the antagonist organisms that have been found to help to reduce *Phytophthora palmivora* are *Trichoderma martiale* that help control black pod disease in cacao (Hanada et al., 2009), and the bacterium *Paenibacillus polymyxa* has been found to help interfere with oomycetes colonization (Timmusk et al., 2009).

2.2.3.2 Chemical Control

Fosetyl aluminum (Aliette®) and metalaxyl (Ridomil®, Subdue®) are systemic fungicides commonly used to control soilborne pathogens such as *Phytophthora* spp. including *Phytophthora palmivora* (Lucas et al., 1991). Application of fosetyl aluminum (Aliette®) as a foliar fungicide can be absorbed by different part of the plants (roots, leaves and stem) (Pscheidt and Ocamb, 2015). Metalaxyl (Ridomil®, Subdue®) applied as a soil drench is transported through seeds and roots to new growth (Drenth and Guest, 2004). Other fungicides used for *Phytophthora* spp. are mefenoxam (Subdue Maxx®) and propomocarb (Elliot et al., 2004). There are many other systemic fungicides introduced recently for the control of *Phytophthora* and downy mildew in ornamentals such as dimethomorph, cyazofamid and fanamidone (Daughtrey and Benson, 2005).

Fungicides that are commonly used for leaf and crown rot of Liriope are mefenoxam (Subdue Maxx®), propamocarb (Banol® 66.5MC), fosetyl aluminum (Aliette®), phosphite fungicides (Leahy and Davison, 1999; Standberg, 2002a; Smith and Cartwright, 2010). These fungicides are recommended to be used as prophylactic applications because structures of *Phytophthora* living in soil will not be affected by fungicides and disease may appear again (Standberg, 2002a).

2.3 *Fusarium* spp.

Fusarium spp. are known to be soilborne pathogens that have been found around the world and mainly by the production of mycotoxins being harmful and pathogenic to humans, animals and plants (Nelson et al., 1994). They are also known to be soil saprophytes that can survive during a long period of time as chlamydospores or hyphae primarily in soil debris (Stoner, 1981). Link (1809) first described banana shaped macroconidia asexual spores as a key character used to be place an organism in the *Fusarium* genus.

Sexual life of *Fusarium* is varied (asexual, homothallic and heterothallic) (Trail, 2013). Asexual spores produced by all *Fusarium* species are macroconidia, microconidia and chlamydospores; and ascospores which are sexual spores not produced by all *Fusarium* species (Trail and Gardiner, 2014), and dispersed long distances by air (Klix, 2007). Macroconidia and microconidia are produced by the asexual state (anamorph) and can be dispersed by wind or wáter, primarily rain splash (Klix, 2007). Dispersal may occur when the pathogen is causing infection to the host and also when the pathogen is in a survival stage as a saprophyte in plant debris or organic matter (Trail and Gardiner, 2014). Macroconidia is septated and has different shapes and sizes that can be formed on sporodochium or also can be produced in monophialides and polyphialides in the aerial mycelium. Microconidia have also several shapes and sizes and are formed in the aerial mycelium in monophialides or polyphialides and can be grouped or in chains. Chlamydopores are also formed by *Fusarium*, and are a thick wall structures which contain lipids and can be produced in various forms such as single, pairs grouped or in chains (Nelson et al., 1994; Moretti, 2009).

Fusarium is classified in the class of Ascomycetes and in the order Hypocreales. Teleomorphs of *Fusarium* spp. are placed mostly within the genus *Giberella* (Moretti, 2009), which belongs to same class and order, and to the Nectariaceae family (Klix, 2007). Anamorphs have been categorized in the Pezizomycotina subphylum, Sordariomycetes class and Hypocreales order (Trail, 2013).

Pathogenic *Fusarium* spp. are known to have a wide range of hosts. It affects most of the important agricultural crops (Esser et al., 2002). It causes disease approximately to 81 hosts out of 101 economically important crops (Chandra et al., 2011). Some of the crops being affected are sugarcane (*Saccharum officinarum*), cereals and grasses, cucurbits, squash (*Cucurbita* spp.), bean (*Phaseolous vulgaris*), pea (*Pisum sativum*), potato (*Solanum tuberosum* L.) and many others

(Esser et al., 2002; Klix, 2007). *Fusarium* can be found in tropical and temperate places, and in deserts, alpine and arctic zones (Stoner, 1981). *Fusarium* is associated with root infection and aerial plant parts (Nelson et al., 1994).

Fusarium spp. causes crown rot, head blight, and scab that affects to some cereal grains such as wheat (*Triticum aestivum*), barley (*Horedeum vulgare*) and oat (*Avena sativa* L.) (Schmale III and Bergstrom., 2010); also causing vascular wilts to many horticultural crops (Nelson et al., 1994; Agrios, 2004), root rots and cankers (Booth, 1971).

Taxonomy of *Fusarium* spp. has greater than 1000 described species which were classified in 16 sections and 65 species (Wollenweber and Reinking, 1935). Characters that were used for classification into sections were mainly presence, absence and shape of microconidia, presence, absence and location of chlamydospores, shape of macroconidia. Other features were used for classification of sections into species. Taxonomy of this genus has been controversial because morphological, cultural and physiological variation in *Fusarium* (Nelson et al., 1994). Classification and identification of *Fusarium* genus relied primarily on morphological characteristics, but recent investigations propose molecular studies as a tool for classification (Klix, 2007; Moretti, 2009). Molecular tools that have been used for *Fusarium oxysporum* identification are RFLP (restriction fragment length polymorphism), AFLP (amplified fragment length polymorphism), PCR and RAPDs (random amplified polymorphic DNA) (Saikia and Kadoo, 2010). The translation elongation factor 1- α (TEF) gene has been used because it is highly informative within *Fusarium*, with the use of EF1 and EF2 primers for amplification (Geiser et al., 2004).

Culture media that are mostly used for *Fusarium* spp. growth and further identification are carnation leaf agar, KCL (potassium chloride medium), potato dextrose agar (PDA) and soil agar (Nelson et al., 1994). Booth (1977), described PDA as a medium used for *Fusarium*

oxysporum growth. Color of colony growth of *F. oxysporum* in PDA can be white or pink or violet to a dark purple colour cottony mycelia growth (Tuite, 1969; Rodrigues and Menezes, 2005; Sharma and Pandey, 2010).

2.3.1 *Fusarium oxysporum*: Biology, Ecology, Taxonomy

Fusarium oxysporum was one of the nine species that were reduced by Snyder and Hansen according to the taxonomic system previously described by Wollenweber and Reinking (1935), and placed in the *Elegans* section (Snyder and Hansen, 1940).

F. oxysporum is within the *Fusarium* species that has a great impact on many important crops (Rehman et al., 2013). Strains of *F. oxysporum* can be found as pathogenic and non-pathogenic (Bao et al., 2002). Pathogenic strains may cause symptoms and diseases such as wilt, root and crown rot (Burgess, 1981). *Fusarium* wilts can affect many important agricultural crops such as vegetables, flowers, perennials specifically herbaceous ornamental plants, and many others primarily caused by *F. oxysporum* (Agrios, 2004), which is known to affect approximately 120 crops (Rehman et al., 2013).

Infection starts in roots and fungi travels through the vascular system. Plants not showing symptoms restrict fungal movement through the vascular system and are unable to cause disease and are non-pathogenic (Olivain and Alabouvette, 1997). It is considered as a saprophytic soilborne fungi found to colonize roots of plants. Non-pathogenic strains are endophytes (Kistler, 2001) and are used as suppressive or biological control caused by pathogenic *F. oxysporum* strains (Postma and Rattink, 1992; Fravel et al., 2003).

F. oxysporum is known to cause disease to several hosts such as humans, animals and plants. Pathogenic *F. oxysporum* causing disease has been classified as *forma specialis* (*f.sp.*) and races (Amstrong and Amstrong, 1981). Some examples of *F. oxysporum* infecting tomato

was designated as *Fusarium oxysporum f. sp. lycopersici*; cotton *f. sp. vasinfectum*; carnation *f. sp. dianthii*; cucurbits *f. sp. conglutinans*; banana *f. sp. cubense*, and many others (Esser et al., 2002).

Fusarium oxysporum belongs to the Fungi kingdom, Phylum Ascomycota, Sordariomycetes class, Hypocreales order and *Nectriaceae* family (Michielse and Rep, 2009). *F. oxysporum* have been found in soils worldwide and reproduces asexually (Kistler, 1997). It produces three asexual spores which are macroconidia, microconidia and chlamydospores. Macroconidia are septated (3 to 4 septas) oval structures that are produced on conidiophores (terminal phialides) or produced by hyphae (intercalary phialides). Microconidia have an elliptic shape with no septations and are produced on short microconidiophores (intercalary phialides) in false heads in the aerial mycelium (Verma and Sharma, 1999; Ohara and Tsuge, 2004; Mace, 2012). Macro and microconidia are commonly situated in aerial plant parts such as stems of plants and known to serve as a secondary inoculum as a source of dispersal inside the plant and also to other uninfected plants (Ohara and Tsuge, 2004; Mace, 2012). In *F. oxysporum* there is frequent presence of chlamydospores which had a globose shape and a thick wall and is produced by conidia and hyphae (Ohara and Tsuge, 2004; Nowrousian, 2014). Chlamydospores are survival spores that can survive without a host for a long period of time and can serve as a primary inoculum in soil (Ohara and Tsuge, 2004; Mace, 2012).

F. oxysporum disease starts the infection throughout the roots by the spore germ tube or mycelium and then by the colonization of the vascular system, and then infecting other plant parts such as stems and crowns (Mourad AM et al., 2004). Some of the symptoms that can occur because of *F. oxysporum* infection are: browning vascular tissue, vein discoloration, wilting, yellowing on lower leaves, stunted growth of the plant, defoliation and plant wilt (Tjamos and Beckman, 1989; Ma et al., 2013).

Sources of dispersal of *F. oxysporum* can be by water or wind (Booth, 1971). Asexual spores are related to be dispersed by flowing water (Grinstein, 1983; Adams et al., 2013), and by air (Rowe, 1977). Contaminated plants and soil can serve as a source of spread of *F. oxysporum* (Mace, 2012), also by sexual (seeds) or asexual (vegetative) propagation (Adams et al., 2013).

Environmental conditions that will favor disease development are primarily in summer when temperatures are approximately at 28 °C (Adams et al., 2013). If temperatures are lower, less than 20 °C, the pathogen will avoid the development and plants will remain without symptoms (Dreistadt et al., 2001). Soils with lower pH, approximately 4.2 (Wilson, 1946), and excessive soil moisture in soil (Verma and Sharma, 1999), will also promote disease development.

2.3.2 Control

Fusarium wilt caused by *F. oxysporum* management relies on chemical, biological control, and sanitation through cultural practices (Gisi et al., 2009). This is a disease which is difficult to eradicate completely, but best management practices may help to reduce disease development. Management of fusarium wilt is best done by using preventative fungicides and propagation of clean stock (Verma and Sharma, 1999; Michielse and Rep, 2009). Chemical control includes methyl bromide and basamid which provides a provisional control (Dreistadt, 2001), and benzimidazoles that are used before transplant (Garibaldi and Gullino, 1990). Methyl bromide is widely used as a preplant soil fumigant, but also known to be harmful to the ozone layer (Bowers and Locke, 2000). There are other fungicide applications for soil such as thiophanate-methyl (Fungoflo®), iprodione (Chipco® 26019) and *Streptomyces griseoviridis* (Mycostop®) as a biofungicide. No chemical treatment completely eradicates the pathogen (Reuveni, 2002).

Sanitation and cultural practices are helpful in managing of *F. oxysporum*. Crop rotation using a tolerant crop, steam sterilization or solarization of the substrate or soil to be used for media, use of propagation material free of disease, and removal of infected plant debris (Garibaldi and Gullino, 1990; Dreistadt et al., 2001; Adams et al., 2013) are helpful. It was found that higher concentrations of free chlorine may help control *F. oxysporum* in irrigation water (Cayanan et al., 2009).

Studies on *F. oxysporum* and *Pseudomonas fluorescens* may represent biocontrol for fusarium wilts (Alabouvette et al., 1993), based on the presence of non-pathogenic strains of *F. oxysporum* and *Pseudomonas fluorescent* in fusarium wilt suppressive soils (Alabouvette and Lemanceau, 1996). Since 1970 non-pathogenic strains of *F. oxysporum* were described as biological controls, but it is difficult to distinguish between non-pathogenic and pathogenic strains (Stirling, 2014). Fravel et al. (2003) reported that not all non-pathogenic strains can control all pathogenic *F. oxysporum* causing fusarium wilt. *Thricoderma* spp. may also serve as a biological agent for *Fusarium* (Koike, 2010).

CHAPTER 3: IDENTIFICATION OF *PHYTOPHTHORA PALMIVORA* AND *FUSARIUM OXYSPORUM* ON SYMPTOMATIC *LIRIOPE* PLANTS

3.1 Introduction

Liriope are herbaceous perennial plants (Fantz, 2008a) that belong to *Ruscaceae* family (Nyffeler and Eggli, 2010), and are considered one of the most common groundcovers used in landscape primarily in the southeastern United States (Broussard, 2007). Three of the most common species used are *L. muscari*, *L. gigantea* and *L. spicata* (Owings, 2012b), but *L. muscari* is most widely used in landscapes (Leahy and Davison, 1999). In the last eight years nursery and landscape *L. muscari* plants have been found to be affected by leaf and crown rot disease (Owings, 2012b). According to Standberg (2002a), all *Liriope* species are susceptible to this disease, however, ‘Evergreen Giant’ seems to be the most susceptible.

Symptoms of leaf and crown of *Liriope* start with infection of the leaves at the base and then turning yellow from the bottom to the tip of the leaf with the leaves being easily pulled off from the crown (Standberg, 2002a). This disease mostly appears in the late spring and early summer where environmental conditions such as higher temperatures and heavy rainfall are optimum for the development of disease (Ferrin, 2011), and once it is established it is difficult to eradicate. Many micro-organisms have been attributed to be the cause of leaf and crown rot of *Liriope*. It was proposed that *Phytophthora palmivora* may be the causal agent of the disease (Standberg, 2002a; Armitage, 2008; Popenoe, 2008; Ferrin, 2011; Owings, 2012b; Russ, 2014), and others who have found *P. palmivora* and others have suggested that *Fusarium oxysporum* and *Rhizoctonia solani* are possible secondary pathogens (Russ, 2014). Smith and Cartwright (2010) proposed that *P. palmivora* and *Pythium* are the causal micro-organisms of the disease.

