Effects of *Pythium* and Cold Storage on the Survival of Southern Pine Seedlings

by

Daniel Paul Jackson

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
August 9, 2010

Keywords: *Pythium dimorphum*, *Pythium irregulare*,
root growth potential, root collar diameter

Approved by

Scott A. Enebak, Co-Chair, Professor of Forest Pathology
Charles H. Gilliam, Co-chair, Professor of Horticulture
David B. South, Professor of Forestry
D. Joseph Eakes, Professor of Horticulture

Abstract

Cold storing bareroot loblolly pine (*Pinus* spp.) seedlings for > 1 week after lifting from October to mid-December has been associated with poor outplanting survival compared to when seedlings are lifted and stored in January. In contrast, container-grown seedling survival is not affected when stored for > 1 week during the same period. The practice of lifting bareroot seedlings can wound root systems, which pathogenic fungi, particularly *Pythium* spp., could use as infection sites. Once seedlings are placed in storage, the cool, moist environment may be conducive for fungal growth which could lead to seedling mortality after outplanting. The objective of this research was to evaluate bareroot and container-grown seedling survival after inoculations with *Pythium dimorphum* Hendrix and Campbell and *Pythium irregulare* Buisman and cold storage.

Bareroot longleaf pine (*Pinus palustris*) survival decreased as a result of the *Pythium* inoculations but bareroot loblolly (*Pinus taeda*) and slash pine (*Pinus elliottii*) were not affected by *Pythium*. Container-grown seedling survival was similar to non-inoculated seedlings even after wounding root systems. When a peat-mix was packed around inoculated bareroot loblolly pine roots and used as a container media, seedling survival did not improve. This suggests that something other than antagonistic fungi in peat may improve container-grown seedling storability.

As a fine feeder root pathogen, *Pythium* can kill the fine feeder roots that are critical for seedling establishment after outplanting, and therefore, the effects of *Pythium*

on seedling root growth potential (RGP) were tested. *Pythium* reduced bareroot loblolly and slash pine RGP after storage, but only *P. irregulare*-inoculated slash pine experienced reductions in survival after outplanting.

Pythium must be present in nursery soils if bareroot seedlings are infected with *Pythium* during fall lifting. Bareroot nursery soils were assayed to quantify *Pythium* populations during the fall and winter seasons. *Pythium* populations were variable between nurseries with more *Pythium* being recovered from samples taken in the fall of Year 1 and winter of Year 2.

It was determined that both *P. dimorphum* and *P. irregulare* act as storage pathogens. If *Pythium* spp. are actively present in the soil at lifting, infect seedling roots through wounds, and grow on seedling roots in cold storage, reductions in seedling root growth potential and survival are possible.

Acknowledgments

First, I would like to thank Dr. Charles Gilliam for agreeing to work with me and Dr. Scott Enebak on this joint project. To my graduate committee, Drs. Scott Enebak, David South, Charles Gilliam, and Joseph Eakes, I thank you for being teachers, listeners, providers of numerous opportunities, and for always having an open door. I am grateful to the Southern Forest Nursery Management Cooperative for funding this research.

I thank Dr. Tom Starkey for his perpetual support and for always lending an ear. To Tommy Hill, Marietjie Quicke, and Barry Brooks, thank you for all of your time and hard work, but more importantly, for your friendship. I also thank Greg Creech, Chris Marble, Rena Miller, and Patrick Jernigan for their assistance. I look forward to the life-long friendships I will have with Dr. George Matusick, David Dyson, Jay Ransom, and Ben Whitaker and will always cherish our camaraderie.

I thank my parents, David and Pamela Jackson, and my in-laws, John and Rebecca Brennan, for driving long hours to Auburn for visits and for their uplifting spirits. To my wife, Missy, the sacrifices you have made are countless, and my hope is that one day I can repay you for being such a kind, loving, and patient person. To my son, Brennan, you have made the last seven months of this journey the best.

This dissertation is dedicated to Dr. James Barnett, a pioneer, who contributed over 45 years to pine regeneration research and who gave me an opportunity on February 26, 2001, which changed my life forever.

Table of Contents

List of Tables

List of Figures

CHAPTER 1

REVIEW OF LITERATURE

1.1. Forest Tree Nurseries in the Southern United States

In the southern US, the forest industry is one of the leading economic drivers in the region. With the demand for forest products and decreased levels of wood production in other areas of the country, pressure is being placed on the South to maintain a high level of timber harvesting and processing (Barnett 2002). The regeneration of the forest is a long-term cyclic process, and to produce high quality timber, one often begins by planting high quality seedlings.

Nursery managers use two methods to produce forest tree seedlings, bareroot and container production. Container-grown seedlings are produced within cavities, and the number and size of cavities can vary among container types. Bareroot seedling production utilizes intensively managed soils, much like any other row crop. Nursery cultural practices such as sowing technique, fertilization, irrigation, root and shoot pruning, and pest management play a critical role in seedling quality at the end of the growing season. Survey results from 58 nurseries across the southeast indicated a total of 949,637,000 bareroot and 69,401,000 container conifer seedlings were produced in the 2007-2008 planting season (Table 1.1) (Enebak 2008).

Table 1.1. Bareroot and container-grown seedling production in the 2007-2008 planting season across the southeastern United States (Enebak 2008).

		Stock Type	
Pine species	Common Name	Bareroot	Container
Pinus taeda	Loblolly	801,745,000	21,128,000
Pinus palustris	Longleaf	8,494,000	42,208,000
Pinus echinata	Shortleaf	1,794,000	2,081,000
Pinus elliottii	Slash	127,230,000	2,961,000
	Other conifers	10,384,000	1,023,000
Total		949,637,000	69,401,000

1.1.1. Lifting Practices

Forest tree nurseries employ two lifting methods: mechanical and hand lifting (May 1984). Preventing damage and seedling root loss, particularly lateral roots, is imperative to ensure survival after outplanting (Wakeley 1954). To prevent root loss, lateral and taproots are pruned several weeks before lifting begins (May 1984). However, soil texture and moisture are key factors that also contribute to root loss and injury (May 1984). Moist sands and loamy sands are best when using machine lifters, but lifting should be delayed if soil conditions are saturated or frozen (May 1984; Carlson 1991). Additional root injury can occur if seedlings are lifted in dry conditions or if the lifter is moving at a high rate of speed in any soil conditions (Wakeley 1954). Loblolly pine seedling survival can be less than 10% when the lateral roots are stripped off during normal mechanical lifting operations (Dierauf et al. 1992). Hand lifting may be as stressful to root systems as machine lifting under similar soil conditions (Brissette 1986). In the past, root pruning was common after hand lifting (Wakeley 1954). Roots were only described as injured when pruning tools were dull or roots were pruned too short. Pruning roots after lifting is not recommended today.

Dormancy is a state in which meristematic growth is inactive even when environmental constraints to growth are absent (Burdett and Simpson 1984). Dormancy occurs when tissue predisposed to elongate does not do so (Wenny et al. 2002). If seedlings are properly hardened off in late summer or fall, loblolly pine buds will become dormant by discontinuing growth and forming a resting bud (Boyer and South 1985). However, weather patterns in the South are unpredictable, making it difficult to determine when bud dormancy occurs (Hebb 1982; Venator 1984). In addition, seedlings

differing in genetics can differ in the onset of bud dormancy (Boyer and South 1989). Some believe that lifting seedlings when buds are dormant will increase outplanting success by reducing transplant shock and enhancing seedling root growth potential (RGP) (Burdett and Simpson 1984; Carlson 1991). During bud dormancy, lateral roots continue to grow, which is important for seedling establishment (May 1984). Fulfilling a "chilling hour" requirement classifies seedlings as being in a state of after-rest in which the bud would resume growth when placed in a favorable environment (Boyer and South 1985).

The traditional planting season in the southern U.S. is from late November to early March and can be dictated by either the demand from planting operations or the conditions at the outplanting site (May 1984). Soil moisture at outplanting sites must be adequate for initial seedling establishment, especially with plantings that occur in March (May 1984). If loblolly pine seedlings are lifted in the fall, they should be outplanted within 2 to 7 days (Dierauf 1976; Carlson 1991) and under favorable site conditions (Carlson 1991), a process known as "hot planting".

1.1.2. Packing and Cold Storage Practices

Due to its high water holding capacity and lower pH, sphagnum peat moss was once popular seedling packing material that provided an unfavorable environment for certain fungi (Wakeley 1954; May 1984). The labor and costs involved in soaking and draining sphagnum peat moss, and the risks from the disease sporotrichosis are reasons this material lost favor as a packing medium (May 1984). Its first replacement, kaolinite clay slurry, provided roots protection when exposed, reduced the need to add water to seedlings in storage, and was less expensive than peat moss (May 1984). Clay was

applied by hand dipping seedling roots into a tank or by directly spraying roots (May 1984). Other packing materials that have been used in the past included wood fibers, felt blankets, cotton bating, excelsior waste, sawdust, shavings, bark peelings, chips, shingle tow, sisal fibers, chopped hay, bagasse, burlap, seaweed, fabric liners, Kim-pac™, and root-rap (May 1984). In the mid-1980's, superabsorbent gel products became available for seedling packing (Erazo 1987) and gel application to roots before storage is now a common practice (personal comm., Dr. Tom Starkey, Research Fellow, Auburn University).

Kraft-polyethylene (KP) bags, U.S.D.A. Forest Service bales or bundles, and wax corrugated cardboard boxes are three commonly used seedling packaging methods in southern nurseries (May 1984; Carlson 1991). Kraft-polyethylene bags are designed to prevent moisture loss and withstand added weight from water (Carlson 1991) but are permeable to some gases (May 1984). Before the use of clay slurries and gels, seedlings in KP bags could be cold stored without peat moss for 2 to 3 months. (May 1984). However, bagging seedlings with > 0.5 liters of wet peat moss could cause seedlings to mold and decay due to water buildup in the bottom of bags (May 1984).

Seedlings packed in Forest Service bales had their root systems treated with clay slurry or gels and wrapped in waterproof paper, and seedling tops protruded out of the bale (Wakeley 1954; May 1984). This packing design was used in temporary cold and ambient storage, but periodic watering was necessary to prevent root dessication. In ambient conditions such as warehouse storage, baled seedlings packed in peat moss needed to be watered every 2-3 days and outplanted within 1-4 weeks to avoid reductions in outplanting survival (May 1984). Years ago, peat moss was packed in between and

around root systems when using Forest Service bales (Wakeley 1954). Getting moist peat moss on part of the shoot extending from the bale would sometimes reduce transpiration and aeration and cause molding of foliage (May 1984). Wax corrugated boxes are used mainly for packaging container-grown seedlings (Dumroese and Barnett 2004) and reduced the need of stacking with pallet shelving at the nursery (Carlson 1991).

When cold storage rooms or coolers are used to hold seedlings, they should be kept between 1 to 5ºC (May 1984). Carlson (1991) claimed these temperatures could reduce cell respiration and loss of stored carbohydrates, particularly, total non-structural carbohydrates (TNC), which are used for energy in dark and cold conditions endured by seedlings in storage (Page-Dumroese et al. 2008). Short periods of cold storage during the fall can result in significant seedling weight loss via respiration and depletion of food reserves (Garber ad Mexal 1980). Loblolly pine seedlings have exhibited 3-10% reductions in dry biomass after 9 weeks in storage (Garber and Mexal 1980). May (1984) reported seedlings with exposed tops, packed in bales for instance, may increase physiological activity in cold storage (May 1984). Maintaining a high relative humidity in the cooler keeps the environment wet and can assist in reducing seedling dessication. However, high humidity coolers that do not allow proper airflow can induce the growth of storage molds such as *Botrytis cinerea* Pers.; Fr. or "gray mold" (Srago and McCain 1989).

Wakeley (1954) stated "too few experiments have been made with cold storage of southern pine nursery stock to warrant any recommendations concerning it." Carlson (1991) claimed that if cold storage was necessary for more than a few days, seedlings

should not be actively growing. May (1984) made packaging recommendations in KP bags based on the state of bud dormancy, and stated that loblolly pine seedlings lifted prior to bud dormancy had reduced storability. Garber and Mexal (1980) insisted that no data exists to link bud dormancy to seedling survival after cold storage. Scientists have yet to determine if chilling hours relate to seedling storability. In fact, Boyer and South (1985) reported that longer periods of cold storage were possible for container-grown loblolly seedlings before reaching the number of chilling hours needed to break bud dormancy. Therefore, successful cold storage might not be related to seedling bud dormancy status at all. In contrast, guidelines involving the storage of container-grown seedlings do not include any information relating bud dormancy to seedling storability (Dumroese and Barnett 2004). Dumroese and Barnett (2004) reported that September and October-lifted container-grown southern pine seedlings should be cold stored for < 1 week but those lifted in November could be stored for 1-2 weeks. They claim container-grown seedlings lifted in January can be stored for 2-3 months. Unfortunately, these recommendations were made based on personal communication with an employee at a container nursery and were not based on experimental data.

1.1.3. Cold Storage and Seedling Survival

Cold storing bareroot seedlings from November to mid-December could extend the traditional planting season and reduce the need to lift seedlings in the spring (Garber and Mexal 1980). Many experiments have concluded that fall lifting of bareroot seedlings results in poor survival after long-term (>1 week) cold storage. Kahler and Gilmore (1961) lifted and stored loblolly pine seedlings once per month from October to

March and outplanted them after March 30. They found survival to be 0-60% for fall-lifted seedlings and > 92% for seedlings lifted after mid-December. Dierauf (1976) found loblolly pine seedlings lifted in October and November and cold stored for two weeks to have 0 and 2% survival, respectively. In contrast, survival without cold storage was 66 and 92%, respectively. Another study showed loblolly pine seedling survival to be 67, 61, and 31% when lifted on December 15 and stored for one, two, and three months, respectively (Venator 1984). Poor survival in these studies was blamed on non-dormant buds at the time of lifting, rather than cold storage per se (Kahler and Gilmore 1961; Dierauf 1976; Venator 1984). However, no measure of bud dormancy or chilling hours was reported. Similar to loblolly pine, sand pine (*Pinus clausa*) seedling survival was reduced when lifted in November and stored for more than 10 weeks (Hebb 1982). Lower seedling survival was reported to be due to storing actively growing seedlings. In contrast, container-grown longleaf pine seedlings were cold stored for 3 weeks, outplanted in October, and survival was acceptable (Pickens and Robbins, unpublished data). Similarly, bareroot loblolly pine seedlings lifted in late October and early November had > 80% survival after 4 weeks of cold storage (Stumpff and South 1991).

To understand why cold storage was successful for seedlings prior to bud dormancy, some researchers turned their attention towards fungi in cold storage. To suppress the incidence of brown-spot needle blight (*Mycosphaerella dearnessii* Barr), researchers added the fungicide benomyl to seedling roots and subjected them to long-term cold storage and increased longleaf pine seedling survival (Cordell et al. 1984; Kais and Barnett 1984). Adding benomyl to clay slurry (Kais and Barnett 1984; Barnett et al. 1988) and peat moss media (Cordell et al. 1984; Barnett et al. 1988) improved bareroot

longleaf pine seedling survival after cold storage. However, when planted in sand, a clay slurry plus benomyl (Cordell et al. 1984) and Viterra® gel slurry (South and Lowenstein 1994) reduced longleaf seedling survival. Longleaf pine seedlings packed with benomyl and clay had less brown-spot needle blight infection after storage (Kais and Barnett 1984). In another trial, Barnett and others (1988) reported improved survival after 6 weeks of cold storage for longleaf and shortleaf pine seedlings packed in clay slurry plus benomyl. Loblolly pine survival in this study was exceptional regardless of packing and storage treatment. Conversely, Boyer and South (1987) found a reduction in seedling survival after 12 weeks of cold storage when loblolly pine roots were treated with clay plus benomyl prior to storage. Seedlings with higher shoot:root ratios, sown earlier in the year, were affected more by the addition of the fungicide (Boyer and South 1987).