Although there have been numerous references to the micro-organisms responsible for leaf and crown rot of *Liriope*, none of these studies have conducted tests for positive

identification. Thus, the objective of this experiment was to identify and verify the presence of the most commonly suspected micro-organisms, *P. palmivora* and *F. oxysporum*, on *Liriope* with leaf and crown rot symptoms.

3.2 Materials and Methods

3.2.1 Isolation and Identification of Causal Organisms

Liriope muscari ‘Big Blue’ plants exhibiting symptoms of leaf and crown rot were collected in April 2013 from the landscape at Louisiana State University AgCenter Botanic Gardens and from containers from a wholesale nursery in Forest Hill, LA. Plants were transferred to the LSU Plant Diagnostic Center, Baton Rouge, LA for isolation of potential causal agents of leaf and crown rot.

3.2.1.1 *Phytophthora* spp.

For isolation of *Phytophthora* spp. from the plants exhibiting leaf and crown rot symptoms, the baiting method was used and modified (Erwin and Ribeiro, 1996) (Figure 1). Soil and infected roots were placed in 800 ml plastic containers. Tap water was added until it covered the soil and roots approximately 520 ml. Five leaf discs of *Camellia*, were floated on the surface of each of the soil and root samples in water. Containers were left for 48 h at room temperature and then disks were retrieved and washed with tap water for 2 h. After washing, leaf discs were blot dried with Kimwipes® (Kimberly-Clark, Profesional) and placed onto 100 × 15 cm Petri plates containing *Phytophthora* selective PARPH media (Jeffers and Martin, 1986) (Appendix A1). Plates were sealed with Parafilm M® (Bemys Company, Inc) and incubated in dark at room temperature for approximately 4 d.

Petri plates were observed under microscope for any mycelia growth. Plugs (1 cm³) of PARPH media with growth specific to *Phytophthora* spp. were transferred to Petri plates

containing V₈ media (20%) (Erwin and Ribeiro, 1996) (Appendix A2). Petri plates were sealed with Parafilm and placed in the dark at room temperature for 7 d.

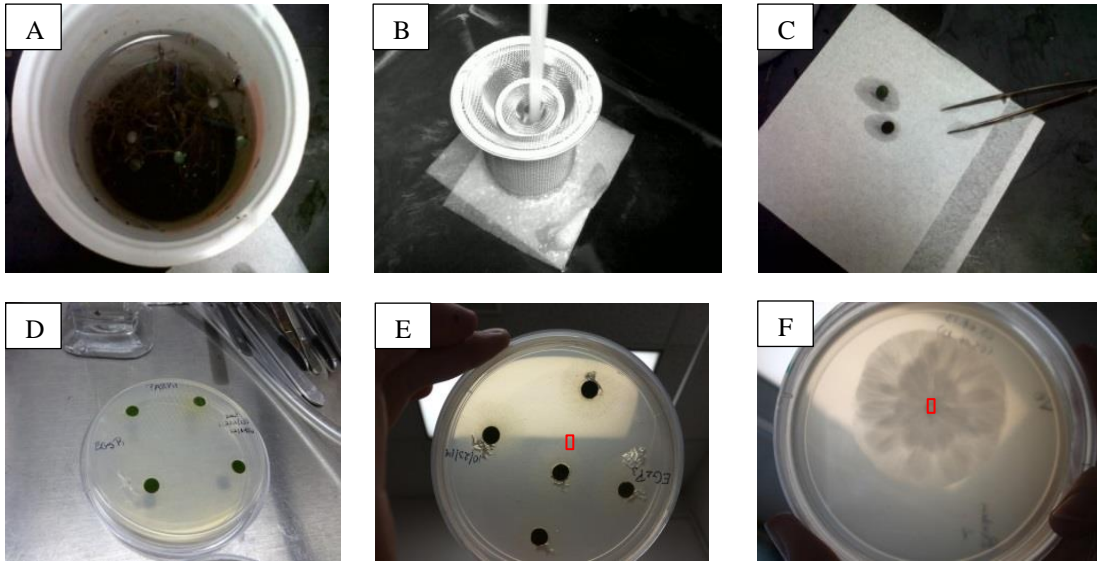


Figure 1. Procedure used for *Phytophthora* spp. isolation. A) soil and infected roots with tap water and camellia leaf disks, B) washing of *Camellia* leaf disks, C) drying of leaf disks, D) leaf discs plated onto PARPH media, E) PARPH media with mycelia growth, F) hyphal tip growth transferred to V₈ media.

3.2.1.2 *Fusarium* spp.

Fusarium spp. was isolated from the same plants that were used for *Phytophthora* spp. isolation. Symptomatic leaves (approximate size) were rinsed with tap water to remove soil and then surface disinfested with 10% sodium hypochlorite for 1 min. The disinfested leaf pieces were rinsed with deionized water and blotted.

Leaves were aseptically cut into three mm² pieces and plated onto ¼ PDA modified media (Ainsworth, 1971) (Erwin and Ribeiro, 1996) (Appendix A3). Petri plates were sealed with Parafilm and incubated at 28°C for five days. Each of the isolates were subcultured in a new ¼ PDA media Petri plate using the hyphal tip method, sealed with Parafilm and placed in a dark incubator at 28 °C (Figure 2). Cultures with mycelia growth were used for morphological identification.

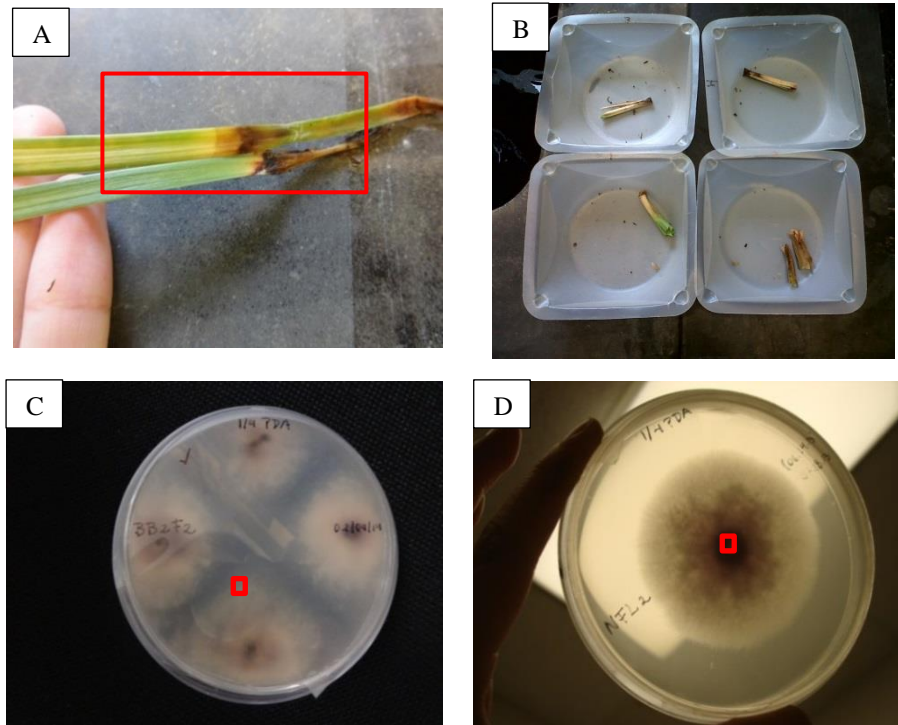


Figure 2. Procedure used for *Fusarium* isolation. A) bottom of leaves with lesions, B) surface disinfected using 10% sodium hypochlorite for 1 min and rinsed in deionized water, C) leaf squares plated onto ¼ PDA media and D) hyphal tip transferred to ¼ PDA media 28 °C, 4 – 5 d.

3.2.2 Long Term Storage of *Phytophthora* and *Fusarium*

Three glass vials (15 × 45 mm) (Kimble-Chase[®]) were filled with 2 ml of autoclaved nuclease free water. Seven isolates, four from nursery and two from the landscape that were previously identified morphologically as *F. oxysporum* were subcultured onto ¼ PDA media. After 3 d each isolate was transferred to a new ¼ PDA media plate and sealed with Parafilm and incubated at 28°C for 7–8 d. A 4 × 4 cm square was cut in small squares approximately 2 mm³ from the center of the media with mycelia growth and placed in each of the three tubes per isolate. Tubes were capped and sealed with Parafilm. A total of 21 tubes were stored at room temperature.

The same procedure was used for long term storage of *Phytophthora*, however V₈ media was used for cultures from six isolates, four nursery and three landscape isolates. Three glass vials per isolates were also used for long term storage isolation resulting in a total of 21 tubes.

3.2.3 DNA Extraction

3.2.3.1 *Phytophthora palmivora*

Seven isolates previously used for long term storage were used for *P. palmivora* DNA extraction, which were four nursery and three landscape isolates. Each isolate was subcultured on V₈ media, and stored for seven days, in dark at room temperature. On 7 June 2013, using a 9 mm sterilized cork borer, three plugs of V₈ media with mycelia growth were taken and placed on a 60 mm Petri plate and suspended in distilled water and placed in dark at room temperature (Figure 3). After 3 d, plugs were cut in half and the section with mycelia growth was used for DNA extraction.

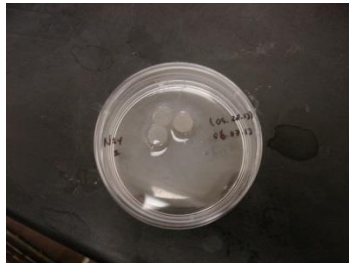


Figure 3. V₈ media plugs with *Phytophthora* mycelia growth in tap water.

3.2.3.2 *Fusarium oxysporum*

Six isolates, three from nursery and three from landscape were used for DNA extraction of *Fusarium*. These isolations were subcultured in May 2013 in a ¼ PDA media from long term storage cultures. Plates were sealed with Parafilm and placed in a dark incubator at 28°C for seven days. To obtain the sample for DNA extraction, a sterilized inoculation loop was used to scrape the mycelia growth (Figure 4).

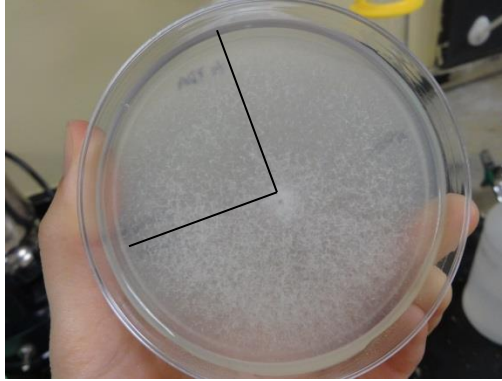


Figure 4. Mycelial growth of *Fusarium oxysporum* on ¼ PDA media plate, 1/4 of sample scraped and used for DNA extraction.

A DNeasy Plant Mini Kit (QIAGEN Inc, Valencia, CA) was used for DNA extraction of *Fusarium* sp. and *Phytophthora* sp. The manufacturer's protocol was followed from step #11. A Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific INC., Wilmington, NC) was used for DNA quantification with absorbance of 260 nm.

3.2.4 PCR (polymerase chain reaction) Amplification and Sequencing

PCR amplification was used to identify both pathogens *Phytophthora* spp. and *Fusarium* spp. from the DNA extractions.

Primers used for amplification of the ribosomal ITS (internal transcribed spacer) region for the identification of *Phytophthora* spp. were ITS 6 (5'-GAAGGTGAAGTCGTAACAAGG-3') and ITS 4 (5'-TCCTCCGCTTATTGA TATGC-3') (Bowman et al., 2007); ITS 6 the forward primer and ITS 4 the reverse primer. The total volume of PCR reaction mixture was of 25 µl. The components used in the mixture were: 12.5 µl GoTaq® Green Master Mix (Promega Co.), 6.5 µl of nuclease free water, 1 µl of each primer (ITS 4 and ITS 6) and 4 µl of DNA template. Tubes with total PCR mixture were placed in a thermo cycler (C 1000 Touch™). The cycle consisted of: DNA denaturation cycle at 94°C for 3 min, 35 cycles at 94 °C for 1 min, 35 cycles at 55°C for 1 min, 35 cycles at 72°C for 1 min, and one final elongation cycle at 72°C for 10 min. PCR products were analyzed in 2% agarose gel electrophoresis using 15 µl of each sample

and 8 µl for the positive control for the DNA marker. Each sample was placed individually in each well of the gel.

Primers used for *Fusarium* spp. identification and to amplify the TEF (translation elongation factor) region were EF1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3'), used as the forward primer, and EF2 (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') used as the reverse primer (Geiser et al., 2004). The total volume of the PCR mixture was the same used for *Phytophthora* sp. (25 µl). The volume of the components used for the PCR mixture were: 12.5 µl of GoTaq® Green Master Mix (Promega Co.), 8.5 µl of nuclease free water, 1 µl of each primer (EF 1 and EF 2) and 2 µl of *Fusarium* spp. DNA. Tubes with the total mixture were placed in a thermocycler. The cycle consisted of 94°C for 2 min, thirty five cycles at 94°C for one min, thirty five cycles at 53°C for one min for annealing, thirty five cycles at 72°C for one min, and one cycle at 72°C for five min for final elongation. Products of PCR were analyzed in 2% agarose gel. The volume used for each sample in each well was 15 µl, nuclease free water was used as a negative control and 8 µl of DNA marker.

The gel was run at 98V for 1 h and placed under UV light (UV Transilluminator, Spectroline®, Select™ series) for data collection. Positive band gel fragments were collected individually and taken to the Gene Lab, located at LSU School of Veterinary Medicine for DNA sequencing. To identify sequenced samples of *Phytophthora* spp. and *Fusarium* spp. a GenBank database (National Center of Biotechnology Information, <http://www.ncbi.nlm.nih.gov/genbank/>) and nucleotide blast (Basic Local Alignment Search tool) was used.

3.3 Results

Each of the isolates from nursery and landscape grown plants were cultured in two different medias for identification; ¼ PDA media was used for *F. oxysporum* identification and PARPH and V₈ media for *P. palmivora*. Both organisms were identified according to mycelia

growth patterns, by observation of morphological characteristics, and a molecular study to confirm the results.

Isolates that were cultured on V₈ media for *P. palmivora* identification had a stellate pattern growth which is typical of *P. palmivora* mycelia growth (Figure 5). It was characterized by the presence of caducous and differentiated papillate in sporangia, and by the higher presence of chlamydospores (Erwin and Ribeiro, 1996) (Figure 6). Identification of *F. oxysporum* was based on mycelia growth in ¼ PDA media having characteristics such as branching, white and cottony typical growth of this species, and a purple pigment primarily found in the center of the culture (Booth, 1971) (Figure 5).

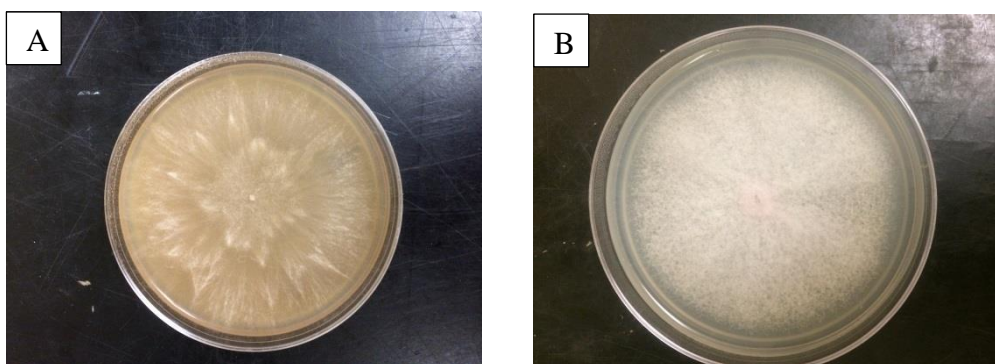


Figure 5. Cultures of A) *Phytophthora palmivora* on V₈ media and B) *Fusarium oxysporum* on ¼ PDA.

Four nursery isolates were morphologically and molecularly identified as *P. palmivora* (Figures 5 and 7). The ITS region was analyzed with the use of ITS 4 and ITS 6 primers with a DNA fragment between 750 - 1000 bp.

3.3.1 *Phytophthora palmivora* Sequence Analysis

P. palmivora and *F. oxysporum* isolates were sequenced to confirm species classification and compared to GeneBank database. ITS region sequences of four nursery isolates, nucleotide sequence identity showed 99% homology with *P. palmivora* strain TARI 24325 (GeneBank Accession No. GU111660.1)

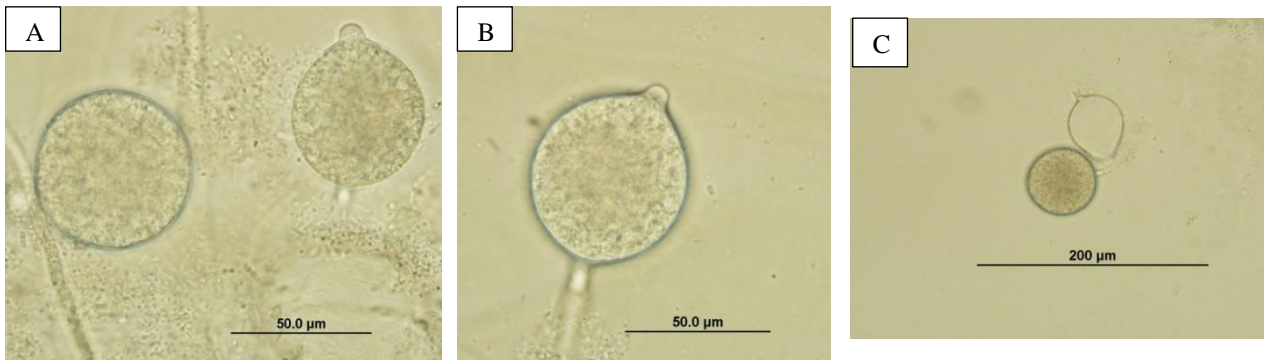


Figure 6. Morphological characteristics of *Phytophthora palmivora*: A) chlamydospore (left) and papillate sporangium (right), B) sporangium with zoospores inside, C) chlamydospore and empty sporangia (zoospores released).

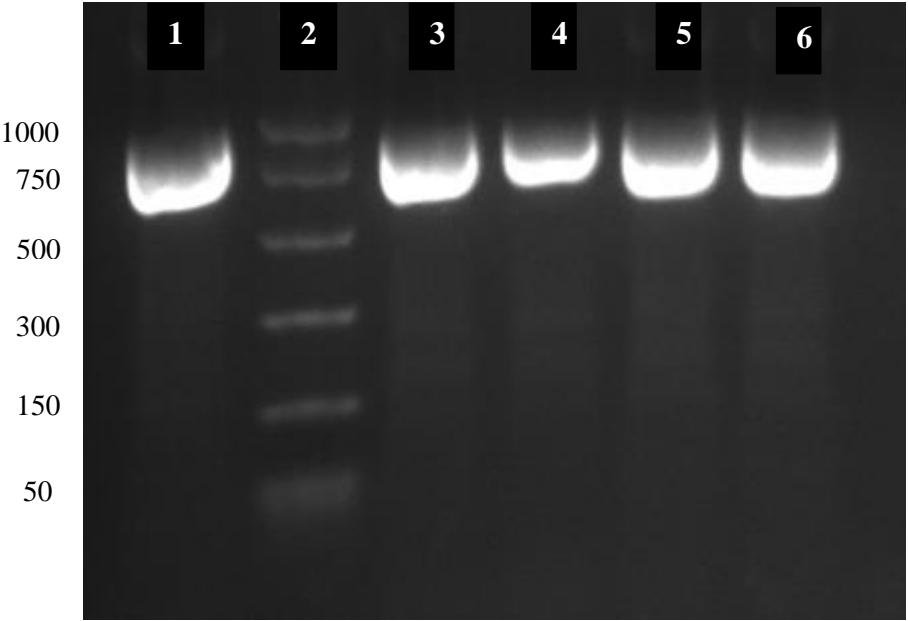


Figure 7. Agarose gel electrophoresis (2 %) of PCR from DNA extraction of *Phytophthora palmivora* isolates. Lane 1, positive control; Lane 2, DNA Marker; Lanes 3-6 nursery isolates.

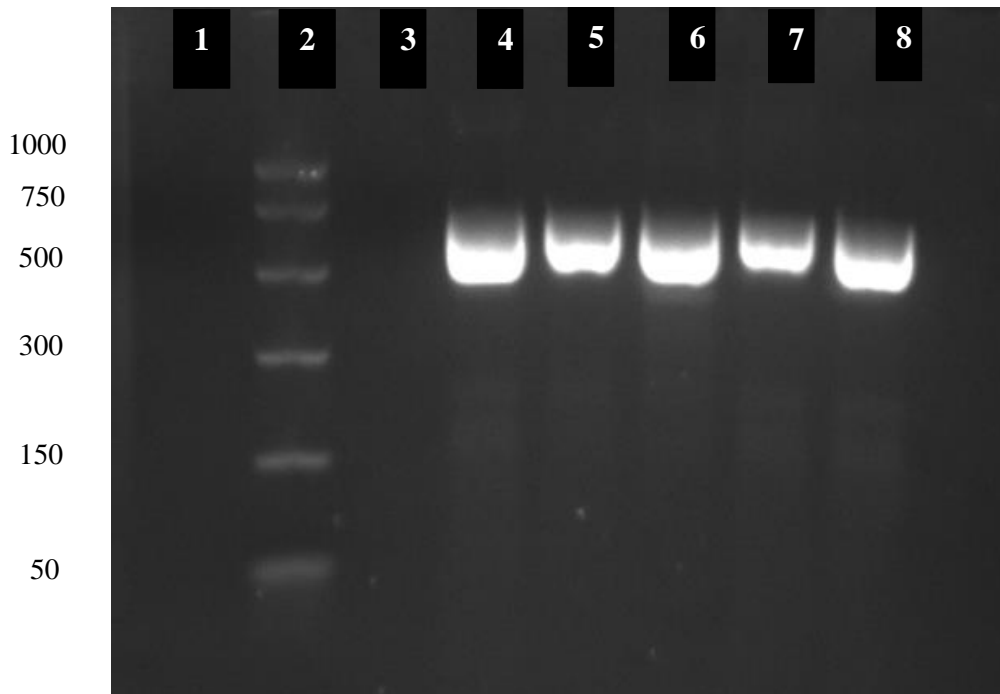


Figure 8. Agarose gel electrophoresis (2 %) of PCR from DNA extraction of *Fusarium oxysporum* mycelia growth on ¼ PDA media. Lane 1, negative control; Lane 2, 1000 kb DNA Marker; Lanes 3-5, nursery isolates; Lanes 6-8, landscape isolates.

Two nursery and three landscape isolates were morphologically and molecularly identified as *F. oxysporum* using a primer α elongation factor ef1 and ef2 with a DNA fragment of 700 bp (Geiser et al., 2004) (Figure 8).

3.3.2 *Fusarium oxysporum* Sequence Analysis

TEF region sequences of *Fusarium oxysporum* from two nursery, and three landscape isolates showed a nucleotide sequence identity matching of 99% with *F. oxysporum* (GeneBank Accession No. DQ837687.1).

3.4 Discussion

Diseases are a major cause of reduced plant growth and death in the nursery industry that can be caused by a number of plant pathogenic (disease-causing) organisms. Fungal diseases are the number one cause of crop loss worldwide (Langor and Sweeney, 2009). There are numerous diseases that may affect ornamentals plants in the landscape and nursery trade. Some of these

diseases include leaf spots and blights that mostly affect the aesthetics of plants, as well as powdery mildews, crown and root rots (Hong, 2014). Most of these diseases manifest various symptoms and affect plant quality which can affect sales. Leaf and crown rot is a disease affecting many nursery and landscape plants. Symptoms may not be present prior to planting in the landscape and can spread once planted, resulting in poor quality plants and plant death. Many organisms have been attributed to leaf and crown rot. This disease has recently been attributed to the decline and death of the popular landscape ground cover, *Liriope*, but the causal agent(s) are still unknown.

P. palmivora has been associated with crown rot symptoms in woody ornamentals nurseries (Donahoo and Lamour, 2008) and found to be the causal disease of several perennial plants causing palm bud rot (Elliott et al., 2004), coconut perennial bud rot (Briton-Jones and Cheesman, 1940) and diseases in citrus such as damping-off (Savita and Nagpal, 2012), and root rot (Ahmed et al., 2014). Similar crown rot symptoms such as chlorotic leaves and defoliation have been observed on *Liriope* (Chliyah et al., 2014a). Standberg (2002a) suggested that *P. palmivora* was the primary pathogen to cause leaf and crown rot disease in ‘Evergreen Giant’ *Liriope* thought to be the most susceptible to this disease and discontinued in the nursery trade. Similar leaf and crown rot symptoms such as yellowing and wilting leaves were observed in plants inoculated with *P. palmivora*. However, these results were based on visual observations and the micro-organisms causing the symptoms were not properly isolated and identified.

Fusarium spp. has also been associated with crown rot (Ploetz, 2006) and found in container grown *Hostas* developing crown rot symptoms (Wang and Jeffers, 2000). *Fusarium*, although suspected of being the pathogen causing leaf and crown rot in *Liriope*, it has not been properly identified and confirmed as the causal agent. Leahy and Davison (1999) suggested that *F. oxysporum* could be a secondary pathogen infecting *Liriope* and partially responsible for leaf

and crown rot symptoms. For example, *P. palmivora* and *F. oxysporum* were isolated from olive trees (*Olea europaea* L.) with symptoms similar to leaf and crown rot in *Liriope* such as wilting, chlorosis and defoliation (Chliyeh et al., 2014b).

This study focused on the isolation and identification of the causal agent of leaf and crown rot on *Liriope*. *P. palmivora* and *F. oxysporum* were isolated from symptomatic 'Big Blue' *Liriope* plants collected from the nursery and landscape. These disease organisms were then systematically identified by morphological observations, growth pattern in cultures and PCR analysis.

P. palmivora was identified by stellate pattern growth of mycelia and by microscopic observation primarily of caducous papillate sporangia, a unique characteristic compared to other *Phytophthora* spp. *F. oxysporum* was identified by characteristic cottony white mycelia growth with a purple color in the center of the culture plate.

P. palmivora and *F. oxysporum* isolates were sequenced to confirm species classification and compared to GeneBank database. ITS region sequences showed 99% homology with *P. palmivora* and the TEF region showed a 99% homology with *F. oxysporum*.

Now that the micro-organisms associated with leaf and crown rot of *Liriope* have been substantiated, research needs to be conducted to determine whether leaf and crown rot disease incidence is caused by one and/or both of these pathogens.

CHAPTER 4: PATHOGENICITY TESTS ON *LIRIOPE* CULTIVARS

4.1 Introduction

Liriope muscari is commonly used as a border or massing plant in landscapes in the United States (Fantz, 2008b). The most widely used *L. muscari* are ‘Big Blue’ and *L. gigantea* ‘Evergreen Giant’ and ‘Emerald Goddess’ (Nesom, 2010). Leaf and crown rot is a disease that affects the health and the aesthetics of *Liriope* used in the landscape. According to Standberg (2002a), all *Liriope* plants are susceptible to this disease. ‘Evergreen Giant’ has been labeled as the most susceptible (Leahy and Davison, 1999; Armitage, 2008; Russ, 2014) and ‘Emerald Goddess’ the most tolerant (Pategas and Pategas, 2011b). Standberg (2002a) inoculated *Liriope* plants with *P. palmivora* which resulted in leaf and crown rot symptoms such as yellowing and wilting leaves. Although *P. palmivora* was not confirmed as the causal organism in that study, Standberg (2002a) suggested that it was the primary micro-organism causing leaf and crown rot.