Improved seedling survival with fungicides shifted attention to other storage pathogens (Boyer and South 1987). From seedling roots tested *in vitro*, *Pythium* spp. was recovered from seedlings cold stored 6 weeks (Barnett et al. 1988). Survival was < 20% and < 65% for seedlings packed in clay slurry and peat moss, respectively, with no addition of fungicide (Barnett et al. 1988). Higher survival in benomyl-treated seedlings suggests that fungi on seedling roots affected viability in long-term cold storage. Coincidently, Stumpff and South (1991) had near 100% survival for control and benomyl-treated loblolly seedlings lifted in January, but seedlings treated with benomyl had < 50% survival in November and December. They also found no interaction between benomyl treatment and storage. Apparently, either pathogenic fungi were not responsible for survival reductions in November and December or the concentration of benomyl used in the study did not control existing pathogens. Treating longleaf pine seedling roots

with metalaxyl (*Pythium* targeting), benomyl, and metalaxyl plus benomyl provided > 90% survival for all treatments of cold storage (Jones et al. 1992). In that study, seedlings with no fungicide had < 20% survival. *Pythium* was recovered as storage time increased, while *Trichoderma* levels sharply declined in cold storage (Jones et al. 1992). However, even though *Pythium* levels were higher after 6 weeks of storage, fungicides improved seedling survival. The fungicide concentrations may have attributed to survival differences. A 5% active ingredient (ai) by weight of benomyl controlled *Pythium* (Jones et al. 1992), which might explain the low survival found by South and Stumpff (1991) when seedlings were lifted in November and December and benomyl only applied at 1.25% ai. When loblolly and longleaf pine were lifted in December and treated with 10 ppm metalaxyl, seedling survival improved compared to non-treated seedlings cold stored for 6 weeks (Brissette et al. 1996). The broad spectrum fungal control of benomyl and the specific activity against *Pythium* for metalaxyl resulted in better overall survival for seedlings lifted in December, January, and February compared to non-treated seedlings after 4 years (Brissette et al. 1996). Benomyl applied to shortleaf seedling roots at 0.25 % ai for December-lifted seedlings and 0.5 % ai for November, March, and April-lifted seedlings resulted in increased survival over non-treated seedlings stored for 4 weeks (Hallgren and Ferris 1995). However, using benomyl in packing material yielded inconsistent survival results with loblolly, longleaf, and shortleaf pine seedlings (Stumpff and South 1991; Jones et al. 1992; Hallgren and Ferris 1995; Brissette et al. 1996). Therefore, seedling survival can be unpredictable, regardless of benomyl level, and the soil texture of the outplanting site (Kais et al. 1986).

To further determine the effects of *Pythium* on seedling storability, Sun (1996) inoculated bareroot longleaf pine seedling roots with 50, 100, or 200 ml of wheat bran which had been inoculated with *Pythium dimorphum* Hendrix and Campbell. After 4 weeks of cold storage, as the levels of inoculum applied to seedling roots increased, seedling survival decreased to 39, 12, and 2%, respectively. This was the first report that direct application of *Pythium* spp. could negatively affect bareroot seedling survival after long-term cold storage. Previously, Jones and others (1992) had only correlated higher populations of *Pythium* from 6-week stored longleaf pine seedlings to low survival. Thus, Sun (1996) proved *P. dimorphum* to be pathogenic to bareroot longleaf pine seedlings.

1.2. *Pythium* in Southern Forest Tree Nurseries

1.2.1. *Pythium* in Bareroot Nurseries

Pythium is a common pathogen found in nursery soils associated with "damping-off" disease (Kelley and Oak 1989). Pre- and post- emergence damping off can cause seed to either not germinate or new germinants to buckle (damp-off) at the hypocotyl area (Wakeley 1954). *Pythium* is also known as a "fine feeder root disease" which infects and kills seedling feeder roots that are essential for water and nutrient absorption (Kelley and Oak 1989). Seedlings up to several weeks old remain susceptible to both damping-off and fine feeder root infection. Patches of either stunted or chlorotic seedlings found throughout the nursery are a symptom of *Pythium* is present in the soil (Dumroese and James 2005).

Pine seed may be treated with fungicides before sowing to prevent pre-emergence damping-off. Unfortunately, *Pythium* can lay dormant in soil for years with fluctuations in fungal activity triggered by temperature and soil moisture. Considered a "water mold", *Pythium* spores move throughout the soil profile and nursery bed after rainfall or irrigation (Kelley and Oak 1989). The use of machinery can cause soil compaction, altering soil drainage and allowing the fungus to become active (Dumroese and James 2005). Controlling irrigation frequencies and establishing good soil drainage are two ways to minimize soil conditions that favor fungal growth (Dumroese and James 2005). Other control methods include maintaining a low soil pH, avoiding the use of contaminated equipment, and periodic applications of soil fumigants (Kelley and Oak 1989).

A survey of 18 nurseries in the southeast US revealed that *Pythium* spp. were present from 1-179 colony forming units per gram (cfu/g) of soil in 70 of 85 samples (Hendrix and Campbell 1968). Specifically, the species *P. irregulare* Buisman, *P. sylvaticum* Campbell and Hendrix, *P. spinosum* Sawada, *P. helicoides* Drechsler, and *P. splendens* Braun were isolated from pine species in five states. Campbell and others (1972) found *Pythium* spp. to be relatively high in nursery soils with an average of 48 cfu/g of soil in 67 samples.

Diseased root symptoms observed in bareroot Noble fir (*Abies procera*) seedlings resulted in an investigation of *Pythium* pathogenicity of several Irish pine species (Shafizadeh and Kavanagh 2005). *Pythium* inoculation produced root rot disease of Noble fir seedlings and outbreaks in lodgepole pine (*Pinus contorta*), Sitka spruce (*Picea sitchensis*), and Norway spruce (*Picea abies*) seedlings. Juzwick and others (1999)

enumerated *Pythium* in three forest tree nurseries and found from 10-20 cfu/g of soil. *Pythium* spp. was isolated in 4% of red pine (*Pinus resinosa*) and white pine (*Pinus strobus*) seedlings in two nurseries and 29% in another nursery which had visible root rot (Juzwick et al. 1999). *Pythium aphanidermatum* (Edson) Fitzp. caused pre- and post-emergence damping-off of slash pine seedlings in a Georgia nursery (Huang and Kuhlman 1990).

1.2.2. Nursery Fumigation and *Pythium* Populations

Nursery soils are fumigated before sowing to control soil-borne fungi, nematodes, insects, bacteria, and weeds. Treatment with methyl bromide has been the most effective fumigant for years (May 1984), but this compound was declared an ozone depleting chemical and is to be phased out under the Montreal Protocol (Cram et al. 2007). Researchers have been testing alternatives to methyl bromide that will hopefully control nursery pests and produce high quality seedlings with minimal environmental impact. Fraedrich and Dwinell (2003) evaluated loblolly and slash pine seedling responses using a standard 67% methyl bromide/33% chloropicrin combination at 393 kg/ha (MC33) and a non-fumigated control, where chemicals were applied annually for three years. Significantly more *Pythium* was isolated from soils in the control plots of slash and loblolly seedlings at the end of each year (October-December) from 1995-1998. *Pythium* abundance decreased for mid-year analyses (July or August) with no significant difference found between either tree species or fumigation treatment during the same three-year span. Another study evaluated dazomet on slash and loblolly pine seedlings during the spring and fall at two rates (280-325 kg/ha and 493-560 kg/ha), MC33, and a

non-fumigated control in two nurseries (Fraedrich and Dwinell 2003). Soils from a Georgia nursery revealed significantly less *Pythium* in both dazomet treatments in the spring while numbers were similar in all treatments in the fall. Root systems in the control plots had more *Pythium* in the fall and spring. North Carolina soils showed no differences in *Pythium* presence among fumigation treatments in the fall. *Pythium* isolates from roots were significantly higher in the control plots in the fall, but no differences were observed in the spring. Despite treatment, *Pythium* was present in all treatments in the fall and spring (Fraedrich and Dwinell 2003).

Spring fumigation with MC33, chloropicrin at 336 kg/ha (CP336), and a combination of metam sodium at 90 kg/ha and chloropicrin at 163 kg/ha (MS/CP) significantly reduced *Pythium* spp. in April (Cram et al. 2007). By November of the same year, *Pythium* levels in all treatments were similar to those in non-fumigated plots. *Pythium* levels in soils of another nursery were reduced in May of the same year of spring fumigation with MC33, chloropicrin at 168 kg/ha (CP168), CP168 plus EPTC herbicide, CP336, and CP336 plus EPTC (Cram et al. 2007). By November of the same year, fumigation with MC33 was the only soil fumigant that significantly reduced *Pythium* populations. Collectively, these results show that chloropicrin can control *Pythium* in nursery soils if methyl bromide becomes unavailable. However, *Pythium* re-colonization was detected in the same year of fumigation with any product or formulation used in this study, excluding EPTC herbicide alone (Cram et al. 2007). Another study done in the Pacific Northwest found MC33 at 360 kg/ha controlled *Pythium* populations in bareroot Douglas-fir (*Psedotsuga menziesii*) beds (Tanaka et al. 1986). However, the next spring following fumigation, *Pythium* levels rebounded, causing root disease in all treatments.

1.2.3. *Pythium* in Container Nurseries

Pythium is one of the major causes of root disease of container-grown pine seedlings (Landis 1989). Sowing seed contaminated with fungal spores is a common way *Pythium* is vectored into containers, especially if seed have not been treated with hydrogen peroxide, bleach, or fungicides (Dumroese et al. 1988). Irrigating with contaminated water is another method of *Pythium* entry into the nursery (Landis 1989). Substrate residue remaining on containers after they are washed and disinfected can provide an inoculum source the following growing season (Sutherland and Dennis 1992; Dumroese et al. 2002). Also, *Pythium* can remain dormant in container media until saturated or poorly drained cavities cause spores to become active (Landis 1989).

Pythium spp. has been isolated from diseased container-grown Scots pine (*Pinus sylvestris*), Norway spruce, and larch (*Larix sibirica*) seedlings (Lilja 1994). In the U.S., another study revealed 20% of white pine seedlings without foliar symptoms were infected with *Pythium* spp. (James 1988). A survey of four container nurseries reported that *Pythium* spp. were present in each nursery on Douglas-fir, white spruce (*Picea glauca*), and Engleman spruce (*Picea englemannii*) seedlings that had no symptoms of root disease (Kope et al. 1996). After inoculating container-grown Fraser fir (*Abies fraseri*) seedlings with isolates of *Pythium vexans* de Bary, no disease symptoms occurred after 10 weeks (Ivors et al. 2008). However, *P. vexans* was recovered from asymptomatic seedlings, suggesting the fungus could persist within the root plug and not cause disease (Ivors et al. 2008).

1.2.4. *Pythium* and Peat Moss Suppressiveness

Peat moss is the standard media used in container seedling nurseries to which vermiculite, perlite, and sand are added (Landis et al. 1990). These media-mix can provide adequate nutrient availability, aeration, and water holding capacity for many species of seedlings to grow, given standard cultural practices are maintained by the nursery manager (Landis et al. 1990). It remains unclear, however, the extent that peat based media may have on suppressing *Pythium* diseases. Peat moss varieties from Sweden, Finland, and Denmark, ranging in pH from 5.3 to 6.3, differed considerably in their ability to suppress *Pythium* disease of cucumber (*Cucumis sativus*) seedlings (Wolffhechel 1988). A light-colored Swedish sphagnum peat (pH = 6.3) was the most effective at suppressing *Pythium* disease, but suppressiveness was not evident when the peat was either heated above 60ºC or treated with benomyl (Wolffhechel 1988). This implied that suppressiveness may be tied to antagonistic fungi present in the media. *Trichoderma* spp. has controlled *Pythium ultimum* Drechsler when tested in peat media of cucumber (*Cucumis sativus*) seedlings (Thrane et al. 2000). Of 39 peat moss samples tested, Hunter and others (2006) found 15 to be conducive, 10 as suppressive, and 14 intermediate to post-emergence damping-off of cress (*Lepidium sativum*) germinants caused by *Pythium sylvaticum* Campbell and Hendrix. Suppression of *P. sylvaticum* increased as levels of the genera *Cryptococcus, Rhodotorula*, and *Bullera* increased within the peat. Thus, disease suppression may have been associated with higher levels of basidiomycetous yeasts (Hunter et al. 2006). Tomato (*Lycopersicon esculentum*) seedlings grown in peat, pine sawdust, and compost had significantly less root rot caused by *P. ultimum* when *Penicillium*, *Pseudomonas*, and *Trichoderma* were added (Gravel et

al. 2006). A peat moss sample amended with pine sawdust and shrimp waste (2:2:1 ratio) and another sample with pine sawdust and cow manure (1:1:1 ratio) significantly improved survival of cucumber seedlings in the presence of *P. ultimum* (Labrie et al. 2001). In the absence of peat, van Os (2001) reported root rot of Iris (*Iris xyphium*) and the growth of *Pythium macrosporum* (Vaartaja and Van der Plaats-Niterink) increased in sterilized sand (van Os and Ginkel 2001). Sterilizing the soil is believed to have killed organisms that were suppressive against *Pythium* disease.

1.3. *Pythium* Taxonomy and Biology

Pythium spp. were grouped under the kingdom Fungi until 40 years ago when mycologists separated them from fungi based on characteristics shared with chlorophyll containing chrysophytes or diatoms (Alexopoulas et al. 1996; van West et al. 2003). The phylogeny of *Pythium* is as follows: kingdom *Stramenopila*, phylum *Oomycota*, order *Peronosporales*, family *Pythiaceae*, and genus *Pythium* (Alexopoulas et al. 1996). Characteristics that set *Pythium* apart from true fungi involve dispersion via zoospores, sexually formed thick-walled oospores, cell walls containing cellulose, movement by a tinsel and whiplash flagellae, and tubular mitochondrial cristae (van West et al. 2003).

Alexopoulas and others (1996) give an excellent explanation of the *Pythium* life cycle. Asexual reproduction of *Pythium* occurs by the production of zoospores in a lemon-shaped sporangium formed along the somatic hyphae. Upon their release, zoospores swarm for a period of time, then rest, encyst, and germinate via a germ tube that develops into mycelia. Encystment involves the detachment of both flagellae into the resting (encysted) zoospore that can later produce either a germ tube or appresorium

that can infect the fine feeder roots of host plants. Zoospore release and encystment occurs in the presence of higher concentrations of calcium ions (Ca^{2+}) (van West et al. 2003). Sexual reproduction involves the conjugation of an antheridium (male reproductive structure) with an oogonium (female reproductive structure), where the exchange of gametes occurs through a fertilization tube. Antheridia are called "monoclinous" if formed on the same hypha as the oogonium or "diclinous" if formed on separate hyphae (Van der Plaats-Niterink 1981). Meiosis occurs within the oosphere of the oogonium. The oosphere develops a thick wall after fertilization and becomes an oospore. A germ tube or appresorium can form directly from the oospore, or the oospore can form a sporangium that will produce zoospores. Once released, zoospores are attracted to roots by an electrochemical gradient produced by ions (H^+, Cl^-, K^+, and Ca^{2+}) as they pass across cell membranes from the root to the soil and vice versa (van West et al. 2003). In addition, root exudates such as sugars and amino acids can stimulate spore germination that can lead to root colonization (Hendrix and Campbell 1973).