Fusarium has not been identified as being the causal pathogen of leaf and crown rot in *Liriope*, however, Leahy and Davison (1999) speculate that both, *Phytophthora* spp. and *F. oxysporum* could be the causal agents of leaf and crown rot with *F. oxysporum* representing an opportunistic or secondary pathogen. Studies need to be conducted to definitively identify the causal micro-organisms of leaf and crown rot on *Liriope* and test these micro-organisms for their effect on disease incidence. *P. palmivora* and *F. oxysporum* were positively identified from *Liriope* manifesting leaf and crown symptoms (Chapter 3). Thus the objective of this study was to determine the effect of *P. palmivora* and *F. oxysporum* on disease incidence and shoot and root growth of three *Liriope* cultivars.

4.2 Materials and Methods

4.2.1 Plant Production and Experiment Design

Three cultivars of *Liriope* were used for pathogenicity test: ‘Emerald Goddess’ and ‘Super Blue’ reported to be highly tolerant, and ‘Big Blue’ reported as susceptible (Allen Owings, personal communications). Disease free bare root liners ‘Emerald Goddess’ and ‘Big Blue’ were provided by Bill Moore & Company Inc, Florida and ‘Super Blue’ from KPS sales Inc, Costa Rica.

‘Emerald Goddess’ and ‘Super Blue’ were planted June 2013 and ‘Big Blue’ July 2013 in 0.49 L pots placed in plastic trays (26.5 cm × 52 cm × 5 cm), eighteen pots per tray. The substrate was composed of 1 cubic yard aged pine bark, 2721.5 g of pelletized dolomitic lime and 680 g of Scotts[®] granular micromax. Plants were grown in a greenhouse at LSU AgCenter’s Botanic Gardens, Ornamental & Turfgrass Research Facility in Baton Rouge, LA.

Plants were fertirrigated with 250 ppm N 20-20-20 (Scotts, Peters Professional[®]) and top dressed with 2.5 g of Scotts[®] Osmocote plus 15-9-12. Plants were treated with DuraGuard[®] ME September 2013 (15 g/ gallon of water) and on December 2013 with Tempo[®] SC Ultra insecticide (1 g/ gallon of water) for control of fungus gnats.

The inoculation experiment was replicated three times. Replicate one was conducted on 12 December 2013 when 60 plants per cultivar were transferred from black propylene pots of 0.49 L to 0.85 L pots, using the same substrate as previously listed. Plants were inoculated on 20 February, 2014. Plants were fertilized with 5 g of Osmocote plus Scotts[®] 15-9-11 as a top dress after inoculation on 3 March, 2014.

On 25 March, 2014, 390 plants (130 plants per variety) for replicates 2 and 3 were transferred to pots of 0.85 L with the same pine bark substrate. Plants used in replicate 2 were

top-dressed with 15 g of Scotts® Osmocote 15-9-11 on 13 May, 2014 and inoculated on 31 May, 2014.

Replicate 3 plants were transferred to 1.60 L propylene pots of on 7 August, 2014 using the same pine bark substrate. Plants were top-dressed with 15 g of Scotts® Osmocote 15-9-11 on 14 August, 2014 and inoculated on 22 August, 2014.

Greenhouse temperatures for each replicate were 20/31°C min/max spring 2014 were; 24°C/39°C min/max summer 2014; 23/32 °C min/max fall 2014.

4.2.2 Experimental Design

The experimental design for each replicate was a randomized complete block design (RBCD) with three blocks per replicate and a total of 60 experimental units per block in a 3 x 4 factorial; three *Liriope* cultivars: ‘Emerald Goddess’, ‘Big Blue’ and ‘Super Blue’ and four inoculations; Water control (WC), *P. palmivora* (PO), *F. oxysporum* (FO), and *P. palmivora* + *F. oxysporum* (PO+FO) for a total of 12 treatments (Table 1). There were five plants for each cultivar/treatment for a total of 45 experimental units per inoculation treatment. Plants were spaced at 33×16.5 cm between inoculation treatments to avoid cross-contamination.

Table 1. Treatments used for the pathogenicity test included three cultivars with four inoculation treatments.

Treatments	Cultivar	Inoculation treatments
1	Emerald Goddess	Water control
2		<i>P. palmivora</i>
3		<i>F. oxysporum</i>
4		<i>P. palmivora</i> + <i>F. oxysporum</i>
5	Big Blue	Water control
6		<i>P. palmivora</i>
7		<i>F. oxysporum</i>
8		<i>P. palmivora</i> + <i>F. oxysporum</i>
9	Super Blue	Water control
10		<i>P. palmivora</i>
11		<i>F. oxysporum</i>
12		<i>P. palmivora</i> + <i>F. oxysporum</i>

4.2.3 Inoculum preparation

Inoculum was prepared based on the total volume needed for all inoculation treatments.

The volume, application date and harvest date for each of the three replicated experiments are listed in (Table 2).

Table 2. Volume used for each inoculation treatment in each replicate with dates of inoculation and harvest.

Replicate	Inoculation treatment (ml)				Date	
	Control	PP	FO	PP + FO	Inoculation	Harvest
1	40	40	30	40 + 40	February 20, 2014	April 15, 2014
2	80	80	80	80 + 80	May 31, 2014	July 23, 2014
3	120	120	120	120 + 120	August 22, 2014	October 20, 2014

4.2.3.1 *Phytophthora palmivora*

One small block (3 mm³) of V₈ media with *P. palmivora* mycelia growth from one of the nursery isolates from long term storage was dried with a Kimwipe, cut in half and subcultured in the center of a new ¼ PDA media Petri plate (100 × 15 mm). The plate was sealed with Parafilm and incubated in the dark at room temperature approximately 5–7 d until mycelia growth reached half of the plate. From the edge of mycelia growth of one of the culture plates, one plug (2 mm³) was halved and transferred to the center of a Petri plate containing a 20% V₈ media, sealed and stored in dark room at room temperature for 8 – 9 d for mycelia growth to cover the entire plate.

Each plate of V₈ media with mycelia growth of *P. palmivora* was blended, one day before inoculation with 200 ml of deionized water for approximately 28–21 s (Standberg, 2006) using a commercial blender (Hamilton Beach®). The mixture of each plate was placed into plastic trays and covered with foil at room temperature for 24 h to stimulate the release of zoospores from sporangia.

4.2.3.2 *Fusarium oxysporum*

F. oxysporum inoculum was prepared from one of the isolates of long term storage. The same procedure for inoculum preparation of *P. palmivora* was followed, but subcultured in ¼ PDA media, and placed in an incubator at 28 °C. Inoculation solution of *F. oxysporum* was prepared in the same manner using ¼ PDA media with mycelia growth.

4.2.4 Inoculation Method

The *Liriope* substrate was saturated just prior to inoculation. The substrate around each crown was moved to the side of the container and a quantity of inoculum, depending on the replicate, was decanted around the crown using a graduated cylinder. The substrate was moved to recover the inoculated crown. Plants were not irrigated for approximately 2 – 3 d after inoculation, to avoid flushing the inoculums. Overhead irrigation was used for all three replicates: 1) 07:00 and 15:00 h for 7 min, 2) 07:00, 13:00 and 15:00 h, for 7 min, 3) 07:00, 10:00, 13:00 and 15:00 h for 7 min.

4.2.5 Data Analysis

Variables measured were fresh and dry weight of leaves and roots, percentage of disease incidence and percentage of root rot infection.

Disease incidence was defined as percentage of plants manifesting chlorosis as an initial symptom of leaf and crown rot. Progression of disease incidence included necrosis starting at the base of the leaves at the crown with chlorotic leaves. Disease incidence was measured every 10 days until plants were harvested on day 50.

Visible root rot, those roots that were brown and sloughed, was rated one day prior to destructive harvest. Root rot was rated according to the following scale: 6: healthy roots, no symptoms of root rot; 5: ≤25 % root rot; 4: ≤50 % root rot; 3: ≤75% root rot; 2: ≤95% root rot; 1: ≤ 100 percent root rot.

Leaf and root fresh weight was measured immediately after harvest. The leaves of each plant were cut just above the crown at the soil line and weighed (Scout® Pro, Ohaus). The substrate was removed from the roots by washing them with tap water. The crown was cut from the roots and was discarded. Roots were blotted with a paper towel and then weighed (Mettler PC 4400, Delta Range®). Leaves and roots were placed in separate # 6 hardware paper bags (Uline), and placed in a forced air dryer (Shel Lab, SM028-2) set at 50 °C. Dry weights were recorded after one week.

4.2.6 Re-isolation of *Phytophthora palmivora* and *Fusarium oxysporum*

Inoculated plants that showed symptoms were randomly selected for re-isolation of both micro-organisms. Samples of soil and roots of symptomatic plants were used for re-isolation of *P. palmivora* using the same baiting method as previously described. Leaf samples of symptomatic plants were used for re-isolation of *F. oxysporum* as previously described (Chapter 3), with the exception of time of surface sterilization. These samples were disinfected for 2 and 3 min with sodium hypochlorite and then disinfected with a Kimwipe sprayed with 85% ethanol. Three to four small infected leaf pieces were placed on a ¼ PDA media plate, and separated by each disinfection time. Time was increased to ensure there was no contamination.

4.2.7 Statistical Analysis

Root rot rating, disease incidence, fresh and dry weight of leaves and roots (dependent variables) were analyzed with SAS® 9.3 program (Statistic Analysis System). Proc Mixed was used for analysis with pdmix800 at $p < 0.05$ level (Saxton, 1998). LSMEANS (Least Square Means) separation and Tukey-Kramer were used for adjustment. Block was considered as random and replicates, treatments and cultivars were considered as fixed effects.

4.3 Results

4.3.1 Leaf and Root Fresh and Dry Weight

‘Emerald Goddess’ leaf fresh and dry weight was not significantly affected by the WC or FO inoculation treatments, however, leaf weights of plants inoculated with PP and PP+FO were similar, but significantly lower ($P < 0.0001$). Similar results were obtained for root fresh and dry weight, in which plants inoculated with C and FO had a similar dry leaf weight while the plants inoculated with PP and PP+FO had the lowest root fresh and dry weight (Table 3).

Table 3. Means of *Liriope gigantea* ‘Emerald Goddess’ leaf and root fresh and dry weights with four inoculation treatments.

Inoculation treatments	Weight (g) ¹			
	Leaf		Root	
	Fresh	Dry	Fresh	Dry
Water control	41.7 ^a	10.4 ^a	29.4 ^a	4.8 ^a
<i>Fusarium oxysporum</i>	42.0 ^a	10.4 ^a	27.9 ^a	4.3 ^{ab}
<i>Phytophthora palmivora</i>	21.6 ^b	6.5 ^b	19.1 ^b	3.4 ^b
<i>P. palmivora</i> + <i>F. oxysporum</i>	22.5 ^b	6.8 ^b	21.5 ^b	3.8 ^b

¹Means within columns followed by the same letter are not significantly different ($P \leq 0.05$).

Inoculation treatments had an effect on leaf fresh and dry weight of ‘Big Blue’, in which plants inoculated with FO had a lower leaf fresh weight than PP inoculated plants (Table 4).

Inoculation treatments had no effect on root fresh and dry weight of ‘Big Blue’ plants.

Table 4. Means of *Liriope muscari* ‘Big Blue’ leaf and root fresh and dry weights with four inoculation treatments.

Inoculation treatments	Weight (g) ¹			
	Leaf		Root	
	Fresh	Dry	Fresh	Dry
Water control	52.8 ^{ab}	13.4 ^{ab}	54.4 ^a	6.9 ^a
<i>Fusarium oxysporum</i>	44.1 ^b	11.7 ^b	45.6 ^a	5.7 ^a
<i>Phytophthora palmivora</i>	53.3 ^a	14.1 ^a	44.2 ^a	6.3 ^a
<i>P. palmivora</i> + <i>F. oxysporum</i>	47.5 ^{ab}	13.2 ^{ab}	50.3 ^a	6.9 ^a

¹Means within columns followed by the same letter are not significantly different ($P \leq 0.05$).

Inoculation treatments had no effect on leaf and root fresh and dry weights of ‘Super Blue’ plants (Table 5).

Table 5. Means of leaf and root fresh and dry weights of *Liriope muscari* ‘Super Blue’ with four inoculation treatments.

Inoculation treatments	Weight (g) ¹			
	Leaf		Root	
	Fresh	Dry	Fresh	Dry
Water control	100.2 ^a	24.1 ^a	59.3 ^a	9.6 ^a
<i>Fusarium oxysporum</i>	100.9 ^a	24.5 ^a	59.6 ^a	9.8 ^a
<i>Phytophthora palmivora</i>	101.0 ^a	26.3 ^a	55.1 ^a	10.0 ^a
<i>P. palmivora</i> + <i>F. oxysporum</i>	99.8 ^a	25.5 ^a	49.4 ^a	8.6 ^a

¹Means within columns followed by the same letter are not significantly different (P≤0.05).

4.3.2 Percent of Disease Incidence

‘Emerald Goddess’ plants inoculated with PP and PP+FO had a higher percentage of disease incidence from day 20 through day 50, with more than 90% of the plants exhibiting leaf and crown rot by day 50 (Table 6). Figure 9 provides a pictorial representation of disease symptom treatment differences indicated in Table 6. Symptoms of leaf and crown rot of plants inoculated with PP began as a slight chlorosis in the crown and in the base of leaves of the plant, followed by yellowing of the leaves from the bottom and moving upwards (Figure 10). For those plants inoculated with PP+FO leaf crown rot symptoms began as a necrosis moving up the leaves (Figure 11).

Table 6. Percentage of disease incidence of *Liriope gigantea* ‘Emerald Goddess’ with four inoculation treatments: water control (WC), *P. palmivora* (PP), *F. oxysporum* (FO), and *P. palmivora* + *F. oxysporum* (PP+FO).

Inoculation Treatments ¹	Days				
	10	20	30	40	50
WC	0 ^a	0 ^b	0 ^b	0 ^b	0 ^b
FO	0 ^a	0 ^b	0 ^b	0 ^b	0 ^b
PP	27 ^a	67 ^a	80 ^a	84 ^a	91 ^a
PP+FO	40 ^a	75 ^a	91 ^a	95 ^a	95 ^a

¹Means within columns followed by the same letter are not significantly different (P≤0.05).

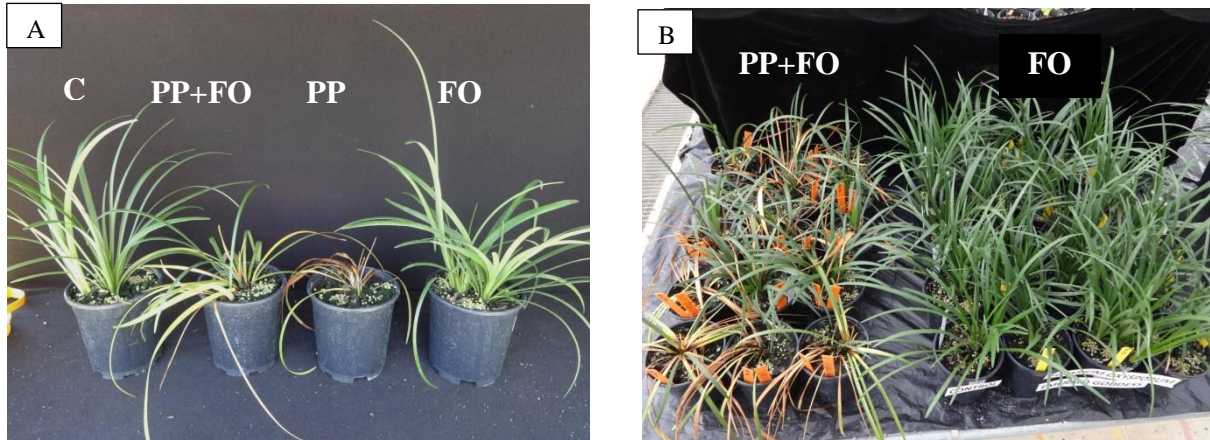


Figure 9. A) Leaf and crown rot symptoms on *Liriope gigantea* 'Emerald Goddess' inoculated with water control (WC), *Phytophthora palmivora* + *Fusarium oxysporum* (PP+FO), *P. palmivora* (PP), and *F. oxysporum* (FO). B) Symptomatic plants inoculated with *P. palmivora* + *F. oxysporum* (PP+FO), and asymptomatic plants inoculated with *F. oxysporum* (FO).

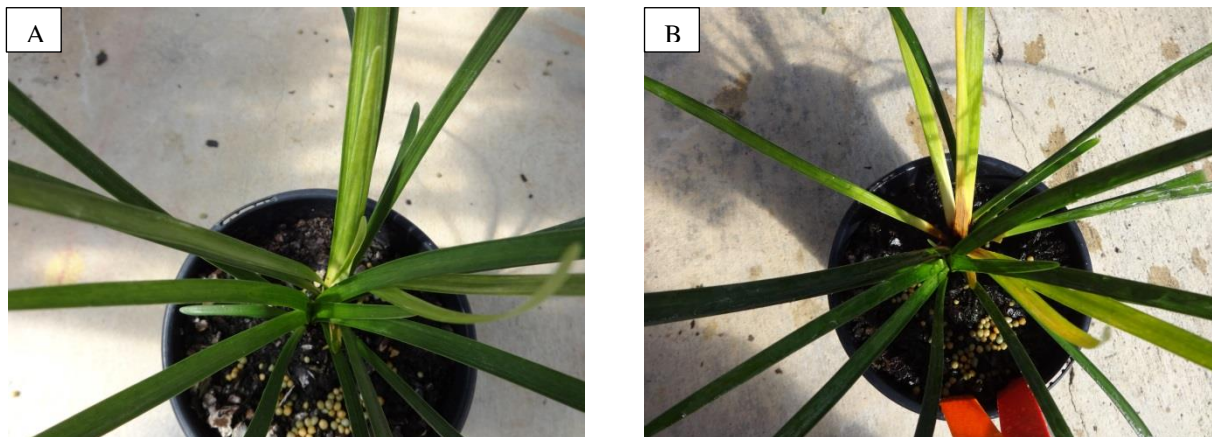


Figure 10. Progression of crown rot symptoms of *Liriope gigantea* 'Emerald Goddess' inoculated with *Phytophthora palmivora*. A) Leaf chlorosis starting at the base of the crown after 18 days of inoculation) and B) after 2 days (20 days after inoculation) with necrosis at the base of the leaves and chlorosis moving upwards.

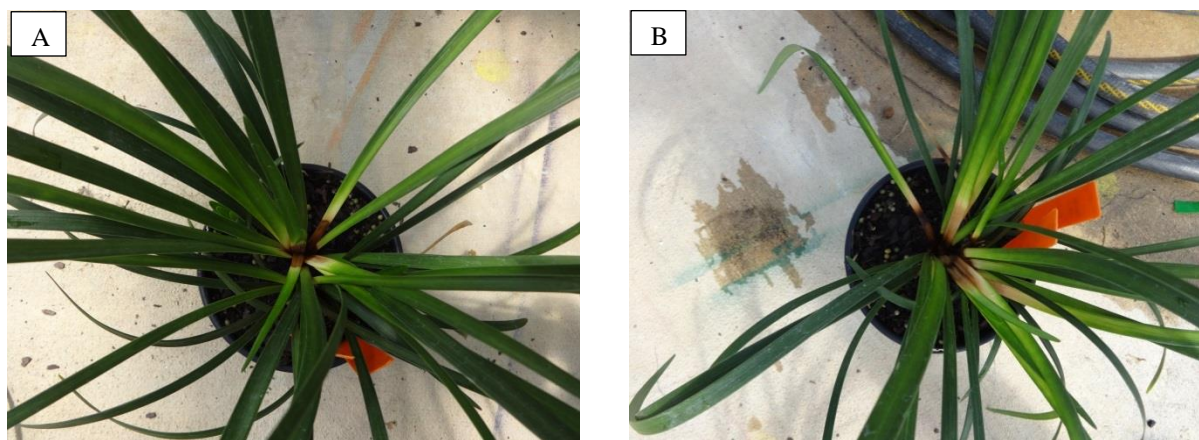


Figure 11. Progression of crown rot symptoms of *Liriope gigantea* ‘Emerald Goddess’ inoculated with *Phytophthora palmivora* + *Fusarium oxysporum*. A) Leaf chlorosis and necrotic tissue at the base of the crown after 14 days of inoculation and B) after 3 days (after 17 days of inoculation) necrosis and chlorosis progressing up the leaves.

‘Big Blue’ plants inoculated with FO had the highest percent of disease incidence compared to all other inoculation treatments after 20 d after inoculation (Table 7). ‘Big Blue’ plants inoculated with FO leaf and crown rot symptoms were manifested as leaf chlorosis followed by necrosis at the crown and leaves turning chlorotic from the bottom to the tip followed by necrosis at the leaf tips (Figure 12). When the infected leaves were completely yellow and/or brown, they began falling off the plant and new leaves started to emerge.

Table 7. Percentage of disease incidence on *Liriope muscari* ‘Big Blue’ inoculated with four inoculation treatments: water control (WC), *P. palmivora* (PP), *F. oxysporum* (FO), *P. palmivora* + *F. oxysporum* (PP+FO).

Inoculation Treatments	Days				
	10	20	30	40	50
WC	0 ^a	0 ^a	0 ^b	0 ^b	0 ^c
FO	0 ^a	2 ^a	29 ^a	35 ^a	42 ^a
PP	0 ^a	0 ^a	0 ^b	2 ^b	5 ^{bc}
PP+FO	0 ^a	2 ^a	9 ^b	16 ^{ab}	20 ^b

¹Means within columns followed by the same letter are not significantly different ($P \leq 0.05$).

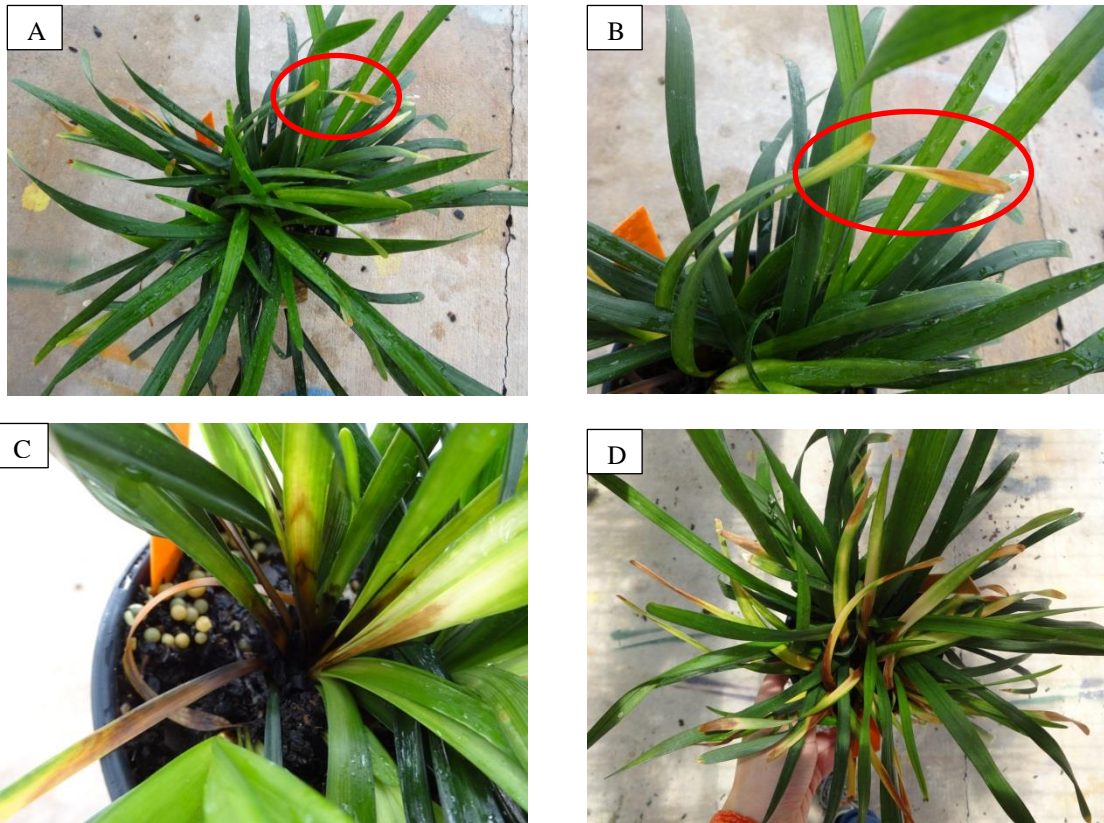


Figure 12. Progression of disease symptoms on *Liriope muscari* ‘Big Blue’ inoculated with *Fusarium oxysporum*. A) and B) Initial symptoms of leaf chlorosis and necrosis of leaf tips and C) necrosis at the crown after 55 days of inoculation. D) The same plant 5 days after initial symptoms (60 days after inoculation), necrosis occurred at the bottom of leaves and chlorosis moving upwards and downwards.

There was no effect of inoculum treatments on disease incidence of ‘Super Blue’ (Table 8). Some plants, however, inoculated with PP and PP+FO did show some disease incidence.

Table 8. Percentage of disease incidence on *Liriope muscari* ‘Super Blue’ inoculated with four inoculation treatments: water control (WC), *P. palmivora* (PP), *F. oxysporum* (FO), and *P. palmivora* + *F. oxysporum* (PP+FO).

Inoculation Treatments ¹	Days				
	10	20	30	40	50
WC	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
FO	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
PP	0 ^a	4 ^a	7 ^a	9 ^a	9 ^a
PP+FO	2 ^a	9 ^a	11 ^a	18 ^a	24 ^a

¹Means within columns followed by the same letter are not significantly different ($P \leq 0.05$).

It is important to note that the plants used for the third replicate were approximately one year older and 40% larger. These larger plants had three growth flushes during the treatment period compared to the first two replicates which had one flush of growth during the treatment period. *Liriope* are rhizomatous and new growth occurs through generation of plantlets or daughter plants. Thus in replicate three, the growth flush that was inoculated manifested disease symptoms while the new growth flushes had no symptoms (Figure 13).

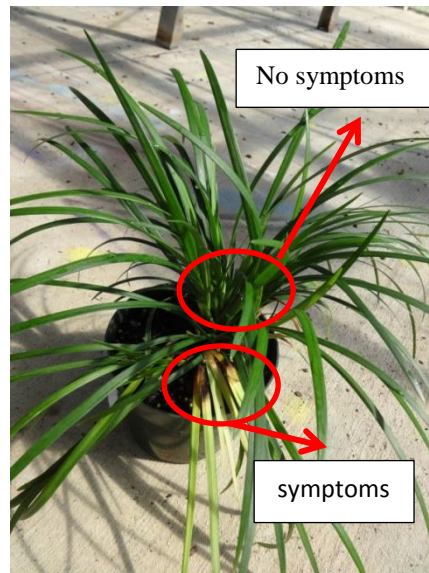


Figure 13. Progression of leaf and crown rot symptoms on one of three growth flushes of *Liriope gigantea* ‘Emerald Goddess’ inoculated with *Phytophthora palmivora* + *Fusarium oxysporum*.

4.3.3 Root Rot Incidence

‘Big Blue’ plants inoculated with FO and PP+FO had greater root rot compared to the control. However, ‘Emerald Goddess’ and ‘Super Blue’ plants inoculated with PP and PP+FO had greater root rot than the control and FO inoculation treatments (Table 9).

Table 9. Means of root rot incidence of three *Liriope* cultivars inoculated with four inoculation treatments.

Inoculation treatments ¹	Root Rot Incidence ²		
	‘Big Blue’	‘Emerald Goddess’	‘Super Blue’
Water control	5.6 ^{2a}	5.4 ^a	5.9 ^a
<i>Fusarium oxysporum</i>	4.8 ^b	5.2 ^a	5.8 ^a
<i>Phytophthora palmivora</i>	5.1 ^{ab}	4.7 ^b	5.4 ^b
<i>P. palmivora</i> + <i>F. oxysporum</i>	4.9 ^b	4.5 ^b	5.4 ^b

¹Means within columns followed by the same letter were not significantly different ($P \leq 0.05$).