1.4. *Pythium dimorphum*

Hendrix and Campbell (1971) were the first to describe *Pythium dimorphum*, which was isolated from loblolly pine feeder roots sampled from symptomatic trees in southwest Louisiana. *Pythium dimorphum* is characterized from other *Pythium* species by having extremely large, thin-walled chlamydospores (resting structures) that were mistakenly identified as oogonia. Chlamydospores average 64 µm in diameter and have been reported up to 80 µm. Chlamydospores and oogonia can develop either intercalary (along middle sections) or terminally (either end) along the same hypha in close

proximity to each other. Oogonia can be accented with conical projections along its perimeter. Antheridia are rarely identified in culture but when present are monoclinous.

Unlike other *Pythium* species, *P. dimorphum* is infrequently recovered from soils. The second known isolation was from diseased roots of rhododendron spp. in New York (Ho 1986). The pathogen was also isolated from diseased Norway spruce seedlings in a forest tree nursery in Norway (Asiegbu et al. 1996). Bridge and others (2008) compared *Pythium* isolated from diseased roots of Antarctic hairgrass (*Deschampsia Antarctica*) to *P. dimorphum* and found a 62-65% similarity between DNA sequences. The authors concluded that the Antarctic *Pythium* isolates would have to be lumped into a clade and labeled as similar to other *Pythium* spp. Using chlamydospore size as a method of identification was deemed fruitless given the abundance of similarly sized chlamydospores formed by many *Pythium* spp. However, Hendrix and Campbell (1971) and Ho (1986) successfully identified *P. dimorphum* using morphological parameters.

To understand the species' potential hosts, Asiegbu and others (1996) examined *P. dimorphum* and determined the fungus contained chitin, which is normally found in true fungal cell walls. However, the chitin was being masked by glucan residues, possibly giving *P. dimorphum* an advantage for infection. Borja (1995) found that after inoculation with *P. dimorphum*, the lignification of Norway spruce seedling roots continued for six days, but the defense response could not defend intruding hyphae.

Host plant inoculations using *P. dimorphum* have been performed by Borja (1995) with Norway spruce seedlings and Sun (1996) with longleaf pine seedlings. Borja (1995) found *P. dimorphum* to significantly reduce shoot lengths when roots were inoculated with only the pathogen or a pathogen plus benomyl treatment. Furthermore, *Pythium* was

re-isolated more often from seedlings treated with benomyl. Survival of longleaf pine seedlings decreased and the percentage of *P. dimorphum* recovered increased as inoculum amount increased after 4 weeks of cold storage (Sun 1996). Sun (1996) also identified strains of *Trichoderma* spp. that effectively controlled *P. dimorphum*.

1.5. *Pythium irregulare*

Van der Plaats-Niterink (1981) gives a description of *Pythium irregulare*. The species was first discovered in the Netherlands from pea roots and cucumber seeds but has since been found throughout the world. Sporangia are seldom produced, but oogonia can form either intercalary or terminally along the hyphae. Antheridia can be monoclinous or diclinous. Oogonia are normally ornamented, but higher temperatures (33ºC) can reduce ornamentation and cause abnormal oogonia formation and reductions in diclinous antheridia. Distinct hyphal swellings are characteristic for identification.

Based on isolations from soil samples from across the US, Hendrix and Campbell (1961) reported *P. irregulare* as one of the most described *Pythium* spp. in the English literature. Although soil moisture is key for *Pythium* spp. to become active, *P. irregulare* relies on lower soil temperatures as a trigger for activation (Hendrix and Campbell 1973). In nurseries, the species is most often associated with damping-off of newly germinated or several-week-old seedlings.

Until Mexican weeping pine (*Pinus patula*) seedlings experienced mortality on previously cultivated sites in South Africa, *P. irregulare* was not associated with pine seedling disease after outplanting (Linde et al. 1994). Soil testing revealed 99 and 87% *P. irregulare* colonization in soil and on roots of diseased seedlings, respectively. In

comparison, testing of outplanted virgin lands resulted in 5 and 0% infestation from soil and roots, respectively. Linde and others (1994) used isolates of *P. irregulare* to make a sand-based inoculum to which 4-month-old Mexican weeping pine seedlings were planted. They also performed a damping-off test by placing seeds next to *P. irregulare* mycelia plugs. Mortality occurred in 76 and 83% of seedlings in the damping-off and transplanting trials, respectively. This was likely the first experiment to link outplanting mortality of pine seedlings to root disease caused by *P. irregulare*. Unlike reports of increased mortality of longleaf pine seedlings inoculated with *P. dimorphum*, no reports exist to link the pathogenicity of *P. irregulare* to pine seedlings that have been held in long-term cold storage.

1.6. Forest Tree Nurseries in the Pacific Northwest Region of the United States

The Pacific Northwest region is another area that produces forest tree seedlings. Both the Pacific Northwest and southern U.S. regions share similar cultural practices to maintain and improve seedling quality. These include sowing techniques, irrigation, fertilization, pest management, top pruning, root pruning, lifting, and packaging. However, seedling storage techniques are vastly different. Seedlings in the Pacific Northwest are routinely held in freezer storage, which can last for several months.

1.6.1. Lifting Practices

Machine lifting is the most common method used to harvest seedlings (Burdett and Simpson 1984). Due to the frequency of sub-freezing temperatures, lifting seedlings from frozen soils should be avoided to reduce root stripping and wounding. Managers of

Pacific Northwest nurseries start the lifting season based on accumulation of chilling hours to ensure bud dormancy (Burdett and Simpson 1984). Ponderosa pine (*Pinus ponderosa*) seedlings in Oregon had accumulated > 2,400 chilling hours at ≤ 10° C when lifted in February (Omi and Schuch 1987). Wenny and others (2002) describe three phases of dormancy that change with season: (1) initial quiescence (pre-dormancy) in late summer that is induced by shorter photoperiods and moisture stress, (2) rest in late fall and winter, and (3) final quiescence (post-dormancy) in spring when shoots are ready to elongate when placed in favorable environmental conditions.

1.6.2. Packing and Cold/Freezer Storage Practices

In order to protect seedling from adverse conditions, seedling packaging methods are similar to southern nurseries with respect to moisture retaining materials, storing, and shipping (Burdett and Simpson 1984). In addition to cooler storage (1-2ºC), nurseries in the Pacific region utilize freezer storage (-1ºC) of seedling stock (Ritchie 2004; Burdett and Simpson 1984). Often, seedlings must be freezer-stored after being lifted from December to early March due to outplanting sites in higher elevations not favorable for outplanting until May or June (Heel 1987). Similar to loblolly pine seedlings in storage (Garber and Mexal 1980), seedling dry weight can diminish over time in freezer or cold storage (Ritchie 1987). Douglas-fir seedlings lost more total nonstructural carbohydrates (TNC) (starch and sugars) in foliage than in either the stem or roots after 2 months of freezer storage (Ritchie 1987). White spruce seedlings lost more TNCs in needles than roots after 7 weeks of cold and freezer storage (Wang and Zwiazek 2001). One-year-old container-grown lodgepole pine (*Pinus contorta*) seedlings that had been hot-planted on a

site with organic matter intact retained significantly more TNCs than seedlings cold and freezer stored for 6 months (Page-Dumroese et al. 2008).

The temperatures of both storage regimes are reported to contribute to further chilling requirements (Ritchie 2004) and slowly promote bud dormancy (Ritchie 2004; Dumroese et al. 2005). Remarkably, no reports exist of cold storage on bud dormancy of southern pine seedlings. Buds exposed to long-term freezer storage (2-6 months) require about 350 chilling hours below 6ºC, while shorter-term cooler storage (1-2 months) requires about 300 chilling hours (Ritchie 2004). Heel (1987) found that seedlings had enough chilling hours and were hardy enough for freezer storage by the second week of December in nurseries west of the Cascades. Describing seedling behavior from different climate regions, Omi and Schuch (1987) concurred with May (1984) in that fall-lifted seedlings could result in unsuccessful storage when lifted prior to deep bud dormancy and on buds that are not responsive to chilling hours. However, Burdett and Simpson (1984) insisted that no data had been linked to bud dormancy and seedling storability. They claimed that dormancy only refers to meristematic tissue and the physiology and growth in areas of the shoot, root, and vascular cambium could persist and were unrelated to the meristematic region. Using this logic, determining an exact number of chilling hours to seedling storability/survival (Heel 1987; Ritchie 2004) would not be recommended.

If freezer storage is utilized, frozen seedlings must be thawed before outplanting. A temperature gradient of < 2ºC from the center to the edge of the package should be maintained to prevent seedlings farthest from the center from thawing too fast (Burdett and Simpson 1984). Container-grown seedlings have been outplanted with frozen root

plugs without affecting survival (Kooistra and Baker 2002). Damage to seedlings by storage molds is reduced with freezer storage (Landis et al. 2010), but molds can become an issue during the thawing process (Heel 1987). Thawing can take a few days to several weeks (Burdett and Simpson 1984).

1.6.3. Cold/Freezer Storage and Seedling Survival

The lifting window for cold or freezer storage in the Pacific Northwest region extends from early December to the end of February (Burdett and Simpson 1984). Other than unfavorable outplanting conditions in higher elevation nurseries (Heel 1987), the potential for having frozen soils during the lifting window can force nursery managers to make decisions on lifting in autumn and over-wintering seedlings in storage until favorable outplanting conditions arrive in March or later (Ritchie et al. 1985). The bulk of seedling cold and freezer storage research in the Pacific Northwest has been focused on this dilemma.

Ponderosa pine seedlings lifted in September had < 30% survival after over-wintering in freezer storage (-1.5°C) (Omi et al. 1993). Seedlings lifted in October and November in the same study had survival of > 70 and 80% after accumulating 249 and 574 more chilling hours, respectively. In another study, November-lifted ponderosa pine seedlings had 16% survival after 19 weeks of freezer storage (Edgren 1972). Conversely, a study of November-lifted ponderosa pine, along with lodgepole pine, Douglas-fir, Engelmann spruce, and western larch (*Larix occidentallis*) resulted in high survival after four months in both cold (0.6°C) and freezer storage (-2.2°C) (Morby and Ryker 1979). Survival after freezer and cold storage averaged 85 and 96%, respectively, for stock held

in either bags or crates. Morby and Ryker (1979) did not discourage fall lifting and cold storing of seedlings until spring. Omi and others (1993) also had 80% survival for March-lifted ponderosa pine seedlings. Douglas-fir seedlings had near 80% survival when lifted in February and March and stored in cold (2°C) and freezer (-2°C) storage (Van den Driessche and Cheung 1979). When lifted in May, Douglas-fir seedlings survival was about 23% after freezer storage. Lifting Douglas-fir seedlings that are not cold hardy and subjecting them to long-term freezer storage could be detrimental to survival (van den Driessche and Cheung 1979).

To determine seedling quality, physiological tests are often performed to determine the performance potential a seedling might express after outplanting. Overall survival of freezer stored container-grown Douglas-fir, lodgepole pine, and western larch seedlings lifted from late September to early December and freezer stored throughout the spring was > 80% when chlorophyll fluorescence, a measure of photosynthetic efficiency, was > 40% FvFm (L'Hirondelle et al. 2007). Similarly, survival was > 80% when stomatal conductance, a measure of gas exchange in the needles, was > 84 mmol m^{-2} s^{-1} (L'Hirondelle et al. 2007). Eight weeks after outplanting, stomatal conductance of white spruce seedlings lifted in the fall and freezer stored for 7 months was about 40 mmol m^{-2} s^{-1} (Jiang et al. 1994). Gas exchange of freezer stored seedlings was similar to white spruce seedlings lifted in April that were not freezer stored, and survival for both lifting times were also similar (90%) (Jiang et al. 1994). Fall-lifted white spruce seedlings overwintered in freezer storage may be more efficient at closing stomates earlier, leading to better drought resistance (Blake 1983). Stomatal conductance for ponderosa pine seedlings was found to be similar between non-stored spring-lifted

seedlings and seedlings lifted and freezer stored in the fall (Omi et al. 1991). Attention to stomatal conductance during cold or freezer storage may affect water relations and/or photosynthetic systems of seedlings trying to adjust to field conditions after outplanting (Grossnickle and Blake 1985). Depending on soil temperatures after outplanting, various storage durations have been linked to differences in white spruce (Grossnickle and Blake 1985; Harper et al. 1989) and jack pine (*Pinus banksiana*) (Grossnickle and Blake 1985) root growth. The amount of organic matter on an outplanting site can cause soil temperatures to rise and affect root growth (Page-Dumroese et al. 2008). Container-grown lodgepole pine seedlings experienced > 90% survival after 7 months of freezer storage on two sites, regardless of whether organic matter had been removed or retained after logging operations (Page-Dumroese et al. 2008). Survival in this study was also better for seedlings cold stored for 7 months when organic matter was removed. Seedlings not placed in storage had decreased survival in the absence of organic matter.

1.7. Cold Storage and Seedling Root Growth Potential

The ability of a seedling to produce new roots is an indicator of seedling performance potential and a reflection of a seedling's physiological status. Testing performance potential based on new root production is done by measuring seedling root growth potential (RGP). Root growth potential has been defined as the ability of a seedling to elongate and produce new roots in an environment conducive for root growth (Rietveld and Tinus 1987). Simpson and Ritchie (1996) similarly define RGP as the ability of a tree seedling to initiate and grow new roots within a prescribed time period in an environment optimal for root growth. Stone (1955) may have performed the first

known RGP experiment by trying to correlate the physiological state (new root growth in pots) of ponderosa pine and red fir (*Abies magnifica*) seedlings to poor outplanting survival. After Stone's work, individuals began attempting to link results from RGP trials to growth and survival after outplanting.

After a pattern of poor survival in the month of December was detected in loblolly pine, South (1999) coined the term "December dip" and mentioned low RGP as a possible reason for increases in seedling mortality. Seedlings that had been cold stored for several weeks following autumn lifting were more inclined to suffer poor survival during this time (South 1999). He also suggested that some loblolly pine genotypes may have inherently lower RGP and poor storability.

In one trial, mid-November and mid-December-lifted loblolly seedlings produced more new roots after 12 weeks of storage than seedlings lifted at the same time and not stored (DeWald and Feret 1988). In another trial, lifting and cold storage had a detrimental effect on RGP. However, the authors deem that autumn lifting and subsequent cold storage had a negative effect on RGP and attribute this to not enough chilling hours (< 400) before lifting. The authors account for RGP gains in that seedlings had been exposed to > 500 chilling hours before autumn lifting and 12 weeks of cold storage (Dewald and Feret 1988). This study concluded that cold storage was not detrimental to RGP and was comparable to the RGP of freshly lifted seedlings. However, while RGP was high for December-lifted loblolly, none of the seedlings were outplanted and monitored for survival to make a correlation between actual field performance and RGP.

Storing December-lifted shortleaf pine seedlings resulted in low RGP, but increases and decreases in RGP for non-stored shortleaf occurred when lifted from December to April (Hallgren et al. 1993). Despite this finding, RGP of stored and non-stored December-lifted seedlings had about 85 new roots and field survival was similar (Hallgren et al. 1993). Brissette and Barnett (1993) also found lower RGP in shortleaf seedlings lifted in December but found that RGP increased over time in storage. Dipping shortleaf seedling roots in a clay-slurry amended with benomyl before storage improved RGP and survival over non-stored seedlings in December (Hallgren and Ferris 1995).

Depending on the date seedlings are lifted and either cold stored or without cold storage, variations in RGP between pine species will exist. Seedling genotype may be the most important reason for this variation (South 1999). Two sources of ponderosa pine produced more new roots in storage compared to non-stored seedlings lifted on November 1, while two additional sources exhibited the opposite behavior (Stone and Schubert 1959). Container-grown Douglas-fir seedlings from a single seed source decreased, increased, or remained the same when exposed to various levels of cold acclimation (Burr and Tinus 1988). These results indicate that the RGP of container stock can be unpredictable, but can be detected between seedling genotypes.

Root growth potential has been examined to determine if cold storage is detrimental to seedling root growth after outplanting. Adding benomyl to clay slurry reduced RGP with increased storage time (Barnett et al. 1988) and first-year survival (Stumpff and South 1991) of loblolly pine. Reductions in longleaf pine RGP have also been reported with additions of benomyl to packing medium (Stumpff and South 1991).

Another study reported that adding benomyl to gel slurry decreased RGP and survival after storage when compared to gel only treatment (South and Lowenstein 1994).

1.8. Central Theme of Research

It is clear that the effects of genotype, species, chilling hours, and lifting play a role in seedling storage and survival. However, many questions still remain unanswered. The research presented in this dissertation was conducted in order to answer two questions related to the cold storage of southern pine seedlings: 1) why do bareroot seedlings exhibit poor survival following lifting and long-term cold storage during the months of October to mid-December and 2) why is container-grown seedling survival not affected by lifting and long-term storage during the same time of year? The questions were approached with the theory that pathogenic fungi may be colonizing bareroot seedlings through wounds sustained to root systems as they are lifted from nursery beds. The cool, moist environment in cold storage may be conducive to fungal growth and subsequent increases in disease leading to seedling mortality. *Pythium* was chosen for seedling inoculations based on a dataset presented by Sun (1996), which reported reductions in longleaf pine seedling survival after inoculations with *Pythium dimorphum* and long-term cold storage. The second question expanded on the theory to include the possibility that antagonistic fungi present in peat moss may suppress *Pythium* and yield it unable to cause disease. The following hypotheses served as a baseline for experimentation and outline subsequent chapters: 1) *Pythium* and cold storage does not affect loblolly, longleaf, shortleaf, and slash pine seedling survival, 2) peat moss used as a packing medium and container substrate does not affect loblolly, longleaf, shortleaf,

and slash pine seedling survival after *Pythium* inoculation and cold storage, 3) *Pythium* and cold storage does not affect loblolly, longleaf, and slash pine seedling root growth potential, 4) *Pythium* populations are similar in soils taken from southern forest tree nurseries during the fall and winter seasons, and 5) fungicide amended agar will not affect the growth rate or survival of *Pythium* species.

1.9. Literature Cited

Alexopoulas, C.J., C.W. Mims, and M. Blackwell. 1996. Introductory Mycology. 4th edition, John Wiley and Sons, Inc.: 869 p.

Asiegbu, F.O., A. Lonneborg, and M. Johansson. 1996. Chitin and glucans detected in the cell walls of *Pythium dimorphum*-an oomycetous fungus. European Journal of Forest Pathology 26: 315-321.

Barden, C.J. and P.P. Feret. 1986. Root growth potential of loblolly pine seedlings held in cold storage under differing moisture treatments. In: Schroeder, R.A., ed., Proc. of Southern Forest Nursery Association Conference, July 22-24, 1986, Pensacola, FL: 218-223.

Barnett, J.P., J.C. Brissette, A.G. Kais, and J.P. Jones. 1988. Improving field survival of southern pine seedlings by treating with fungicides before storage. Southern Journal of Applied Forestry 12: 281-285.

Barnett, J.P. 2002. Trends in nursery research and production. In: Dumroese, R.K., Riley, L.E., Landis, T.D., tech. coords., National Proceedings: Forest and Conservation Nursery Associations-1999, 2000, and 2001. Proc. RMRS-P-24. Ogden, UT: USDA Forest Service, Rocky Mountain Research Station: pp 97-100.

Blake, T.J. 1983. Transplanting shock in white spruce: effect of cold-storage and root pruning on water relations and stomatal conditioning. Physiologia Plantarum 57(2): 210-216.

Borja, I. 1995. Root dieback and defense responses in Norway spruce seedlings infected with the fungal pathogen, *Pythium dimorphum*. PhD Dissertation, Agricultural University of Norway: 126 p.

Boyer, J.N. and D.B. South. 1985. Dormancy, chilling requirements, and storability of container-grown loblolly pine seedlings. Auburn University, Southern Forest Nursery Management Cooperative Research Note No. 23: 12 p.

Boyer, J.N. and D.B. South. 1987. Excessive seedling height, high shoot-to-root ratio, and benomyl root dip reduce survival of stored loblolly pine seedlings. Tree Planters' Notes 38(4): 19-22.

Boyer, J.N. and D.B. South. 1989. Seasonal changes in intensity of bud dormancy in loblolly pine seedlings. Tree Physiology 5(3): 379-385.

Brissette, J.C. 1986. Lifting forest tree nursery crops-An overview. In: Proc. Southern Forest Nursery Association Meeting, Pensacola, FL: 141-145.

Brissette, J.C. and J.P. Barnett. 1993. Lifting and storage duration affect root growth potential and field survival of shortleaf pine seedlings. In: Proc. of seventh biennial southern silvicultural research conference, GTR-SO-93, New Orleans, LA, USDA Forest Service, Southern Forest Experiment Station: 263-268.

Brissette, J.C., J.P. Barnett, and J.P. Jones. 1996. Fungicides improve field performance of stored loblolly and longleaf pine seedlings. Southern Journal of Applied Forestry 20(1): 5-9.

Burdett, A.N. and D.G. Simpson. 1984. Lifting, grading, packaging, and storing. Chapter 21, Forest Nursery manual, M.L. Duryea and T.D. Landis, eds., Martinius Nijhoff/Dr W. Junk Publishers, The Hague, The Netherlands: 385 p.

Burr, K.E. and R.W. Tinus. 1988. Effect of the timing of cold storage on cold hardiness and root growth potential of Douglas-fir. In: Landis, TD, ed., Proc. Combined Meeting of Western Nursery Association, Gen Tech. Rep. RM-167, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 133-138.

Campbell, W.A., F.F. Hendrix, and W.M. Powell. 1972. Pythium and nematode species implicated in root rot. Tree Planters' Notes 23(1): 3 p.

Carlson, W.C. 1991. Lifting, storing, and transporting southern pine seedlings. Chapter 16, Forest Regeneration Manual, M.L. Duryea and P.M. Dougherty, eds., Kluwer Academic Publishers, Norwell, MA. 433 p.

Cordell, C.E., A.G. Kais, J.P. Barnett, and C.E. Affeltranger. 1984. Effects of benomyl root storage treatments on longleaf pine seedling survival and brown-spot disease incidence. In: Proc. Southern Nursery Conference, Western Session, June 11-14, 1984, Alexandria, LA: 84-88.

Cram, M.M., S.A. Enebak, S.W. Fraedrich, L.D. Dwinell, and S.J. Zarnoch. 2007. Evaluation of fumigants, EPTC herbicide, and *Paenibacillus macerans* in the production of loblolly pine seedlings. Forest Science 53(1): 73-83.

DeWald, L.E. and P.P. Feret. 1988. Changes in loblolly pine seedling root growth potential, dry weight, and dormancy during cold storage. Forest Science 34(1): 41-54.

Dierauf, T.A. 1976. Fall planting and storage using non-dormant seedlings. In: Proc. Southeastern Area Nurseryman's Conference-SC Eastern: 31-36.

Dierauf, T.A., L.A. Chandler, and D.L. Hixson. 1992. Stripping lateral roots from loblolly pine seedlings during lifting-effect on field performance. Occasional Report No. 105, Virginia Department of Forestry: 3 p.

Dumroese, R.K., R.L. James, D.L. Wenny, and C.J. Gilligan. 1988. Douglas-fir seed treatments: effects on seedborne organisms and germination. In: Landis, T.D., tech. coords., Proc. Combined Meeting of the Western Forest Nursery Association, GTR-RM-167, Fort Collins, CO, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 155-160.

Dumroese, R.K., R.L. James, and D.L. Wenny. 2002. Hot water and copper coatings in reused containers decrease inoculum of *Fusarium* and *Cylindrocarpon* and increase Douglas-fir seedling growth. HortScience 37(6): 943-947.

Dumroese, R.K. and J.P. Barnett. 2004. Container seedling handling and storage in the Southeastern states. In: Riley, L.E., Dumroese, R.K., and Landis, T.D., tech. coords., National Proc.: Forest and Conservation Nursery Association-2003, RMRS-P-33. Fort Collins, CO, USDA Forest Service, Rocky Mountain Research Station: 22-25.

Dumroese, R.K. and R.L. James. 2005. Root diseases in bareroot and container nurseries of the Pacific Northwest: epidemiology, management, and effects on outplanting performance. New Forests 30: 185-202.

Dumroese, R.K., D.F. Jacobs, and T.D. Landis. 2005. Successful stock production for forest regeneration: What foresters should ask nursery managers about their crops (and vice versa). In: Columbo, S.J. ed., The Thin Green Line: A symposium on the state-of-the-art in reforestation Proc., July 26-28, 2005, Ontario Forest Research Institute, Sault Ste. Marie, Ontario, Forest Research Information Paper No. 160: 14-20.

Edgren, J.W. 1972. Field survival poor for stored and heeled-in ponderosa pine seedlings. Tree Planters' Notes 23(1): 2 p.

Enebak, S.A. 2008. Forest tree seedling production in the southern United States for the 2007-2008 planting season. Auburn University Southern Forest Nursery Management Cooperative, Technical Note 08-01: 10 p.

Erazo, F. 1987. Superabsorbent hydrogels and their benefits in forestry applications. In: Landis, T.D., tech coords., Proc. Intermountain Forest Nursery Association, August 10-14, Oklahoma City, OK. GTR-RM-151, Fort Collins, CO, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 14-17.

Feret, P.P., R.E. Kreh, and L.E. DeWald. 1985. Root growth potential of stored loblolly pine seedlings. In: Shoulders, E. ed., Proc. of the third biennial southern silvicultural research conference, Atlanta, GA, Gen. Tech. Rep. SO-54. New Orleans, LA: USDA Forest Service, Southern Forest Experiment Station: 18-24.

Fraedrich, S.W. and L.D. Dwinell. 2003. The effects of soil fumigation on pine seedling production, weeds, foliar and soil nutrients, and soilborne microorganisms at a South Georgia (U.S.A.) forest tree nursery. Canadian Journal of Forest Research 33: 1698-1708.

Fraedrich, S.W. and L.D. Dwinell. 2003. An evaluation of Dazomet incorporation methods on soilborne organism and pine seedling production in southern nurseries. Southern Journal of Applied Forestry 27(1): 41-51.

Garber, M.P. and J.G. Mexal. 1980. Lift and storage practices: their impact on successful establishment of southern pine plantations. New Zealand Journal of Forest Science 10(1): 72-82.

Gravel, V., C. Martinez, H. Antoun, and R.J. Tweddell. 2006. Control of greenhouse tomato root rot[*Pythium ultimum*] in hydroponic systems, using plant-growth-promoting microorganisms. Canadian Journal of Plant Pathology 28: 475-483.

Hallgren, S.W., C.G. Tauer, and D.L. Weeks. 1993. Cultural, environmental, and genetic factors interact to affect performance of planted shortleaf pine. Forest Science 39(3): 478-498.

Hallgren, S.W. and D.M. Ferris. 1995. Benomyl applied to roots improves second-year survival and growth of shortleaf pine. Southern Journal of Applied Forestry 19(1): 36-41.

Hebb, E.A. 1982. Cold storage of Choctawhatchee sand pine seedlings: Effect of lifting date and length of storage. Southern Journal of Applied Forestry 6(4): 229-231.

Heel (Hee), S.M. 1987. Freezer storage practices at Weyerhaeuser nurseries. Tree Planters' Notes 38(3): 7-10.

Hendrix, F.F., Jr. and W.A. Campbell. 1961. Distribution of *Phytopthora* and *Pythium* species in soils in the continental United States. Canadian Journal of Botany 48: 377-384.

Hendrix, F.F., Jr. and W.A. Campbell. 1968. Pythiaceous fungi isolated from southern forest nursery soils and their pathogenicity to pine seedlings. Forest Science 14(3): 292-297.

Hendrix, F.F., Jr. and W.A. Campbell. 1971. A new species of *Pythium* with spiny oogonia and large chlamydospores. Mycologia 63: 978-982.

Hendrix, F.F., Jr. and W.A. Campbell. 1973. *Pythiums* as plant pathogens. Annual Reviews Phytopathology 11: 77-98.

Ho, H.H. 1986. *Pythium dimorphum* from rhododendron. Mycopathologia 93: 141-145.

Huang, J.W. and E.G. Kuhlman. 1990. Fungi associated with damping-off of slash pine seedlings in Georgia. Plant Disease 74: 27-30.

Hunter, P.J., G.M. Petch, L.A. Calvo-Bado, T.R. Pettitt, N.R. Parsons, J.A.W. Morgan, and J.M. Whipps. 2006. Differences in microbial activity and microbial populations of peat associated with suppression of damping-off disease caused by *Pythium sylvaticum*. Applied and Environmental Microbiology 72(10): 6452-6460.

Ivors, K.L., Z.G. Abad, and D.M. Benson. 2008. Evaluating the pathogenicity of *Pythium vexans* isolates from Fraser fir in North Carolina. Online. Plant Health Progress doi: 10.1094/PHP-2008-1006-01-RS.

James, R.L. 1988. Root disease of containerized conifer seedlings. Western Forest Systems Nursery, Lewiston, ID, Report 88-3, USDA Forest Service, Northern Region: 5 p.

Jiang, Y., J.J. Zwiazek, and S.E. Macdonald. 1994. Effects of prolonged cold storage on carbohydrate and protein content and field performance of white spruce bareroot seedlings. Canadian Journal of Forest Research 24: 1369-1375.

Jones, J.P., D.J. Pantone, J.P. Barnett, and J.C. Brissette. 1992. The relationship between fungal population development and root damage of cold-stored longleaf pine seedlings. Louisiana State University Agricultural Center, Bulletin No. 832: 23 p.

Juzwick, J., K.M. Gust, and R.R. Allmaras. 1999. Influence of cultural practices on edaphic factors related to root disease in *Pinus* nursery seedlings. Plant and Soil 207: 195-208.

Kahler, L.H. and A.R. Gilmore. 1961. Field survival of cold stored loblolly pine seedlings. Tree Planters' Notes, Issue 45: 15-16.

Kais, A.G, C.E. Cordell, and C.E. Affeltranger. 1986. Benomyl root treatment controls brown spot disease on longleaf pine in the southern United States. Forest Science 32: 506-511.

Kais, A.G. and J.P. Barnett. 1984. Longleaf pine growth following storage and benomyl root-dip treatment. Tree Planters' Notes 35(1): 30-33.

Kelley, W.D. and S.W. Oak. 1989. Forest Nursery Pests. In: Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, Jr., R.S., and Harvey, T.V, tech. coords., USDA Forest Service, Agricultural Handbook No. 680: 184 p.

Kooistra, C.M. and J.D. Baker. 2002. Planting frozen conifer seedlings: warming trends and effects on seedling performance. New Forests 23: 225-237.

Kope, H.H., P.E. Axelrood, J. Sutherland, and M.S. Reddy. 1996. Prevalence and incidence of the root-inhabiting fungi, *Fusarium*, *Cylindrocarpon*, and *Pythium* on container-grown Douglas-fir and spruce seedlings in British Columbia. New Forests 12: 55-67.

Labrie, C., P. Leclerc, N. Cote, S. Roy, R. Brzezinski, R. Hogue, and C. Beaulieu. 2001. Effect of chitin-based composts produced by two-phase composting on two oomycete plant pathogens. Plant and Soil 235: 27-24.