²Disease scale: 6 = healthy roots, no symptoms of root rot; 5 = ≤ 25 % root rot; 4 = ≤ 50 % root rot; 3 = ≤ 75 % root rot; 2 = ≤ 95 % root rot; 1 = ≤ 100 percent root rot.

4.3.4 Re-isolation of *Phytophthora palmivora* and *Fusarium oxysporum*

Plants showing symptoms of leaf and crown rot were randomly chosen for re-isolation of both micro-organisms. Leaf samples from inoculated plants were analyzed for *F. oxysporum* by mycelia growth on ¼ PDA, and using PARPH media for determination of *P. palmivora*.

P. palmivora and *F. oxysporum* were recovered from all three cultivars previously inoculated with *P. palmivora* and *F. oxysporum* respectively. Both micro-organisms were recovered from all three cultivar with *P. palmivora* + *F. oxysporum* (Table B1).

4.4 Discussion

Pathogenicity tests were conducted on *Liriope* cultivars ‘Emerald Goddess’, ‘Big Blue’ and ‘Super Blue’ to confirm results of morphological and molecular identification. ‘Emerald Goddess’ and ‘Super Blue’ were used for this study because they are new cultivars which are thought to be more resistant to disease (Owings, 2012a). ‘Big Blue’ was chosen because it is the most popular cultivar sold by nurseries for the landscape and appears to be the most susceptible to leaf and crown rot disease. *Phytophthora palmivora* and/or *Fusarium oxysporum* were the micro-organisms used for the pathogenicity tests in this study based on a previous experiment that identified them as causal agents of leaf and crown rot. These tests confirmed that ‘Emerald Goddess’ inoculated with *P. palmivora* and *P. palmivora* + *F. oxysporum*, were susceptible to

infection and leaf and crown rot symptoms were similar to those reported in the nursery and landscape industry. *P. palmivora* has been found to cause similar symptoms in palms, in which the leaf spear becomes necrotic at the base with chlorosis moving up the leaf (Garofalo and McMillan, 1999). General observations by nursery and landscape professionals indicated that ‘Emerald Goddess’ appeared to be the most tolerant to leaf and crown rot in that the typical symptoms were not observed. This type of qualitative observation although helpful, can sometimes be inaccurate. ‘Emerald Goddess’ plants inoculated with isolates of *P. palmivora* and *P. palmivora* + *F. oxysporum* had the highest percentage of disease incidence. The leaf and crown rot symptoms were manifested as a necrotic or rotted crown with leaf chlorosis moving from the base to the tip of leaves, compared to symptomless control. Water content of fresh tissue is often used as a measure of disease infection in which infected leaves have a lower fresh weight (Harrison, 1970). In this study, leaf fresh and dry weight of inoculated ‘Emerald Goddess’ plants was significantly lower than the control plants whereas ‘Big Blue’ and ‘Super Blue’ showed no significant differences.

‘Big Blue’ appeared to be most susceptible to *F. oxysporum* with a greater disease incidence and lower leaf fresh and dry weights than the control or those plants inoculated with *P. palmivora*. *F. oxysporum* disease infects plant roots by a spore germ tube or mycelium and then colonizes the vascular system moving to other plant parts such as stems and crowns (Mourad AM et al., 2004). Symptoms include browning of the vascular tissue, vein discoloration, wilting, yellowing of lower leaves, stunted growth of the plant, defoliation and plant wilt (Tjamos and Beckman, 1989; Ma et al., 2013). The leaf and crown rot symptoms on ‘Big Blue’ observed in this study were bright yellow, chloritic leaves (Figure 14) compared to the leaves of ‘Emerald Goddess’ infected with *P. palmivora* which were also chloritic but had a lighter yellow appearance. The difference in appearance of leaf and crown rot symptoms between these two

cultivars could be attributed to the type of micro-organism causing the disease. *F. oxysporum* infects the vascular system and was manifested as a brighter yellow leaf leading to necrosis whereas *P. palmivora* infects all leaf and root tissue which caused a more gradual decline and yellowing leading to necrosis. This is an extremely important observation that is validated by the micro-organism causing the leaf and crown rot, producers can use this information to help identify the causal agent and adapt cultural practices that are targeted to the specific micro-organism. This should help reduce the amount of leaf and crown rot disease in both the nursery and landscape setting.

‘Super Blue’ was the least susceptible *Liriope* cultivar to leaf and crown rot in that there was no significant disease incidence after inoculation. Although the inoculation treatments caused no significant disease incidence, leaf and crown rot symptoms were detected during the experiment. Due to the vigorous growth habit of ‘Super Blue’ it appears that this cultivar was able to grow more quickly than the disease could spread from a single inoculation.

P. palmivora has been found to cause similar symptoms in palms, in which the leaf spear becomes necrotic at the base with chlorosis moving up the leaf (Garofalo and McMillan, 1999).

Results from this study suggests that *P. palmivora* was the primary microorganism causing leaf and crown rot in ‘Emerald Goddess’, ‘Big Blue’ being the most susceptible to *F. oxysporum*, and ‘Super Blue’ being the most tolerant cultivar to both pathogens. Standberg (2002a) indicated that he suspected that the primary micro-organism causing leaf and crown rot was *P. palmivora* in ‘Evergreen Giant’ *Liriope*. Some *Phytophthora* spp. are known to be the primary microorganism causing infection and *F. oxysporum* as a secondary infection (Erwin and Ribeiro, 1996). Based on the results from this experiment, producers of *Liriope* can manage cultural practices that will help minimize or eradicate these two disease causing micro-organisms.

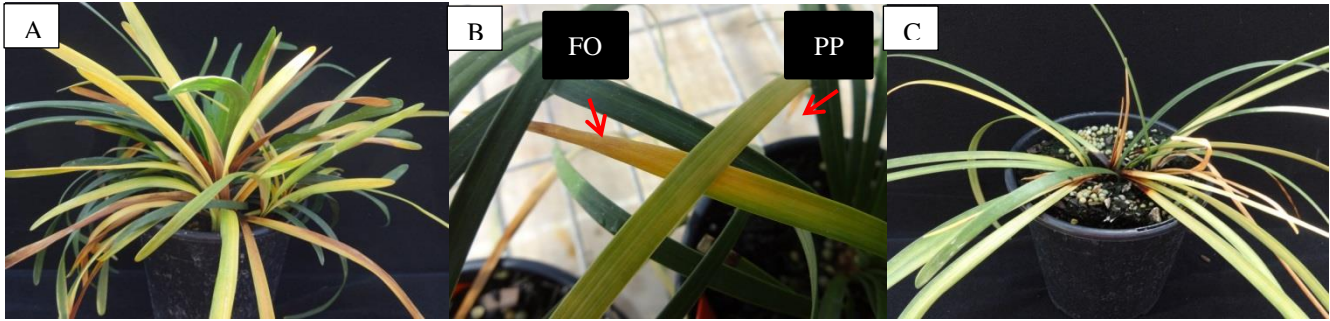


Figure 14. Progression of leaf and crown rot symptoms on *Liriope muscari* 'Big Blue' with A and B) bright yellow chlorotic leaves when inoculated with *Fusarium oxysporum* (FO) compared to the lighter yellow chlorotic of leaves of plants inoculated with *Phytophthora palmivora* (PP). C) *Liriope gigantea* 'Emerald Goddess' with light yellow leaves when inoculated with *P. palmivora*.

CHAPTER 5: SURVEY OF THE OCCURRENCE OF *LIRIOPE* LEAF AND CROWN ROT IN NURSERY SETTINGS

5.1 Introduction

The value of wholesale nursery production in Louisiana was \$166.9 million in 2014 (LSU AgCenter, 2014). Ground covers ranked fifth among all nursery crops in 2006 and represented \$359 million of gross sales, a 31% increase since 2003 and 8% of all nursery sales in the United States. The plant category ground covers and vines represented the greatest percentage of sales (17.4 %) in Louisiana, in 2005 (Brooker et al., 2005).

Liriope has been widely used as a ground cover or border grass and it is one of the most used ground covers in the southeastern United States including Louisiana (Winter, 2003; Harrison, 2005; Gill et al. 2012). The percentage of the total sales of ground covers in wholesale production was 18.4% in the Southeast and in Louisiana it was the 13.5%, in 2005 (Broussard, 2007).

Liriope appeared to be tolerant of most diseases and insects, however, leaf and crown root rot has become a devastating disease of *Liriope* in the landscapes of the southeastern United States (Standberg, 2002a). The symptoms are described as leaves at the bottom of the crown turning brown to yellow from the bottom to the tip with the entire infected leaf becoming yellow and necrotic. Infected leaves becomes rotted at the crown and can be easily pulled from the crown (Popenoe, 2008). Roots may become sloughed and look discolored (Russ, 2014). It appears primarily in late spring and early summer when there is heavy rainfall or with overhead irrigation and high temperatures (Ferrin, 2011). When this disease becomes established, there is no control measure (Owings, 2012b). There were several micro-organisms thought to be the cause of leaf and crown rot of *Liriope* but because the causal agents of the disease had not been identified and tested, producers had to rely on general best management practices to help control

the spread of disease. There are several best management practices that can be followed to avoid or reduce the development of soil borne pathogens in nurseries (Handreck and Black, 2002a). Reliable sources use cultural practices that include sanitation protocol to maintain disease-free stock (Drenth and Guest, 2004; Ferrin, 2011). These practices include the use of sterile substrate (Koike, 2010), plants situated in places with proper drainage, spacing, and removal of plant debris that could serve as a source of inoculum (Erwin and Ribeiro, 1996; Daughtrey and Benson, 2005; Ferrin, 2011).

The objective of this study was to determine what best management practices are being used by the nursery industry that produce and sell *Liriope* plants to prevent development or dispersal of leaf and crown rot disease. Based on these finding recommendations could be made to implement specific best management practices that could help to reduce disease incidence in nurseries.

5.2 Materials and Methods

To confirm that leaf and crown rot of *Liriope* plants was a significant problem in Louisiana, a nursery survey was conducted with retail and wholesale nurseries known to produce or sell *Liriope* plants. A total of eleven nurseries situated in the Forest Hill and Folsom nursery growing areas were visited April through August 2014. Seven retail nurseries in Baton Rouge, LA were visited in June, 2014. The surveys were conducted in the spring and summer because high temperatures and heavy rainfall predispose plants to leaf and crown rot disease.

The survey consisted of questions related to cultural, biological and chemical practices, and observation of disease severity and incidence in production or sale of *Liriope* (Appendix C). Diseased *Liriope* plants were taken as samples to be isolated for identification for presence of *P. palmivora* and/or *F. oxysporum*.

Inspection of *Liriope* for symptoms of leaf and crown rot was conducted in a defined block in which plants with leaf and crown rot disease symptoms were counted. *Liriope* cultivars showing leaf and crown rot symptoms and collected for analysis were ‘Super Blue’, ‘Jeanerette’ and ‘Evergreen Giant’. Roots and soil were used for *P. palmivora* identification cultured in PARPH and infected leaves for *F. oxysporum* using the same procedure as mentioned in the previous chapters. Statistical analysis was not conducted because most of the questions were subjective and not always applicable to retail nurseries.

5.3 Results

All retail nurseries were <10 acres in size and 64 % of wholesale nurseries ranged in area between 10 – 80 acres and the remaining were more than 80 acres. Both retail and wholesale nurseries specialized in *Liriope* production but also grew many other types of plants. All retail nurseries in the group sold between 1 – 5 different *Liriope* cultivars with wholesale nurseries producing between 6 – 15 *Liriope* cultivars.

All but one of the wholesale nurseries produced ‘Big Blue’ *Liriope*. In the case of ‘Super Blue’, 55% of wholesale nurseries produced this cultivar and only one produced ‘Emerald Goddess’. For retail nurseries 88% sold ‘Big Blue’ while none of them sold ‘Super Blue’ or ‘Emerald Goddess’.

With respect to propagation, the source of propagation material used by wholesale nurseries came from both, outside and in-house production (91%). One of the retail nurseries propagated plants from clumps that customers brought to them. Most of suppliers of retail nurseries are from Forest Hill, but none of them knew if their suppliers were reliable sources. For wholesale nurseries 55% knew that their supplier was reliable and 27% did not know if their source was reliable. The remaining nurseries assumed they were reliable because of state inspection. However, state inspection does not guarantee disease free plant material. All

wholesale nurseries that propagated in house, had propagation areas located far from the production area and the retail nursery propagated next to production.

In the case of sanitation, a majority of wholesale nurseries (60%) disinfected tools and propagation areas as a preventative sanitation practice, 20% mentioned the use of fungicides, 10% did not use any sanitation techniques, specifically disinfestation because they didn't see any improvement in disease symptoms. Only 10% of the nurseries surveyed indicated that weeding and sweeping residual matter was part of their best management practices. Retail nurseries had no sanitation protocol because plants are for a short period of time; one of the nurseries indicated that *Liriope* are "tough plants" and another mentioned that they discard diseased plants and thus sanitation practices were not necessary

In wholesale nurseries (90%) workers in the propagation area get training in regards to sanitation practices at the propagation site. The most used propagation method was division (90%) while the remaining nurseries bought liners. Seventy percent of the wholesale nurseries responded that new bibs were treated with a fungicide before planting.

A majority of wholesale nurseries used 0.07 L, 3.78 L or 11.35 L (or both) container sizes to grow *Liriope* plants (64%), others used 1 gallon (18%) and the remaining used 4 inch containers. Some of wholesale nurseries (45%) used new pots, but the other 45% used both new and reused pots, with 9% using only reused pots. Most of the nurseries that reused pots did not disinfect them (55%). Other nurseries indicated that they didn't reuse pots to grow 'Big Blue' cultivar and another indicated that new pots were used for outside source plants. Only 9% of the wholesale nurseries disinfected reused pots. The product that was primarily used for disinfestation was Clorox™. In the case of retail nurseries, pots were not reused by 57% of the nurseries, while others reused pots, but only 29% used a disinfectant.