Landis, T.D., R.W. Tinus, S.E. McDonald, and J.P. Barnett. 1989. The Container Tree Nursery Manual, Volume 5, The Biological Component: Nursery Pests and Mycorrhizae. Agricultural Handbook 674. Washington D.C., USDA Forest Service: 171 p.

Landis, T.D., R.W. Tinus, S.E. McDonald, and J.P. Barnett. 1990. The Container Tree Nursery Manual, Volume 2, Containers and Growing Media. Agricultural Handbook 674. Washington D.C., USDA Forest Service: 87 p.

Landis, T.D., R.W. Tinus, and J.P. Barnett. 1998. The Container Nursery Manual, Volume 6, Seedling Propagation. Agricultural Handbook 674. Washington D.C., USDA Forest Service: 166 p.

Landis, T.D., R.K. Dumroese, and D.L. Haase. 2010. The Container Tree Nursery Manual, Volume 7, Seedling Processing, Storage, and Outplanting. Agricultural Handbook 674. Washington D.C., USDA Forest Service: 192 p.

L'Hirondelle, S.J., D.G. Simpson, and W.D. Binder. 2007. Chlorophyll fluorescence, root growth potential and stomatal conductance as estimates of field performance potential in conifer seedlings. New Forests 34: 235-251.

Lilja, A. 1994. The occurrence and pathogenicity of uni- and binucleate *Rhizoctonia* and *Pythiaceae* fungi among conifer seedlings in Finnish forest nurseries. European Journal of Forest Pathology 24(3): 181-192.

Linde, C., G.H.J. Kemp, and M.J. Wingfield. 1994. *Pythium irregulare* associated with *Pinus* seedling death on previously cultivated lands. Plant Disease 78: 1002-1005.

May, J.T. 1984. Southern Pine Nursery Handbook. U.S. Department of Agriculture, Forest Service, Southern Region.

Morby, F.E. and R.A. Ryker. 1979. Fall-lifted conifers successfully spring planted in Southwest Idaho. Tree Planters' Notes 30(3): 27-29.

Omi, S.K. and U.K. Schuch. 1987. Fall lifting: Its effects on dormancy intensity of ponderosa pine seedlings-a preliminary investigation. In: Landis, T.D., tech. coord., Proc. Intermountain Forest Nursery Association, August 10-14, 1987; Oklahoma City, OK, GTR-RM 151, Fort Collins, CO, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 93-97.

Omi, S.K., B. Yoder, and R. Rose. 1991. Fall lifting and long-term freezer storage of ponderosa pine seedlings effects on post-storage leaf water potential, stomatal conductance, and root growth potential. Tree Physiology 8: 315-325.

Omi, S.K., R. Rose, and T.E. Sabin. 1993. Fall lifting and long-term freezer storage of ponderosa pine seedlings: effects on starch, root growth, and field performance. Canadian Journal of Forest Research 24: 624-637.

Page-Dumrose, D.S., R.K. Dumrose, M.F. Jurgenson, A. Abbott, and J.J. Hensiek. 2008. Effect of nursery storage and site preparation techniques on field performance of high-elevation *Pinus contorta* seedlings. Forest Ecology and Management 256: 2065-2072.

Rietveld, W.J. and R.W. Tinus. 1987. Alternative methods to evaluate root growth potential and measure root growth. In: Landis, T.D., tech. coord.. Proc., Intermountain Forest Nursery Association, Oklahoma City, OK. Gen. Tech. Rep., USDA. Forest Service, Rocky Mountain Forest and Range Experiment Station: 70-76.

Ritchie, G.A., J.R. Roden, and N. Kleyn. 1985. Physiological quality of lodgepole pine and interior spruce seedlings: effects of lift date and duration of freezer storage. Canadian Journal of Forest Research 15: 636-645.

Ritchie, G.A. 1987. Some effects of cold storage on seedling physiology. Tree Planters' Notes 38(2): 11-15.

Ritchie, G.A. 2004. Container seedling storage and handling in the Pacific Northwest: answers to some frequently asked questions. In: Riley, L.E., Dumroese, R.K., and Landis T.D., tech cords. National proceedings: Forest and Conservation Nursery Associations. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station: 3-7.

Shafizadeh, S. and J.A. Kavanagh. 2005. Pathogenicity of *Phytopthera* species and *Pythium undulatum* isolated from *Abies procera* Christmas trees in Ireland. Forest Pathology 35: 444-450.

Simpson, D.G. and G.A. Ritchie. 1996. Does RGP predict field performance? A debate. New Forests 13: 249-273.

South, D.B. 1987. Economic aspects of nursery seed efficiency. Southern Journal of Applied Forestry 11: 106-109.

South, D.B., H.S. Larsen, J.N. Boyer, and H.M. Williams. 1990. Seed spacing and seedling biomass: Effect on root growth potential of loblolly pine (*Pinus taeda*). New Forests 4: 179-192.

South, D.B. 1993. Rationale for growing southern pine seedlings at low seedbed densities. New Forests 7: 63-92.

South, D.B. and N.J. Lowenstein. 1994. Effects of Viterra® root dips and benomyl on root growth potential and survival of longleaf pine seedlings. Southern Journal of Applied Forestry 18(1): 19-23.

South, D.B. 1998. Needle-clipping longleaf pine and top-pruning loblolly pine in bareroot nurseries. Southern Journal of Applied Forestry 22(4):235-240.

South, D.B. 1999. The December dip of loblolly pine. In: Landis, T.D., Barnett, J.P., tech. coords., National Proc: forest and conservation nursery associations –1998. Gen. Tech. Rep. SRS-25. Asheville, NC, USDA Forest Service, Southern Research Station: 14-17.

Srago, M.D. and A.H. McCain. 1989. Forest Nursery Pests. In: Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, Jr., R.S., and Harvey, T.V, tech. coords., USDA Forest Service, Agricultural Handbook No. 680: 184 p.

Stone, E.C. 1955. Poor survival and the physiological condition of planting stock. Forest Science. 1(2): 90-94.

Stone, E.C. and G.H. Schumbert. 1959. The physiological condition of ponderosa pine (*Pinus ponderosa* Laws) planting stock as it affects survival after cold storage. Journal of Forestry 57: 837-841.

Stumpff, N.J. and D.B. South. 1991. Benomyl root dips adversely affect first-year performance of stored loblolly pine seedlings. Southern Journal of Applied Forestry 15(3) 133-137.

Sutherland, J. and J. Dennis. 1992. *Pythium* water mould in British Columbia nurseries: British Columbia Ministry of Forests. Seed and Seedling Extension Topics 5(1): 9-10.

Sun, X. 1996. Biological control of Pythiaceous fungi causing death of longleaf pine seedlings during cold storage. PhD dissertation, Louisiana State University: 135 p.

Tanaka, Y., K.W. Russell, and R.G. Linderman. 1986. Fumigation effect on soilborne pathogens, mycorrhizae, and growth of Douglas-fir seedlings. In: Landis, T.D., ed., Proc. Western Forest Nursery Council, GTR-RM-137, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 147-152.

Thrane, C, D.F. Jenson, and A. Tronsmo. 2000. Substrate colonization, strain competition, enzyme production *in vitro*, and biocontrol of *Pythium ultimum* by *Trichoderma* spp. isolates P1 and T3. European Journal of Plant Pathology 106: 215-225.

Van den Driessche, R. and K-W Cheung. 1979. Relationship of stem electrical impedance and water potential of Douglas-fir seedlings to survival after cold storage. Forest Science 25(3): 507-517.

Van der Plaats-Niterink, A.J. 1981. Monograph of the Genus Pythium. Studies in Mycology, No. 21: 242 p.

Van Os, G.J. and J.H. van Ginkel. 2001. Suppression of *Pythium* root rot in bulbous *Iris* in relation to biomass and activity of the soil microflora. Soil Biology and Biochemistry 33: 1447-1454.

Van West P., A.A. Appiah, and N.A.R. Gow. 2003. Advances in research on oomycete root pathogens. Physiological and Molecular Plant Pathology 62: 99-113.

Venator, C.R. 1984. Evidence of the presence of a lifting window for loblolly pine nursery seedlings. In Proc: Southern Nursery Conference-Eastern Session, Asheville, NC: 184-190.

Wakeley, P.C. 1954. Planting the southern pines. USDA Agricultural Monograph No.18: 233 p.

Wang, Y. and J.J. Zwiazek. 2001. Physiological characteristics and carbohydrate contents of spring-lifted *Picea glauca* bareroot seedlings following low-temperature storage. Scandinavian Journal of Forest Research 16: 415-421.

Wenny, D.L., D.J. Swanson, and R.K. Dumroese. 2002. The chilling optimum of Idaho and Arizona ponderosa pine buds. Southern Journal of Applied Forestry 17(3): 117-121.

Wolffhechel, H. 1988. The suppressiveness of sphagnum peat to *Pythium* spp. Acta Horticulturae 221: 217-222.

CHAPTER 2

EFFECTS OF *PYTHIUM* AND COLD STORAGE ON LONGLEAF, LOBLOLLY, SLASH, AND SHORTLEAF PINE SURVIVAL

2.1. Abstract

Cold storing bareroot southern pine (*Pinus* spp.) seedlings for > 1 week after lifting from October to mid-December can lead to poor outplanting survival when compared to seedlings that are lifted and stored in January. In contrast, container-grown seedlings do not experience adverse effects from lifting and storing for periods > 1 week. The practice of lifting bareroot seedlings can cause wounds to root systems, which pathogenic fungi, particularly *Pythium* spp., could use as infection sites. Once seedlings are placed in storage, the cool, moist environment may be conducive for fungal growth and subsequent outplanting failure. Container-grown seedlings roots are injured less during lifting and may not become infected. The effect of storage and *Pythium* on seedling survival was examined on bareroot and container-grown longleaf pine (*Pinus palustris*), loblolly pine (*Pinus taeda*), and slash pine (*Pinus elliottii*) and container-grown shortleaf pine (*Pinus echinata*). Seedlings were inoculated with either *P. dimorphum* or *P. irregulare*, cold stored for 3, 4, 6, or 12 weeks, and outplanted. Seedling survival was monitored for 6 months, and *Pythium* populations quantified from

seedling roots after each storage period. *Pythium* spp. reduced survival of bareroot longleaf pine but not bareroot slash pine. *Pythium* spp. did not affect the survival of container-grown seedlings. Length of storage decreased survival for both seedling stock types, especially for seedlings outplanted after 12 weeks of storage. *Pythium irregulare* was recovered more from seedling roots than *P. dimorphum*, and fungal populations were higher from injured roots.

2.2. Introduction

After southern pine (*Pinus* spp.) seedlings are lifted, nursery managers commonly hold seedlings in cold storage (< 5ºC) for days, weeks, or even months. Cold storage is necessary when weather conditions impose harvesting, planting operations do not need seedlings, or when adequate soil moisture is lacking. Bareroot seedlings lifted and held in long-term cold storage (> 1 wk) from October to mid-December can result in poor seedling survival compared to when seedlings are lifted and stored in January. Kahler and Gilmore (1961) reported loblolly pine (*Pinus taeda*) seedling survival to be < 60% when lifted and cold stored in October and November and > 90% when lifted and cold stored after mid-December. Loblolly pine seedlings lifted in October and November and stored for two weeks had < 3% survival, while survival without storage ranged between 66 and 92% (Dierauf 1976). Poor seedling survival in these studies was attributed to non-dormant buds at the time of lifting and not from cold storage effects. However, no measure of bud dormancy was mentioned to document poor outplanting survival.

Chilling hours is the exposure of seedlings to above-freezing temperatures (> 0ºC) for a specific period of time. After exposure to a certain number of chilling hours, seedling buds are classified as either dormant (May 1984) or in a state of “after-rest” in

which the bud would resume growth (Boyer and South 1985). Carlson (1991) claimed that if cold storage was necessary for more than a few days, seedlings should not be actively growing, which is why seedlings are generally outplanted within 2 to 3 days after lifting from October to mid-December (known as "hot planting"). However, Boyer and South (1985) reported that periods of cold storage were possible for container-grown loblolly pine without the required number of chilling hours. Therefore, successful survival after cold storage might not be related to the status of seedling bud dormancy.

Container-grown seedlings generally do not experience the adverse effects (poor survival) from long-term storage during the fall (October to mid-December) as do bareroot seedlings. Container-grown longleaf pine (*Pinus palustris*) seedlings cold stored for three weeks and outplanted in October had > 94% survival (Pickens and Robbins, unpublished data). One explanation for this difference in survival is that as container-grown seedlings are lifted, seedling roots remain intact within a plug of organic media, which lessens the chance of root injury. In contrast, bareroot seedling roots can be damaged during lifting, causing wounds that could serve as infection sites for soilborne fungi.

Applying fungicides to seedling roots in storage can increase seedling survival after outplanting (Barnett et al. 1988; Jones et al. 1992). In their trials, *Pythium* populations from seedlings increased over time in cold storage and were lowered by adding benomyl, metalaxyl, or combinations of each to seedlings before storage. *Pythium* is a common soilborne pathogen found in nursery soils and is associated with "damping-off" on seed and emerging germinants and "fine feeder root disease" in older seedlings (Kelley and Oak 1989). Classified as a "water mold", cold storage is an ideal

environment for *Pythium* to thrive given the cool, moist environment. Once established on roots, the fungus could increase in numbers and result in seedling mortality after outplanting. The elimination of water, normally added to loblolly pine roots prior to storage increased root growth after 20 weeks when compared to seedlings dipped in water (Barden and Feret 1986). Perhaps environments lacking moisture could affect *Pythium* levels in cold storage and decrease the amount of outplanting mortality. The ability to store bareroot seedlings from November to mid-December, without the risk of seedling mortality, could extend the traditional planting season and ensure that the lifting season ends before spring (Garber and Mexal 1980).

The objectives of these studies were to 1) test the effects of *Pythium* and long-term cold storage on survival of bareroot and container-grown loblolly, longleaf, and slash pine (*Pinus elliottii*) and container-grown shortleaf pine (*Pinus echinata*) seedlings and 2) to quantify *Pythium* populations from seedling roots in cold storage. The null hypothesis tested is that inoculating loblolly, longleaf, slash, and shortleaf pine seedlings with *Pythium* prior to storage does not affect seedling survival after outplanting.

2.3. Materials and Methods

2.3.1. *Pythium* Inoculum

Pythium dimorphum Hendrix and Campbell and *Pythium irregulare* Buisman were obtained from American Type Culture Collection (ATCC®, Manassas, VA) and were aseptically transferred to oatmeal agar (Kim et al. 2005). From the advancing margin of the mycelium, three 0.5 cm disks of each *Pythium* spp. were transferred to oatmeal agar to use later as seedling root inoculum. Prior to inoculation, 1,190 g of oatmeal and 400 ml of distilled water were combined in two autoclavable bags, mixed

thoroughly, and autoclaved. The sterilized oats were allowed to cool for 24 h and one autoclaved oatmeal bag received three plates of *P. dimorphum* and the other bag received three plates of *P. irregulare*. The oatmeal-*Pythium* inoculum was mixed every 12 h and stored at room temperature for 10 days prior to seedling root inoculations (Figure 2.1).

2.3.2. Seedling Inoculations

Two separate experiments were conducted using: 1) bareroot and container-grown longleaf pine (2008) and 2) bareroot and container-grown loblolly and slash pine and container-grown shortleaf pine (2009). Bareroot seedlings were obtained from Smurfit-Stone Corporation's Rock Creek Nursery near Brewton, AL and container-grown seedlings from International Forest Company in Moultrie, GA. Prior to inoculations, bareroot and container-grown pine seedlings remained in cold storage (4-5ºC) for 6 and 8 days, respectively. Inoculation of seedling roots with *Pythium* involved dipping seedlings into a bucket that was filled with 11 liters of water (controls) or 11 liters of water plus the oatmeal/*Pythium* inoculum (Figure 2.1). Bareroot seedlings were treated with one of seven treatments: 50 g, 100 g, and 200 g of *P. dimorphum* oatmeal inoculum, 50 g, 100 g, and 200 g of *P. irregulare* oatmeal inoculum. Control seedlings were dipped in water without the *Pythium* oatmeal inoculum. Three replications of each treatment were used for longleaf (30 seedlings/rep) and loblolly and slash pine (20 seedlings/rep). Container-grown seedlings received five treatments: 200 g of *P. dimorphum* oatmeal inoculum and root wounding or without root wounding and 200 g of *P. irregulare* oatmeal inoculum and root wounding or without root wounding. Control seedlings received neither inoculum nor root wounding. Wounding was done by cutting down two

sides of the container root plug with a knife (Figure 2.1). Three replications of each container-grown seedling treatment were inoculated with longleaf (30 seedlings/rep), loblolly and slash (20 seedlings/rep), and shortleaf pine (15 seedlings/rep). After each seedling replication was inoculated, buckets were emptied, rinsed, and filled with a new oatmeal inoculum mixture. Inoculated seedlings were placed in 49 liter plastic bags and put in cold storage (4-5ºC) for 3, 6, and 12 weeks (Experiment 1) and 4, 6, and 12 weeks (Experiment 2).

Figure 2.1. A. *Pythium* oatmeal inoculum. B. *Pythium* oatmeal inoculum being weighed prior to mixing in water. C. Wounding a container-grown longleaf pine seedling root plug with a knife. D. Inoculating longleaf pine seedlings in the *Pythium* oatmeal water mixture.

2.3.3. Seedling Survival

After each storage period, seedlings were outplanted into sand in a completely randomized block design at 0.3 X 0.3 m spacing near Auburn University (Figure 2.2). Each block represented 17 m^2 of planting area with 1,890 bareroot and 1,350 container-grown longleaf pine seedlings outplanted. In the second trial, 1,260 bareroot and 900 container-grown each of loblolly and slash pine and 675 container-grown shortleaf pine seedlings were outplanted. Seedling survival was monitored for 6 months, and a seedling was considered dead when foliage was no longer green.

2.3.4. *Pythium* Populations

To quantify *Pythium* populations, roots from three seedlings in each replication/treatment of each stock type were assayed after each storage period. After rinsing soil from the roots, lateral roots and fine feeder roots (5 cm from the taproot) were removed from seedlings. Root sub-samples were surface sterilized in a 10% bleach solution and rinsed two times in sterile, distilled water. After sterilization, ten 2.5-cm root segments were randomly chosen and placed onto *Pythium* selective media (Feng and Dernoeden 1999) (Figure 2.3). Each longleaf sample contained a total of 50 cm of roots on two agar plates, whereas loblolly, slash, and shortleaf pine had 25 cm of roots on one agar plate for each replication. *Pythium* recovery was the number of colony forming units (CFUs) identified on the selective media.

Figure 2.2. A randomized complete block of loblolly and slash pine seedlings.

Figure 2.3. Seedling root segments plated onto *Pythium* selective media.

2.3.5. Statistical Analyses

Statistical analyses were conducted using a General Linear Model (GLM) in SAS statistical software (9th ed., SAS Institute, Cary, NC). An experimental unit consisted of 30 longleaf, 20 loblolly and slash, or 15 shortleaf pine seedlings. Means of each experimental unit for each dependant variable were analyzed using Analysis of Variance (ANOVA) in a factorial design ($\alpha = 0.05$). Data for longleaf, loblolly, and slash pine bareroot seedlings were analyzed separately, and contrast analyses were performed using combined levels of each *Pythium* treatment. Data for container-grown loblolly, slash, and shortleaf pine were analyzed together. Due to temporal factors, container-grown longleaf pine data was analyzed separately from the other container-grown species.

2.4 Results

2.4.1. Bareroot Seedlings

2.4.1.1. Longleaf Pine

Inoculating bareroot longleaf pine roots with either *P. dimorphum* or *P. irregulare* prior to 12 weeks of storage reduced longleaf pine survival (Table 2.1). Time in storage also affected seedling survival after outplanting ($P = 0.0098$) with *Pythium*-inoculated seedling survival at 12% at 3 weeks and 3% at 12 weeks. Non-inoculated seedling survival at 3 and 12 weeks was 34% and 22%, respectively (Table 2.2). Due to mis-identification, *Pythium* populations were not quantified from longleaf pine roots.

2.4.1.2. Loblolly Pine

When the three inoculum levels were combined for each *Pythium* spp., bareroot loblolly pine survival was not affected by either *P. dimorphum* (P = 0.1826) or *P. irregulare* (P = 0.1741) (Table 2.1). However, seedlings treated with 100 g of *P. irregulare* oatmeal inoculum decreased seedling survival (P = 0.0026) (LSD = 16) (Table 2.3). Loblolly pine seedlings inoculated with *P. dimorphum* had greater survival than seedlings inoculated with *P. irregulare* (P = 0.0004). Time in storage decreased loblolly pine survival (Table 2.1) at 12 weeks of storage with non-inoculated loblolly pine survival at 37%, compared to 42% for 4 weeks in storage. Seedling survival during the same period for seedlings inoculated with *P. dimorphum* and *P. irregulare* was 43% and 23%, respectively, (Table 2.5).

Higher levels of *Pythium* (CFUs) were recovered from loblolly pine inoculated with *P. irregulare* than those inoculated with *P. dimorphum* (Table 2.4). *Pythium* was not recovered from any of the non-inoculated (control) seedlings (Table 2.4). While *P. dimorphum* was recovered from seedling roots, the numbers were not different from non-inoculated seedlings (P = 0.0609). Generally, the number of *Pythium* CFUs increased as seedlings remained in storage.

Table 2.1. Analysis of variance for bareroot longleaf pine survival (2008) and loblolly and slash pine survival and *Pythium* colony forming units (CFUs) (2009).

		P > F				
		Longleaf	Loblolly		Slash	
	DF	Survival (%)	Survival (%)	*Pythium* (CFUs)	Survival (%)	*Pythium* (CFUs)
Replication	2	0.7257	0.1441	0.3191	0.4504	0.1579
Treatment	6	0.0018	0.0026	0.0001	0.2544	0.0001
Storage	2	0.0098	0.0091	0.0298	0.0147	0.0001
Treatment*Storage	12	0.9766	0.9238	0.9093	0.5923	0.3816
Control vs *P. dimorphum*	1	0.0001	0.1826	0.0609	0.6396	0.0007
Control vs *P. irregulare*	1	0.0001	0.1741	0.0001	0.4832	0.0001
P. dimorphum vs *P. irregulare*	1	0.5806	0.0004	0.0001	0.1031	0.0001
Error	40					

Table 2.2. Bareroot longleaf pine survival for 3, 6, and 12-week stored (4-5°C) seedlings six months after outplanting in 2008.

Treatment[z]	Rep	Survival (%) 3 wks	6 wks	12 wks	Total
Control	1	17	3	27	
	2	23	30	3	
	3	63	13	37	
	Mean	34	15	22	24
P. dimorphum 50 g	1	3	20	3	
	2	30	7	7	
	3	7	10	3	
	Mean	13	12	4	10
P. dimorphum 100 g	1	10	7	0	
	2	3	0	7	
	3	20	0	0	
	Mean	11	2	2	5
P. dimorphum 200 g	1	3	0	0	
	2	0	0	0	
	3	13	7	10	
	Mean	6	2	3	4
P. irregulare 50 g	1	17	7	10	
	2	20	3	3	
	3	7	3	3	
	Mean	14	4	6	8
P. irregulare 100 g	1	27	0	7	
	2	7	13	0	
	3	13	10	3	
	Mean	16	8	3	9
P. irregulare 200 g	1	20	3	10	
	2	13	7	0	
	3	0	3	0	
	Mean	11	4	3	6
LSD[y]		*(25)*	*(12)*	*(13)*	*(9)*

[z] = *Pythium* inoculation treatments took place on December 17, 2007
[y] = least significant difference ($\alpha = 0.05$)

Table 2.3. Bareroot loblolly pine survival for 4, 6, and 12-week stored (4-5°C) seedlings six months after outplanting in 2009.

Treatment[z]	Rep	Survival (%) 4 wks	6 wks	12 wks	Total
Control	1	50	45	35	
	2	25	35	25	
	3	50	30	50	
	Mean	42	37	37	38
P. dimorphum 50 g	1	75	60	55	
	2	80	35	70	
	3	55	30	55	
	Mean	70	42	60	57
P. dimorphum 100 g	1	50	45	15	
	2	75	20	35	
	3	45	50	50	
	Mean	57	38	33	43
P. dimorphum 200 g	1	55	65	30	
	2	25	50	45	
	3	65	15	30	
	Mean	48	43	35	42
P. irregulare 50 g	1	50	55	25	
	2	30	50	20	
	3	75	5	40	
	Mean	52	37	28	39
P. irregulare 100 g	1	30	25	45	
	2	20	5	10	
	3	25	5	15	
	Mean	25	12	23	20
P. irregulare 200 g	1	80	30	5	
	2	20	25	0	
	3	35	10	50	
	Mean	45	22	18	28
LSD[y]		*(33)*	*(31)*	*(28)*	*(16)*

[z] = *Pythium* inoculation treatments took place on December 18, 2008
[y] = least significant difference ($\alpha = 0.05$)

Table 2.4. Number of colony forming units (CFUs) for bareroot loblolly pine after 4, 6, and 12 weeks of storage (4-5°C) in 2009.

		CFUs			
Treatment[z]	Rep	4 wks	6 wks	12 wks	Total
Control	1	0	0	0	
	2	0	0	0	
	3	0	0	0	
	Mean	0	0	0	0
P. dimorphum 50 g	1	0	8	12	
	2	0	0	0	
	3	0	0	1	
	Mean	0	2.6	4.3	2.3
P. dimorphum 100 g	1	0	3	15	
	2	0	4	4	
	3	0	6	13	
	Mean	0	4.3	10.6	5
P. dimorphum 200 g	1	0	0	0	
	2	0	4	6	
	3	0	10	6	
	Mean	0	4.6	4	2.8
P. irregulare 50 g	1	5	5	15	
	2	7	12	0	
	3	7	7	10	
	Mean	6.3	8	8.3	7.5
P. irregulare 100 g	1	9	19	4	
	2	9	15	14	
	3	5	1	13	
	Mean	7.6	11.6	10.3	9.8
P. irregulare 200 g	1	19	13	21	
	2	11	19	14	
	3	5	11	8	
	Mean	11.6	14.3	14.3	13.4
LSD[y]		*(5.0)*	*(9.0)*	*(10.1)*	*(4.3)*

[z] = *Pythium* inoculation treatments took place on December 18, 2008
[y] = least significant difference ($\alpha = 0.05$)

(Table 2.1). *Pythium dimorphum* was not recovered from inoculated seedlings until 6 weeks in storage with an average of 6 CFUs recovered by 12 weeks (Table 2.4). In contrast, *P. irregulare* treatments had an average of 8 CFUs by week 4 and 11 CFUs after 12 weeks (Table 2.4).

2.4.1.3. Slash Pine

Inoculation of bareroot slash pine seedlings with *Pythium* spp. did not affect seedling survival after storage and outplanting (Tables 2.1 and 2.5). However, seedling survival decreased as seedlings remained in storage to 12 weeks (Table 2.1). After 4 weeks, *P. dimorphum* and *P. irregulare*-inoculated seedlings had 72% and 55% survival, respectively, which was similar to non-inoculated seedlings (72%) (Table 2.5). The decrease in survival for seedlings inoculated with *P. dimorphum* was greater than for *P. irregulare* between 4 and 12 weeks of storage (Table 2.5). However, at 12 weeks of storage, seedling survival was similar for all *Pythium* treatments (LSD = 43) (Table 2.5).

Inoculated seedlings had more CFUs of *Pythium* recovered from their roots than non-inoculated seedlings (Table 2.1). Non-inoculated seedlings had 4 *Pythium* isolates recovered at 12 weeks (Table 2.6). Higher levels of *P. irregulare* were recovered from roots than *P. dimorphum* when 100 g or 200 g of inoculum was used (P = 0.0001) (Table 2.6). *Pythium* recovery levels increased with storage length (Table 2.1) with *P. dimorphum*-inoculated slash pine having 4 CFUs compared to 15 CFUs for *P. irregulare*-inoculated seedlings at 4 weeks (Table 2.6). However, *P. dimorphum* numbers increased by 14 CFUs between weeks 4 and 12, which was twice the increase for *P. irregulare* (7 CFUs) (Table 2.6).

Table 2.5. Bareroot slash pine survival for 4, 6, and 12-week stored (4-5°C) seedlings six months after outplanting in 2009.

Treatment[z]	Rep	Survival (%) 4 wks	6 wks	12 wks	Total
Control	1	70	65	35	
	2	75	60	20	
	3	70	30	65	
	Mean	72	52	40	54
P. dimorphum 50 g	1	75	75	50	
	2	95	65	5	
	3	80	40	65	
	Mean	83	60	40	61
P. dimorphum 100 g	1	85	30	50	
	2	100	85	30	
	3	90	65	70	
	Mean	92	60	50	67
P. dimorphum 200 g	1	60	65	40	
	2	20	20	65	
	3	45	50	50	
	Mean	42	45	52	46
P. irregulare 50 g	1	70	25	40	
	2	60	50	65	
	3	30	45	45	
	Mean	53	40	50	48
P. irregulare 100 g	1	70	55	55	
	2	50	50	65	
	3	55	25	40	
	Mean	58	43	53	52
P. irregulare 200 g	1	55	60	55	
	2	35	15	0	
	3	70	50	85	
	Mean	53	42	47	47
LSD[y]		*(25)*	*(36)*	*(43)*	*(19)*

[z] = *Pythium* inoculation treatments took place on December 18, 2008
[y] = least significant difference ($\alpha = 0.05$)

Table 2.6. Number of colony forming units (CFUs) for bareroot slash pine after 4, 6, and 12 weeks of storage (4-5°C) in 2009.

		CFUs			
Treatment[z]	Rep	4 wks	6 wks	12 wks	Total
Control	1	0	0	0	
	2	0	0	0	
	3	0	0	4	
	Mean	0	0	1.3	0.4
P. dimorphum 50 g	1	4	1	14	
	2	3	10	30	
	3	0	11	12	
	Mean	2.3	7.3	18.6	9.4
P. dimorphum 100 g	1	7	7	27	
	2	1	0	19	
	3	0	0	2	
	Mean	2.6	2.3	16	7
P. dimorphum 200 g	1	0	0	14	
	2	10	24	28	
	3	8	1	14	
	Mean	6	8.3	18.6	11
P. irregulare 50 g	1	7	14	19	
	2	6	19	12	
	3	0	14	21	
	Mean	4.3	15.6	17.3	12.4
P. irregulare 100 g	1	22	27	31	
	2	19	20	24	
	3	19	22	25	
	Mean	20	23	26.6	24.3
P. irregulare 200 g	1	25	28	30	
	2	14	12	25	
	3	24	20	17	
	Mean	21	20	24	21.6
LSD[y]		*(7.0)*	*(12.4)*	*(13.2)*	*(4.6)*

[z] = *Pythium* inoculation treatments took place on December 18, 2008
[y] = least significant difference ($\alpha = 0.05$)

2.4.2. Container-grown Seedlings

2.4.2.1. Longleaf Pine

Neither inoculation with *Pythium* spp. nor root wounding of container-grown longleaf pine roots affected the survival of longleaf pine after storage and outplanting (Table 2.7). However, storage length did reduce seedling survival (Table 2.7) as seedling survival for all treatments was 96% at 6 weeks but decreased to 73% at 12 weeks of storage (Table 2.8). Overall, bareroot longleaf pine survival was < 25% for all treatments (Table 2.2), whereas container-grown longleaf pine survival > 85% for all treatments (Table 2.8).