For chemical control of leaf and crown rot in *Liriope* plants, each of the wholesale nurseries used several fungicides. Boscalid + pyraclostrobin (Pageant[®]) and thiophanate methyl (Zyban[®]) were the fungicide that were most widely used (4 nurseries); thiophanate-methyl (Transom[™]) and mefenoxam (Subdue[®]) were also mentioned by some of the nurseries (3 nurseries); azoxystrobin (Heritage[®]), fluopicolide (Adorn[®]) and chlorothalonil (Daconil[®]) were used by 2 nurseries; thiophanate- methyl + etridiazole (Banrot[®]) was used by one nursery, however, it has the same active ingredient thiophanate-methyl (Transom[™], Zyban[®] and 3336[®]), and etridiazole (Truban[®]). Mancozeb (Clevis[™]) was used by one nursery, azoxystrobin (Heritage[®]) and fosetyl-al (Aliette[®]) were applied by different nurseries just once. Six fungicides used by wholesale nurseries are broad spectrum fungicides and ten fungicides are described to help in the control of *Phytophthora* spp. and related diseases. Two nurseries indicated that fungicide applications were made in the morning every 10 d. One nursery responded that fungicides were applied every 2 weeks and one answered that applications were made later in the day, once every 2 weeks or once every week if disease symptoms appeared.

Most of the wholesale nurseries grew the newly propagated liners in the propagation site 3 months or more (60%). The majority of nurseries answered that liners were moved to an outdoor growing area after propagation (89%), and the remaining placed them in a greenhouse. Some wholesale nurseries grew *Liriope* plants only outside (55%) while the remaining nurseries grew plants in both greenhouse and outside (45%). All retail nurseries grew plants outside.

A majority of wholesale nurseries (91%) did not have benches in the production area. Most of the wholesale nurseries used ground cover and gravel in the production area (64%), the remaining had only ground cover (36%), Retail nurseries (71%) use both ground cover and gravel. The most common ground cover used was black cloth.

A majority of retail nurseries (71%) grow pot-to-pot in the production area and the minority (29%) had containers spaced. Containers mostly used in the production area by retail nurseries were 0.07 L and 3.78 L or 11.35 L and the rest use just 4 inch containers. Majority of wholesale nurseries also used primarily 0.07 L inch and 3.78 L or 11.35 L in the production area (55%) and few used just 0.07 L containers (18%), count and liters (18.18%); and counts, 0.07 L and liter containers (9.09%). All of wholesale nurseries use overhead sprinkler irrigation and in retail nurseries majority use same irrigation system (86%), and the remaining hand water the plants.

Two of the nurseries surveyed irrigated plants twice a day in summer during hot temperatures. One nursery irrigated for 10 minutes and the other irrigated for 30 minutes. Three nurseries irrigated once a day for 30 minutes during the summer and once a week the remaining times of the year. Most of the retail nurseries responded that irrigation was dependent on the weather. Three of the retail nurseries watered plants every day. One irrigated 2 - 3 times in the morning, for 35 min; the other irrigated once a day for 40 minutes in the summer and the other in the evening for 30 min.

Eighty-two percent of wholesale nurseries run-off drains directly to retaining ponds and 55% used recycled water for irrigation purposes. Most of wholesale nurseries use well water as a source of irrigation (55%); others use surface water and well water (27%). In retail nurseries the primary source of irrigation water was city water (71%), and the remaining used well water (14%) or surface water and well water combined. A majority of the wholesale nurseries did not treat the irrigation water (82%), and all retail nurseries did not treat irrigation water.

Substrate used for *Liriope* production was different among nurseries some (36%) used pine bark and lime, others (27%) used pine bark, lime and fertilize, the other (27%) of nurseries used pine bark, peat moss and fertilize and just a few used pinebark, lime fertilize and subdue

(9%). Substrate is primarily stored on concrete (82%) while 18% stored substrate on bare ground downhill from the production area (27%). The majority of wholesale nurseries cull piles were located far away from production, propagation and media storage sites and that (82%) water from cull piles does not run into production area, retention or irrigation ponds.

All wholesale nurseries fertilized *Liriope* plants by numerous methods and different types of fertilizer. Most of the wholesale nurseries used a top dress or incorporated fertilizer in the mix and 36% used liquid fertilizer. Most of wholesale nurseries used slow release fertilizers, the majority of which used a 6 month (27%) or one year slow release fertilizer (27%). A higher number of wholesale nurseries used fertilizer that had from 15 to 21% N. The fertilizer most mentioned was Osmocote and others used Nutricote and Florikan.

Time of inspection for disease symptoms in plants varied between nurseries. In wholesale nurseries most of them inspected plants every week (36%) while others inspected daily (27%) or longer than a week (27%). Only 43% of the retail nurseries inspected daily (43%) and 24% inspected weekly (29%). Suspected plants were removed immediately after inspection in the majority of wholesale nurseries (91%) and retail nurseries (71%). In wholesale nurseries plants were removed from a block once per week (44%) or when symptoms were observed. In retail nurseries (50%) plants were removed every three months.

Most of the wholesale nurseries disposed of diseased plants and pots by burning (44%) the remaining nurseries disposed the plants in a cull pile (44%). In retail nurseries a majority used a dumpster to dispose diseased plants (86%). Pots containing disease plants were either disposed of into dumpsters (30%), while others stored them (30%) or others burned them (20%). In retail nurseries most disposed of pots in the dumpster (50%).

A majority of wholesale nurseries sell within and outside the state (73%), 18% outside the state only and only 9% within the state only. A majority of wholesale sales are to retail,

landscapers, re-wholesales (73%) while others sell to re-wholesalers only (18%) and the remaining just to landscapers (9%).

Five of the eleven wholesale nurseries were asked about the percentage of loss in 2013. One of the nurseries indicated 10% loss of ‘Big Blue’ plants, two nurseries responded they had a 20 - 30% loss, and the two remaining nurseries had up to a 50 - 60% loss.

Inspection for leaf and crown rot in wholesale nurseries on ‘Big Blue’ and ‘Super Blue’ indicated that 11% of ‘Big Blue’ plants were symptomatic plants. For retail nurseries, ‘Big Blue’, ‘Super Blue’, ‘Jeanerette’ and ‘Evergreen Giant’ cultivars were analyzed and mean percentage of all retail nurseries with disease was approximately of 12.5% (Table C1).

Disease severity, which is the percentage of infected leaves, is indicated in (Table C2). Percentage of symptomatic leaves and roots in most of wholesale nurseries plants were found to be >25%. All symptomatic plants that were isolated were positive for *P. palmivora* and *F. oxysporum* based on mycelia growth on PARPH and ¼ PDA media (Figure 15) (Table 10).

Table 10. Re-isolation of *Fusariumoxysporum* and *Phytophthora palmivora* from leaf and crown rot symptomatic *Liriope* cultivars collected from different nurseries in Louisiana.

Nursery Type	Nursery #	Cultivar	<i>F. oxysporum</i> (1/4 PDA)	<i>P. palmivora</i> (PARPH)
Wholesale	3	Big Blue	+	+ slow growth
Retail	1	Super Blue	+	+
		Big Blue	+	+
	2	Jeanerette	+	-
	3	Evergreen Giant	+	+
	5	Big Blue	+	+ slow growth
	6	Big Blue	+	+ slow growth

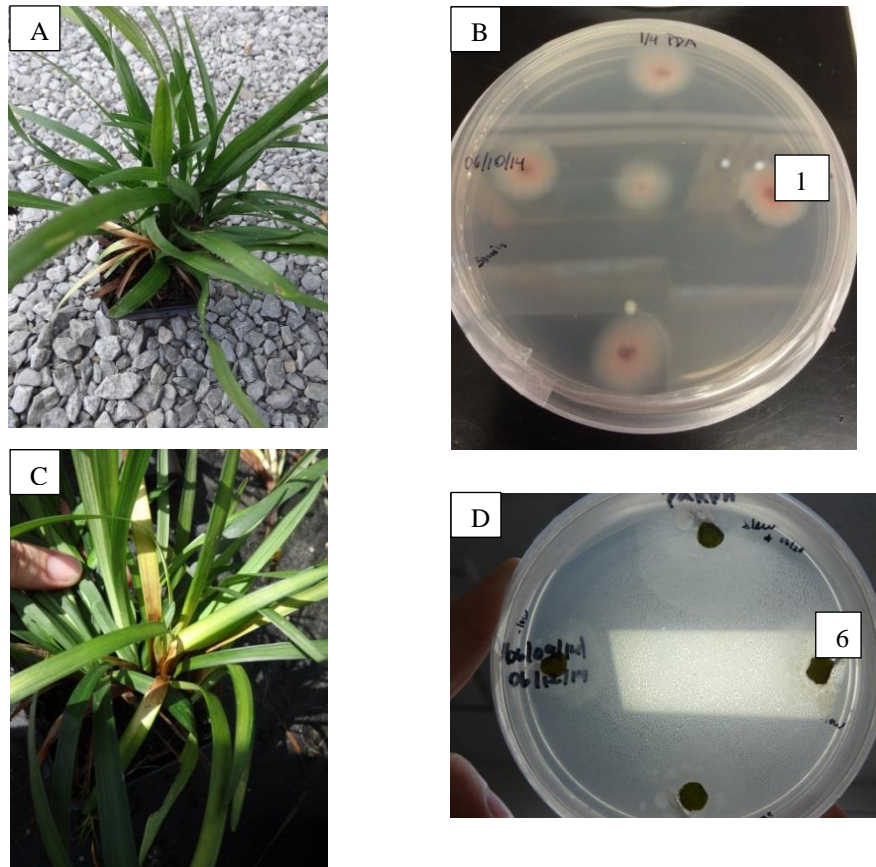


Figure 15. Isolation of symptomatic ‘Big Blue’ plants collected as samples from nurseries. A) Symptomatic plant from a retail nursery. B) Mycelia growth of *Fusarium oxysporum* in ¼ PDA media isolated from the retail nursery. C) Symptomatic plant from a different retail nursery. D) *Phytophthora palmivora* growth in PARPH media isolated from that retail nursery.

5.4 Discussion

This study demonstrated that leaf and crown rot in *Liriope* plants was present in both retail and wholesale nurseries visited in Louisiana. Some of the cultural practices used in these nurseries may have lead to the spread or development of the micro-organisms causing disease. The nursery trade is often correlated to the introduction and spread of pathogens causing diseases (Parke and Grünwald, 2012). Thus, this study focused on cultural practices including disease management and sanitation practices of nurseries to determine if leaf and crown rot disease in *Liriope* could be reduced or eliminated.

Integrated pest management (IPM), includes different practices that help in pest control that create unfavorable environmental conditions to balance or reduce the pest population. These practices includes cultural practices, biological and chemical control (Uneke, 2007). There are practices that surveyed nurseries can use to reduce disease introduction or incidence and with it reduce economical loss (Maloy, 2005).

This survey indicated that most wholesale nurseries did not know if their suppliers were certified disease free and others assumed certification because of state inspections. Most retail nurseries did not know if their suppliers were certified or not. Certification of disease free plant material has been used to avoid pathogen entrance or reduce disease incidence into or through the nursery industry (Drenth and Guest, 2004). However, diseased plants may be unnoticed because symptoms have not been manifested (Parke and Grünwald, 2012). Thus use of sanitation practices for propagation and growth are imperative to help maintain disease free plants.

Growing surfaces, irrigation, containers and substrate are all potential sources for introduction and dispersal of diseases such as *Phytophthora palmivora* (Parke and Grünwald, 2012).

One of the most common cultural practices found in wholesale nurseries visited was reusing containers that had been previously used to grow plants. One half of wholesale nurseries surveyed reused containers without disinfecting them. *Phytophthora* spp. has been found to be spread by reused containers throughout the nursery industry (Lucas et al., 1991; Parke, 2010; Parke and Grünwald, 2012; Osterbauer et al., 2013). If nurseries want to reuse containers it is recommended to disinfect them with 0.5% of sodium hypochlorite (Handreck and Black, 2002a).

One wholesale nursery indicated that a continual cultural practice was removal of plant debris as a sanitation practice; however the remaining wholesale and retail nurseries did not include this practice. The continued removal of plant debris is a highly important sanitation

practice in that diseases such as *Phytophthora* are known to survive as chlamydospores and oospores in plant debris, and thus serve as an inoculum source (Drenth and Guest, 2004; Osterbauer et al., 2013).

Specific irrigation frequency and quantity based on different plant species and container sizes was rarely observed for most nurseries. Amount and frequency of irrigation (Moorman and Gwinn, 2013) and many other factors such as size of container, substrate used, type of plant and season can be detrimental for disease development or necessary to know the required amount of water to be applied (Yeager et al., 1997). Proper irrigation and drainage are one of the most important factors reducing the severity of the diseases such as *Phytophthora* and *Fusarium* in the nursery. Cultural practices such as proper drainage of water from nursery containers and reducing irrigation can help reduce the spread or development of pathogens (Erwin and Ribeiro, 1996; Daughtrey and Benson, 2005; Koike, 2010; Pscheidt and Ocamb, 2015).

The majority of wholesale nurseries surveyed that used recycle water did not treat the water prior to irrigation. Previous studies have shown presence of *Phytophthora* spp. in recycled irrigation water (MacDonald et al., 1994; Charlton, 2001; Bush et al., 2003; Stewart-Wade, 2011), specifically *Phytophthora palmivora* (Bush et al., 2007). One wholesale nursery treated their irrigation water with chlorine prior to use which is a widely accepted practice for recycled irrigation water for eliminating incidence of *Phytophthora* spp. (Hong et al., 2003; Cayanan et al., 2009).

Some of nurseries surveyed produced plants on black polypropylene mesh with no method of drainage (gravel) beneath which is not recommended because it makes easier dispersal of disease to neighbor pot especially if standing water is accumulated (Handreck and Black, 2002b).

Most of wholesale nurseries use division as a propagation method, but it can also be a source and dispersal of disease if propagated plant is infected (Standberg, 2002a). Propagation by division of 'Evergreen Giant' *Liriope* plants infected with leaf and crown rot has been found to cause disease dispersal (Leahy and Davison, 1999). Thus it is recommended that tools are disinfected as a cultural practice to avoid disease dispersal. Most of nurseries that disinfect tools used alcohol or fungicides as disinfectant, but it is recommended at the end of each day tools being disinfected with 2000 ppm quaternary ammonium solution (Handreck and Black, 2002c)

Some of the surveyed wholesale nurseries stored potting media on bare ground which is known to be a possible source of disease primarily of *Phytophthora* species (Osterbauer et al., 2013).