2.4.2.2. Loblolly, Slash, and Shortleaf Pine

Inoculation of seedling roots with *Pythium* did not affect seedling survival (Table 2.9), but storage length and root wounding reduced seedling survival ($P = 0.0311$) (Figure 2.4). Excluding the non-wounded, non-inoculated treatments in the analysis eliminated the variation due to the unbalanced design (no wounded, non-inoculated treatment), but the reduction in seedling survival from storage length and root wounding remained significant ($P = 0.0349$) (Table 2.10).

Seedling survival was 87% for both wounded and non-wounded seedlings at 4 weeks but decreased to 6% at week 12 (Figure 2.4). Container-grown loblolly, slash, and shortleaf pine had 54%, 55%, and 54% survival, respectively, for all treatments (Tables 2.11, 2.12, and 2.13).

Table 2.7. Analysis of variance for container-grown longleaf pine survival in 2008.

		P > F
	DF	Survival (%)
Replication	2	0.0472
Pythium	2	0.6718
Wounding	1	0.8186
Storage	2	0.0001
*Pythium**Wounding	1	0.5673
*Pythium**Storage	4	0.8601
Wounding*Storage	2	0.9867
*Pythium**Wounding*Storage	2	0.4517
Error	28	

Table 2.8. Container-grown longleaf pine survival for 3, 6, and 12-week stored (4-5°C) seedlings six months after outplanting in 2008.

		Survival (%)			
Treatment[z]	Rep	3 wks	6 wks	12 wks	Total
	1	93	100	60	
Control	2	90	97	83	
No Wounding	3	97	97	60	
	Mean	93	98	68	86
	1	97	97	57	
P. dimorphum 200 g	2	90	97	80	
No Wounding	3	93	100	80	
	Mean	93	98	72	88
	1	100	77	43	
P. dimorphum 200 g	2	97	97	90	
Wounding	3	97	100	80	
	Mean	98	91	71	87
	1	97	90	63	
P. irregulare 200 g	2	100	100	97	
No Wounding	3	97	87	63	
	Mean	98	92	74	88
	1	93	100	80	
P. irregulare 200 g	2	97	97	73	
Wounding	3	97	100	80	
	Mean	96	99	78	91
LSD[y]		*(5.4)*	*(9.5)*	*(19.1)*	*(9.2)*

[z] = *Pythium* inoculation treatments took place on December 17, 2007

[y] = least significant difference ($\alpha = 0.05$)

Table 2.9. Analysis of variance (including non-inoculated seedlings) for cold stored (4-5°C) container-grown loblolly, slash, and shortleaf pine survival and *Pythium* colony forming units (CFUs) in 2009.

		P > F	
	DF	Survival (%)	*Pythium* (CFUs)
Replication	2	0.0002	0.2579
Pythium	2	0.2416	0.0001
Wounding	1	0.0917	0.0007
Storage	2	0.0001	0.2239
Pine	2	0.9052	0.0001
*Pythium**Wounding	1	0.8708	0.0135
*Pythium**Storage	4	0.0836	0.4046
*Pythium**Pine	4	0.4804	0.0213
Storage*Pine	4	0.4242	0.6070
Wounding*Storage	2	0.0311	0.4821
Wounding*Pine	2	0.3896	0.2824
*Pythium**Wounding*Storage	2	0.7170	0.3813
*Pythium**Wounding*Pine	2	0.6831	0.0969
Wounding*Storage*Pine	4	0.2046	0.2668
*Pythium**Wounding*Storage*Pine	12	0.4631	0.9676
Error	88		

Table 2.10. Analysis of variance (excluding non-inoculated seedlings) for cold stored (4-5°C) container-grown loblolly, slash, and shortleaf pine survival and *Pythium* colony forming units (CFUs) in 2009.

		P > F	
	DF	Survival (%)	*Pythium* (CFUs)
Replication	2	0.0002	0.2585
Pythium	1	0.1467	0.0001
Wounding	1	0.0967	0.0024
Storage	2	0.0001	0.2246
Pine	2	0.5181	0.0001
*Pythium**Wounding	1	0.8725	0.0271
*Pythium**Storage	2	0.7288	0.2735
*Pythium**Pine	2	0.7679	0.1699
Storage*Pine	4	0.6592	0.6065
Injury*Storage	2	0.0349	0.5572
Injury*Pine	2	0.3999	0.3629
*Pythium**Injury*Storage	2	0.7232	0.4617
*Pythium**Injury*Pine	2	0.6899	0.1540
Injury*Storage*Pine	4	0.3579	0.4114
*Pythium**Injury*Storage*Pine	8	0.2127	0.9068
Error	70		

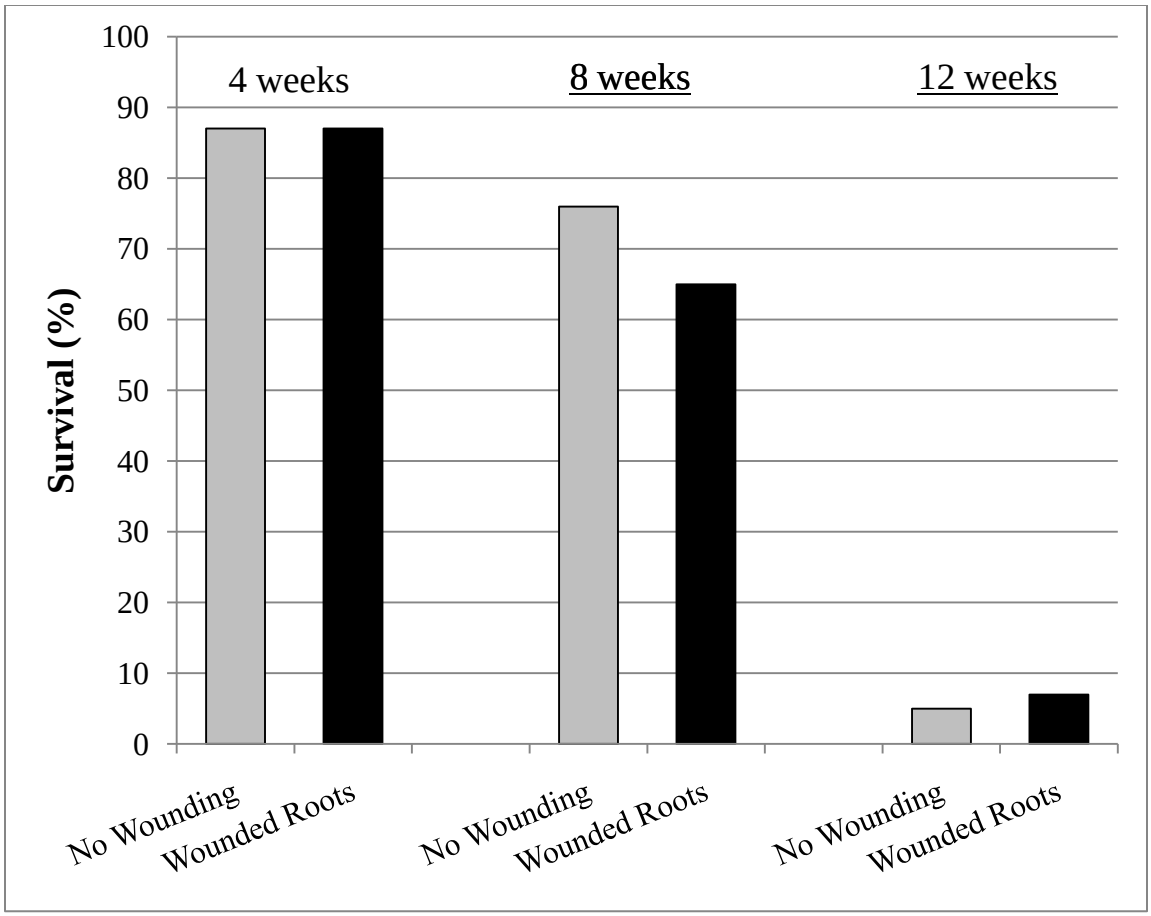

Figure 2.4. Seedling survival for root injury by storage time interaction for container-grown loblolly, slash, and shortleaf pine (P = 0.0311) in 2009.

Table 2.11. Container-grown loblolly pine survival for 4, 6, and 12-week stored (4-5°C) seedlings six months after outplanting in 2009.

Treatment[z]	Rep	Survival (%) 4 wks	6 wks	12 wks	Total
	1	90	45	0	
Control	2	75	80	10	
No Wounding	3	80	90	5	
	Mean	82	72	5	53
	1	90	50	5	
P. dimorphum 200 g	2	100	85	5	
No Wounding	3	90	50	5	
	Mean	93	62	5	53
	1	85	70	0	
P. dimorphum 200 g	2	90	80	5	
Wounding	3	85	45	0	
	Mean	87	65	2	51
	1	85	85	10	
P. irregulare 200 g	2	90	85	10	
No Wounding	3	90	75	10	
	Mean	88	82	10	60
	1	100	65	5	
P. irregulare 200 g	2	95	95	5	
Wounding	3	80	40	10	
	Mean	92	67	7	55
LSD[y]		*(12)*	*(37)*	*(5)*	*(37)*

[z] = *Pythium* inoculation treatments took place on December 19, 2008
[y] = least significant difference ($\alpha = 0.05$)

Table 2.12. Container-grown slash pine survival for 4, 6, and 12-week stored (4-5°C) seedlings six months after outplanting in 2009.

		Survival (%)			
Treatment[z]	Rep	4 wks	6 wks	12 wks	Total
	1	70	70	0	
Control	2	70	75	5	
No Wounding	3	75	70	10	
	Mean	72	72	5	50
	1	80	75	0	
P. dimorphum 200 g	2	95	80	5	
No Wounding	3	85	70	15	
	Mean	87	75	7	56
	1	65	55	10	
P. dimorphum 200 g	2	90	75	10	
Wounding	3	75	80	25	
	Mean	77	70	15	54
	1	90	70	10	
P. irregulare 200 g	2	90	85	0	
No Wounding	3	75	80	5	
	Mean	85	78	5	56
	1	90	65	0	
P. irregulare 200 g	2	95	85	15	
Wounding	3	100	70	5	
	Mean	95	73	7	58
LSD[y]		*(15)*	*(16)*	*(13)*	*(35)*

[z] = *Pythium* inoculation treatments took place on December 19, 2008

[y] = least significant difference ($\alpha = 0.05$)

Table 2.13. Container-grown shortleaf pine survival for 4, 6, and 12-week stored (4-5°C) seedlings six months after outplanting in 2009.

		Survival (%)			
Treatment[z]	Rep	4 wks	6 wks	12 wks	Total
	1	67	87	0	
Control	2	73	93	7	
No Wounding	3	93	80	7	
	Mean	78	87	4	56
	1	53	80	0	
P. dimorphum 200 g	2	87	93	0	
No Wounding	3	93	73	15	
	Mean	78	82	4	55
	1	80	40	7	
P. dimorphum 200 g	2	87	80	0	
Wounding	3	100	53	0	
	Mean	89	58	2	50
	1	80	67	0	
P. irregulare 200 g	2	100	100	0	
No Wounding	3	100	73	7	
	Mean	93	80	2	58
	1	80	47	20	
P. irregulare 200 g	2	80	93	7	
Wounding	3	87	33	0	
	Mean	82	58	9	50
LSD[y]		*(24)*	*(35)*	*(12)*	*(38)*

[z] = *Pythium* inoculation treatments took place on December 19, 2008

[y] = least significant difference ($\alpha = 0.05$)

Container loblolly, slash, and shortleaf pine inoculated with *Pythium* had higher levels of *Pythium* spp. recovered from their roots (Table 2.9) than non-inoculated seedlings (Tables 2.14, 2.15, and 2.16). Higher numbers of *Pythium* were recovered from wounded roots compared to non-wounded roots (Figure 2.5). *Pythium irregulare* was recovered more often than *P. dimorphum* regardless of root wounding (Figure 2.5).

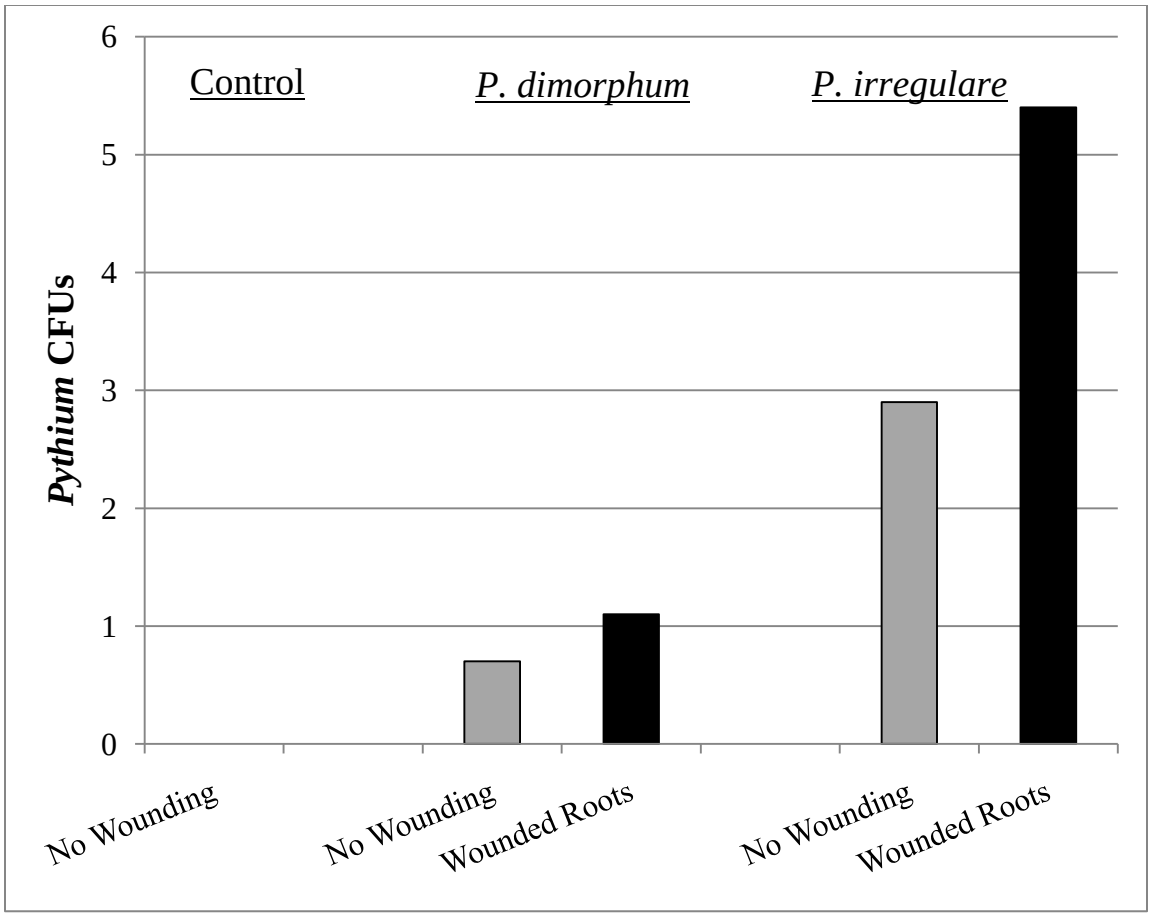

Figure 2.5. *Pythium* colony forming units (CFUs) by root injury interaction for loblolly, slash, and shortleaf pine seedlings (P = 0.0271) in 2009.