Most of the nurseries surveyed grew *Liriope* plants in containers that were not spaced. Disease dispersal occurs much more easily when containers are not spaced (Berger, 1975; Huang, 1980). High density growing conditions can lead to high humidity, an important factor in growth and development of *Phytophthora* species in plant material (Drenth and Guest, 2004). Most of nurseries did not have a specific protocol for disease inspection with only observations made when new plants were introduced. This survey found that fungicides were used to control fungal diseases after disease symptoms were found. Most fungicides can only protect uninfected plants from disease and should not be used as a curative once disease has been identified. It is also important to note that all diseases caused by fungi cannot be adequately controlled by fungicides and therefore proper cultural practices are highly important in disease suppression. Most wholesale nurseries in this survey used broad spectrum fungicides for disease prevention because they did not know the pathogen that was causing the disease. If the disease is properly identified the proper fungicide can be used against the specific pathogen increasing the control of the disease and reducing the economic and environmental impact.

CHAPTER 6: CONCLUSIONS

Liriope is a herbaceous perennial plant commonly used as a ground cover and as a mass plant in landscapes. *Liriope muscari* cultivars are the most popular in the southeastern United States. In the past few years these plants have been affected by leaf and crown rot disease in the landscape and in nursery production. Once the disease is established, there is no control measure to eradicate it and more importantly, the causal pathogen has not been isolated and pathogenicity tested. This research was performed to isolate the causal pathogen(s) of leaf and crown rot in *Liriope*, assess disease incidence and determine what best management practices could be adopted to lead to minimizing infection and spread of this disease.

The first study was conducted to positively identify *Phytophthora palmivora* and *Fusarium oxysporum* as the possible causal agents of the disease. The presence of both micro-organisms was found in the landscape and nursery on *Liriope* plants with symptoms of leaf and crown rot disease. These two micro-organisms were positively identified according to morphological observations from cultures and then confirmed by molecular analysis.

The second study was conducted to evaluate disease incidence, through a pathogenicity tests, the susceptibility of three different *Liriope* cultivars ‘Emerald Goddess’, ‘Big Blue’ and ‘Super Blue’, inoculated with *Phytophthora palmivora* and/or *Fusarium oxysporum* and measured by multiple variables such as fresh and dry weight of leaves and roots, and by visual disease rating. Results from this study showed that ‘Emerald Goddess’, the cultivar believed to be the most tolerant to leaf and crown rot, was the most affected by *P. palmivora* and *P. palmivora* + *F. oxysporum* inoculation. ‘Big Blue’ was susceptible to *F. oxysporum* with a greater disease incidence and lower leaf fresh and dry weights than the control or those plants inoculated with *P. palmivora*. ‘Super Blue’ was the least susceptible *Liriope* cultivar to leaf and

crown rot and it appears that this cultivar was able to grow more rapidly than the disease could spread from a single inoculation.

The third study was a survey conducted at several nurseries in Louisiana to ascertain *Liriope* production practices used by nurseries for *Liriope* that could lead to dispersal of disease and the increase of disease incidence. Results of this study showed that many of the practices used by both retail and wholesale nurseries in Louisiana could serve as a source of dispersal of inoculum and thus provide for spread of the disease.

Combined, these three studies indicated that producers of *Liriope* should focus disease management on preventing infection and spread of *P. palmivora* and/or *F. oxysporum*. These practices include identifying best management practices that can help prevent the infection and spread of these two diseases and using preventative fungicides that are labeled for control of *P. palmivora* and/or *F. oxysporum*.

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APPENDIX

A) Plant Diagnostic Center Media Recipes

A.1. PARPH media:

Deionized water 500 ml

Corn Meal Agar 8.5 gr

Autoclave at 121 °C for 20 minutes at 15 psi.

Cool the media approximately 45-50°C, and then add the following antibiotics:

Pimaricin..... 5 mg

Amicpillin..... 125 mg

Rifampicin..... 5 mg

*PCNB (Penta-chloro-nitro-benzen..... 10ml

Hymexazol..... 25 mg

*PCNB: 2.0 g PCNB + 400 ml Ethanol (95%). Heat the 95% ethanol in a water bath and then add PCNB to it. Store the solution in refrigerator.

A.2. V₈ juice agar media (20%):

Deionized water..... 400 ml

*V₈ supernatant..... 100 ml

Agar..... 10 g

Autoclave at 121 °C for 21 min at 15 psi.

*V₈ supernatant: mix 300 ml of V₈ juice + 5 g of CaCO₃ in a beaker. Centrifuge the mixture at 4000 rpm for 20 minutes. Drain the supernatant into clean beaker and discard the solid portion (pellet) of the mixture. Supernatant can be frozen for future juice.

A.3. ¼ PDA (Potato Dextrose Agar) media:

Deionized water..... 500 ml

Potato Dextrose Agar..... 4.875 gr

Agar..... 5.625 gr

Autoclave 121 °C for 20 minutes at 15 psi

Table B 1. Re-isolation of *Phytophthora palmivora* (PO) and *Fusarium oxysporum* (FO) from three *Liriope* cultivars inoculated with four inoculation treatments.

Inoculation treatments	Treatments ¹	Isolated pathogen ²	FO ³	PP
<i>Water control</i>	BB2	FO	-	d/a
	BB5	FO	+	d/a
	BB4	FO and PP	+	-
	SB1	FO and PP	+	-
	SB2	FO and PP	+	+
	EG5	FO and PP	-	-
	EG2	FO and PP	+	-
	EG3	PP	d/a	-
<i>Fusarium oxysporum</i>	BB3	FO	+	d/a
	BB3	FO	+	d/a
	BB1	FO	-	d/a
	BB2	FO	+	d/a
	BB2	FO	+	d/a
	BB4	FO	+	d/a
	SB4 no symp	FO	+	d/a
	EG1 no symp	FO	-	d/a
	EG2	FO	+	d/a
	EG5	FO	+	d/a
<i>Phytophthora palmivora</i>	BB4	PP	d/a	+
	SB1	PP	d/a	+
	SB5	PP	d/a	+
	EG3	PP	d/a	+
	EG1	PP	d/a	+
	EG2	PP	d/a	+
	EG2	PP	d/a	+
	<i>Phytophthora palmivora + Fusarium oxysporum</i>	BB4	FO and PP	+
BB2		FO and PP	+	+
BB5		FO and PP	+	+
BB1		FO and PP	+	+
SB1		FO and PP	+	+
EG3		FO and PP	+	+
EG1		FO and PP	+	+
EG5		FO and PP	-	+
EG4		FO and PP	-	+
EG3		FO and PP	+	+
EG2		FO	+	d/a
EG3		FO	-	d/a
EG4		FO	-	d/a
EG2		FO	+	d/a
EG3		FO	+	d/a
EG4	FO	-	d/a	

¹BB, EG, SB = ‘Big Blue’, ‘Emerald Goddess’ and ‘Super Blue’ cultivars.

²FO and PP = *Fusarium oxysporum* (¼ PDA media) and *Phytophthora palmivora* (PARPH media)

³(+) = positive re-isolation of the pathogen, mycelia growth in media (-) = no growth of pathogen.

C) Nursery survey questions

Survey of Management Practices of Leaf and Crown Rot of *Liriope* in Louisiana Nurseries

- 1. Size of the nursery in acres.**
- 2. Do you specialize in ground covers?**
- 3. What *Liriope* cultivars do you produce?**
- 4. What is the source of the propagation material?**
in-nursery outside source
- 5. If outside source, is the source certified?**
- 6. If in home production, how far is the propagation site from production area?**
- 7. What kind of sanitation practices being used in the propagation site?**
- 8. Do the workers working in the propagation area get any training regarding the sanitation practices in the propagation site?**
- 9. How do you propagate the new liners or plants?**
- 10. What sizes of container do you use to grow *Liriope*?**
- 11. Are new pots used to start new plants?**
- 12. Do you treat the new bibs with fungicides before planting?**
- 13. What chemicals are being used in *Liriope* production?**
Fungicides (any combinations) Trade names and active ingredient:
Formulations:
Dose:
Application frequency:
Time of application:
- 14. How long the new liners stay in the propagation site?**
- 15. Where are the plants transferred from propagation site?**
- 16. Are plants grown in:**
Greenhouse outside

17. Does the production area have benches?
18. Are the new liners kept on ground in the production area?
19. Does any ground covers or gravels being used in the production area?
20. What is the spacing between containers in the production area?
21. What container size is used in the production area?
22. What irrigation practices are being used in the production area?

Overhead sprinkler Drip Irrigation Other

23. What is the frequency of irrigation?

Amount:

Time per day:

24. How is the run-off water from production area regulated?
25. Is run-off water directed to retaining ponds?
26. Is this run-off water recycled and reused for irrigation purposes?
27. What is the source of irrigation water?

Irrigation pond well water re-circulated water

28. Is irrigation water treated?
29. What kind of potting mix is used for Liriope production?

Concentration of peat in the potting mix

30. Is potting mix stored on concrete surface or on the ground?
31. Is potting mix stored downhill from the production area?
32. Does the run-off water come in contact with the potting mix?
33. Where is the cull pile located in the nursery?

Away from production propagation and media storage sites

34. Are pots being recycled?

YES NO

If yes, are the pots disinfested before being reuse? What method of disinfestations?

35. Does water from cull piles run into the production area or irrigation ponds or retention ponds?

36. How often the plants are inspected for disease symptoms?

37. Are the suspected plants removed immediately after inspection?

38. How often disease plants removed from a block?

39. How are the diseased plants and the pots containing disease plants disposed?

40. Do the infected plants have root knot nematodes?

YES

NO

41. Are symptoms worse on plants infected with root knot nematodes than infected plants with no nematodes?

YES

NO

42. What type of fertilization you use and how often you fertilize?

43. Where do you sale your plants or ship? (within the state or outside of the state)

44. Where are they sold: retail nursery Landscapers Re-wholesale.

45. Inspect for Leaf and Crown rot symptoms in each nursery?

Observation:

Container size:

Number of Symptomatic plants in a block:

Number of Asymptomatic plants in a block:

Total number of completely dead plants:

Total number of plants in a block:

Number of leaves rotted or yellowed from the base per plant

Number of total leaves per plant:

Reddish brown lesions present or absent on symptomatic plants

Roots sloughed or not:

Root Knot nematodes present or absent:

Approximate Root Rot:

0: no root rot, 1: 25% rotting, 2: 50% rotting, 3: 75% rotting and 4: 100% root rot

Table C 1. Number of infected *Liriope* plants with leaf and crown rot symptoms from surveyed nurseries in Louisiana.

Nursery type	Cultivar	Nusery #	# plants/block	Container size	# dead plants	no symp	symp
Wholesale	Big Blue	1	33	mum pan	6	26	7
	Big Blue	2	100	1 gal	0	86	14
	Big Blue	3	36	36 counts	0	35	1
			--	1 gal	--	--	1
	Super Blue	4	100	1 gal	0	0	0
	Big Blue	6	--	1 gal	0	-	2
			18	4 inch, 1 flat	0	12	6
	Big Blue	7	18	4 inch, 1 flat	0	17	1
	Big Blue	9	36	4 inch, 2 flats	0	34	2
			500	1 gal	0	498	2
	Big Blue	10	18	4 inch 1 flat	0	15	3
	Big Blue	11	30	1 gal	0	30	0
Retail	Big Blue	1	20	4 inch, 1 flat	0	18	2
	Super Blue		8	1 gal	0	7	1
	Jeanerette	2	18	4 inch, 1 flat	0	14	4
	Evergreen Giant	3	20	1 gal	0	15	5
	Big Blue	4	18	1 gal	0	18	0
	Big Blue	5	36	4 inch, 2 flats	0	34	2
	Big Blue	6	20	4 inch, 1 flat	0	18	2
Evergreen Giant	7	13	1 gal	0	11	2	

Table C 2. Percentage of infected leaves and rotted roots in symptomatic *Liriope* plants with leaf and crown rot in surveyed nurseries in Louisiana.

Nursery #	Plant #	Cultivar	Container size	Leaves		Roots			
				Rotted or yellow	total #	Redish brown lesion	Sloughed	Nematodes	Rot %
1	1	Big Blue	Mum pan	38	38	present	yes	absent	40
	2			43	60	present	yes	absent	30
	3			38	38	present	yes	absent	70
	4			46	46	present	yes	absent	100
	5			40	54	present	yes	absent	95
	6			11	65	present	yes	absent	25
2	1	Big Blue	1 gallon	75%		present and absent	yes	absent	50
	2			30%					
	3			25%					
	4			50%					
	5			75%					
	6			75%					
	7			50%					
	8			50%					
	9			25%					
	10			50%					
	11			50%					
	12			75%					
	13			25%					
	14								
3	1	Big Blue	36 counts	16	18	present and absent	yes	present and absent	5
	1		1 gallon	50%	--	--	--	--	--
4		Super Blue	no disease						
6	1	Big Blue	1 gallon	12	41	present	yes	--	25
7	1	Big Blue	4 inch	15	31	present			
	1	Big Blue	Trade gal in a row	20	20				
1	1	Big Blue	4 inch	10	59	present	yes, little	absent	20
	2			4	87	present	yes, little	absent	10
	1	Super Blue	1 gallon	12	195	present	yes, little	absent	10
2	1	Jeannette	4 inch	7	20	present	yes	absent	90
	2			5	36	present and absent	yes	absent	95
	3			8	23	present	yes	absent	98
	4			14	44	present and absent	yes	absent	80
3	1	Evergreen Giant	gal	28	88	present and absent	yes	absent	20
	2			22	90	present			
	3			13	100	present			
	4			12	85	present			
	5			14	83	present			
4		Big Blue	gal	no disease					
5	1	Big Blue	4 inch	13	20	present and absent	yes	absent	15
	2			27	27	present	yes	absent	30
6	1	Big Blue	4 inch	57	74	present and absent	yes	absent	90
	2			10	48	present	yes	absent	30
7	1	Evergreen Giant	gal	50	120	present and absent	yes	absent	40
	2			33	81	present and absent	yes	absent	40

VITA

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