Table 2.14. Number of *Pythium* colony forming units (CFUs) for container-grown loblolly pine after 4, 6, and 12 weeks of storage (4-5°C) in 2009.

		CFUs			
Treatment[z]	Rep	4 wks	6 wks	12 wks	Total
	1	0	0	0	
Control	2	0	0	0	
No Wounding	3	0	0	0	
	Mean	0	0	0	0
	1	0	0	0	
P. dimorphum 200 g	2	0	0	0	
No Wounding	3	0	0	0	
	Mean	0	0	0	0
	1	0	0	4	
P. dimorphum 200 g	2	0	0	0	
Wounding	3	0	0	0	
	Mean	0	0	1.3	0.4
	1	0	0	2	
P. irregulare 200 g	2	0	0	0	
No Wounding	3	0	0	0	
	Mean	0	0	0.6	0.2
	1	3	9	3	
P. irregulare 200 g	2	5	1	7	
Wounding	3	0	1	10	
	Mean	2.6	3.6	6.6	4.3
LSD[y]		*(2.0)*	*(3.7)*	*(3.5)*	*(1.7)*

[z] = *Pythium* inoculation treatments took place on December 19, 2008

[y] = least significant difference ($\alpha = 0.05$)

Table 2.15. Number of *Pythium* colony forming units (CFUs) for container-grown slash pine after 4, 6, and 12 weeks of storage (4-5°C) in 2009.

		CFUs			
Treatment[z]	Rep	4 wks	6 wks	12 wks	Total
	1	0	0	0	
Control	2	0	0	0	
No Wounding	3	0	0	0	
	Mean	0	0	0	0
	1	0	0	0	
P. dimorphum 200 g	2	0	0	0	
No Wounding	3	0	1	0	
	Mean	0	0.3	0	0.1
	1	0	0	0	
P. dimorphum 200 g	2	0	0	0	
Wounding	3	0	0	0	
	Mean	0	0	0	0
	1	2	0	3	
P. irregulare 200 g	2	3	5	3	
No Wounding	3	1	4	4	
	Mean	2	3	3.3	2.8
	1	1	1	4	
P. irregulare 200 g	2	1	16	5	
Wounding	3	6	6	11	
	Mean	2.6	7.6	6.6	5.6
LSD[y]		*(2.5)*	*(6.6)*	*(3.1)*	*(2.3)*

[z] = *Pythium* inoculation treatments took place on December 19, 2008
[y] = least significant difference ($\alpha = 0.05$)

Table 2.16. Number of *Pythium* colony forming units (CFUs) for container-grown shortleaf pine after 4, 6, and 12 weeks of storage (4-5°C) in 2009.

		CFUs			
Treatment[z]	Rep	4 wks	6 wks	12 wks	Total
	1	0	0	0	
Control	2	0	0	0	
No Wounding	3	0	0	0	
	Mean	0	0	0	0
	1	0	0	2	
P. dimorphum 200 g	2	7	4	4	
No Wounding	3	0	1	1	
	Mean	2.3	1.6	2.3	2.1
	1	6	5	2	
P. dimorphum 200 g	2	3	2	5	
Wounding	3	1	1	2	
	Mean	3.3	2.6	3	8.9
	1	5	8	7	
P. irregulare 200 g	2	6	3	7	
No Wounding	3	5	3	8	
	Mean	5.3	4.6	7.3	5.7
	1	4	3	5	
P. irregulare 200 g	2	8	12	4	
Wounding	3	6	9	5	
	Mean	6	8	4.6	6.2
LSD[y]		*(4.2)*	*(5.0)*	*(2.0)*	*(2.0)*

[z] = *Pythium* inoculation treatments took place on December 19, 2008

[y] = least significant difference ($\alpha = 0.05$)

2.5. Discussion

2.5.1. Seedling Survival

2.5.1.1. Bareroot Seedlings

Inoculating bareroot longleaf pine seedlings with either *P. dimorphum* or *P. irregulare* before storage reduced seedling survival to < 11% for all inoculum levels. While survival of non-inoculated longleaf pine was only 24%, it was significantly greater than inoculated seedlings at 12 weeks. These studies are the first to show that *P. irregulare* can affect longleaf pine during storage and after outplanting. In 1996, Sun reported a similar decrease in bareroot longleaf pine survival after inoculating seedlings with *P. dimorphum* and 4 weeks of storage. His study is the only one to report that *P. dimorphum* affects longleaf pine. Sun also reported similar seedling survival for stored (95%) and non-stored (96%) longleaf pine which were not inoculated with *Pythium*. For this reason, we did not include a non-inoculated, non-stored treatment in our studies. In hindsight, this data may have assisted in explaining whether poor non-inoculated bareroot longleaf pine survival (24%) was due to either poor outplanting conditions or storage effects.

Slash pine seedling survival was not affected by either *Pythium* spp. used. In contrast, *P. irregulare* reduced loblolly pine survival after outplanting. While both *Pythium* spp. were virulent to longleaf pine, *P. irregulare* was more virulent to loblolly pine than was *P. dimorphum*. *Pythium irregulare* is known primarily as a damping-off pathogen (Hendrix and Campbell 1973) but has been associated to mortality of Mexican weeping pine (*Pinus patula*) seedlings after outplanting in South Africa (Linde et al. 1994) and the decline of loblolly pine stands in Louisiana (Lorio 1966). Our results are

the first to indicate that *P. irregulare* can reduce outplanting survival and that bareroot longleaf pine is more sensitive to the fungus than loblolly and slash pine.

2.5.1.2. Container-grown Seedlings

Neither inoculating seedlings with *Pythium* spp. nor wounding seedling roots before storage affected seedling survival. However, as storage length increased, seedling survival decreased for both wounded and non-wounded loblolly, slash, and shortleaf pine seedlings. Wounding roots was an attempt to simulate injury of bareroot seedling roots as they are lifted. A root wounded, non-inoculated treatment was not included in the trial, which may have indicated the extent only wounded roots would have on seedling survival. By excluding the treatment, existing *Pythium* spores that may have been present on container-grown seedlings were not able to utilize a wound for infection. Non-inoculated (control) bareroot longleaf, loblolly, and slash pine survival was 24%, 38%, and 54%, respectively, for seedlings that may have been wounded during lifting. *Pythium* may have infected the seedlings through wounds and caused the reductions in survival.

Wounding seedling roots affected survival at 6 weeks of storage, and by 12 weeks, wounded and non-wounded seedlings had similar survival (< 8%). Seedling survival declined after each storage period, but the most pronounced reduction in survival occurred after 12 weeks of storage. When seedlings are outplanted out of the traditional planting season (December-February), it is possible that droughty conditions could occur with increases in temperature and cause seedling stress. Wakeley (1954) reported declines in longleaf, loblolly, and slash pine survival (< 40%) after seedlings were

outplanted in March. In our study, reductions in survival after 12 weeks was not due to *Pythium* inoculation (longleaf, P = 0.6718 and loblolly, slash, and shortleaf, P = 0.2416) but more likely from adverse outplanting conditions.

2.5.2. *Pythium* Populations

2.5.2.1. Bareroot Seedlings

More *P. irregulare* CFUs than *P. dimorphum* were recovered from inoculated seedling roots. The amount of *P. irregulare* increased as the level of inoculum and time in storage increased. The higher number of CFUs may explain the lower loblolly and slash pine survival of *P. irregulare*-inoculated seedlings. The recovery of *P. dimorphum* and *P. irregulare* from loblolly and slash pine roots indicates that the pathogen can persist on bareroot seedlings placed in cold storage. As the level of inoculum increased, more fungal spores were recovered. The cool, moist conditions in storage may have provided an environment conducive for fungal growth. In other studies, *Pythium* was recovered > 33% more from longleaf pine roots that did not receive benomyl or metalaxyl treatment prior to storage (Jones et al. 1992). Their data suggests the fungicide controlled the soilborne pathogen that may have been present on the roots. In our study, only 4 *Pythium* CFUs were recovered from non-treated seedlings (bareroot slash pine).

It is not surprising that *Pythium* spp. was not recovered from non-inoculated seedlings. Sun (1996) did not recover *Pythium* from non-inoculated longleaf pine after 4 weeks of storage, whereas Jones and others (1992) recovered *Pythium* spp. from non-inoculated longleaf pine seedlings. The distribution of soilborne fungi is dependent upon many factors, which include species aggressiveness and motility, physical and biological

factors, environmental influences, and interactions among these factors (Campbell and Noe 1985). In response to the environment, *Pythium* can become more active around root exudates or areas of high moisture (Hendrix and Campbell 1973). In addition, *Pythium* can live as a saprophyte for several years in areas of the nursery where seedlings do not exhibit disease symptoms. Thus, it is entirely possible that the fungus may not have been present in the area of the nursery from which non-inoculated seedlings were lifted. For instance, soil surveys conducted in bareroot nurseries have revealed three times the number of *Pythium* CFUs recovered during November than in January (Chapter 5).

2.5.2.2. Container-grown Seedlings

Pythium spp. was recovered more often from seedlings with wounded roots than non-wounded roots. *Pythium irregulare* grew in higher numbers on wounded loblolly and slash pine roots (4.3 and 5.6 CFUs, respectively) compared to *P. dimorphum* (0.4 and 0 CFUs, respectively). In contrast, *P. dimorphum* recovery occurred more on wounded shortleaf pine roots (8.9 CFUs) compared to *P. irregulare* (6.2 CFUs). *Pythium* shares an electrostatic relationship with seed and roots, where ions and cations exchanges (exudates) serve as nutrients for spore germination (Hendrix and Campbell 1973). When roots are wounded, root exudates may become available for the pathogen's growth. For example, exudates from red pine (*Pinus resinosa*) seed increased spore germination of *P. irregulare* (Agnihotri and Vaartaja 1970). In addition, *P. irregulare* responds to temperatures over moisture (Biesbrock and Hendrix 1970). Temperatures in storage were < 5ºC and seedlings were outplanted into sand, which has a lower water holding capacity.

It is possible that roots exposed to the low temperatures in storage and moisture after outplanting caused *P. irregulare* to become more active.

2.5.3. Summary

Inoculation of pine seedlings with *P. dimorphum* and *P. irregulare* before storage reduced bareroot longleaf pine survival. Bareroot slash and container-grown longleaf, loblolly, slash, and shortleaf pine were unaffected by *Pythium* inoculations. However, as storage time increased, seedling survival decreased. Wounding container-grown seedling roots reduced seedling survival after 6 weeks, but seedling survival was similar to non-wounded seedlings at 12 weeks. *Pythium* CFUs were recovered more often from wounded roots, and *P. irregulare* growth was more prolific than *P. dimorphum*. Fungal growth of each species may have been stimulated from increases in root exudates. The null hypothesis that inoculating loblolly, longleaf, slash, and shortleaf pine seedlings with *Pythium* prior to storage does not affect seedling survival after outplanting can only be rejected based on the bareroot longleaf pine seedling survival.

In the future, experiments that incorporate mycorrhizae (symbiont) and *Pythium* (pathogen) treatments may indicate the behavior of each fungus in cold storage and the effect on seedling survival. Also, studies using fungicide applications to seedlings before lifting may yield insight to the dynamics of *Pythium* distribution in the soil and the utilization of root injuries.

2.6. Acknowledgements

The author would like to thank Doug Shelburne at Smurfit-Stone's Rock Creek Nursery in Brewton, AL and Wayne Bell and Mike Croyle at International Forest Company in Moultrie, GA for providing the seedlings used in the experiments. Special thanks go out to Tommy Hill, Marietjie Quicke, Barry Brooks, and Rena Miller for assistance with outplanting seedlings.

2.7. Literature Cited

Agnihotri, V.P. and O. Vaartaja. 1970. Effect of seed exudates of *Pinus resinosa* on the germination of sporangia and on the population of *Pythium irregulare* in the soil. Plant Soil 32: 246-249.

Barden, C.J. and P.P. Feret. 1986. Root growth potential of loblolly pine seedlings held in cold storage under differing moisture treatments. In: Schroeder, R.A., ed., Proc. of Southern Forest Nursery Association Conference, July 22-24, 1986, Pensacola, FL: 218-223.

Barnett, J.P., J.C. Brissette, A.G. Kais, and J.P. Jones. 1988. Improving field performance of southern pine seedlings by treating with fungicides before storage. Southern Journal of Applied Forestry 12(4): 281-285.

Biesbrock, J.A. and F.F. Hendrix. 1970. Influence of soil water and temperature on root necrosis of peach caused by *Pythium* spp. Phytopathology 60: 880-882.

Borja, I. 1995. Root dieback and defense responses in Norway spruce seedlings infected with the fungal pathogen, *Pythium dimorphum*. PhD Dissertation, Agricultural University of Norway: 126 p.

Boyer, J.N. and D.B. South. 1985. Dormancy, chilling requirements, and storability of container-grown loblolly pine seedlings. Auburn University, Southern Forest Nursery Management Cooperative Research Note No. 23: 12p.

Campbell, C.L. and J.P. Noe. 1985. The spatial analysis of soilborne pathogens and root diseases. Annual Review of Phytopathology 23: 129-148.

Carlson, W.C. 1991. Lifting, storing, and transporting southern pine seedlings. Chapter 16, Forest Regeneration Manual, M.L. Duryea and P.M. Dougherty, eds., Kluwer Academic Publishers, Norwell, MA. 433 pp.

Dierauf, T.A. 1976. Fall planting and storage using non-dormant seedlings. In: Proc. Southeastern Area Nurseryman's Conference-SC Eastern: 31-36.

Feng, Y. and P.H. Dernoeden. 1999. *Pythium* species associated with root dysfunction of creeping bentgrass in Maryland. Plant Disease 83: 516-520.

Garber, M.P. and J.G. Mexal. 1980. Lift and storage practices: their impact on successful establishment of southern pine plantations. New Zealand Journal of Forest Science 10(1): 72-82.

Hallgren, S.W. and D.M. Ferris. 1995. Benomyl applied to roots improves second-year survival and growth of shortleaf pine. Southern Journal of Applied Forestry 19(1): 36-41.

Hendrix, F.F., Jr. and W.A. Campbell. 1973. *Pythiums* as plant pathogens. Annual Review of Phytopathology 11: 77-98.

Jones, J.P., D.J. Pantone, J.P. Barnett, and J.C. Brissette. 1992. The relationship between fungal population development and root damage of cold-stored longleaf pine seedlings. Louisiana State University Agricultural Center, Bulletin No. 832: 23p.

Kahler, L.H. and A.R. Gilmore. 1961. Field survival of cold stored loblolly pine seedlings. Tree Planters' Notes, Issue 45: 15-16.

Kelley, W.D. and S.W. Oak. 1989. Forest Nursery Pests. In: Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, Jr., R.S., and Harvey, T.V, Tech. Coords., USDA Forest Service, Agricultural Handbook No. 680: 184 pp.

Kim, Y.K., C.L. Xiao, and J.D. Rogers. 2005. Influence of culture media and environmental factors on mycelia growth and pycnidial production of *Sphaeropsis pyriputrescens*. Mycologia 97(1): 25-32.

Linde, C., G.H.J. Kemp, and M.J. Wingfield. 1994. *Pythium irregulare* associated with *Pinus* seedling death on previously cultivated lands. Plant Disease 78: 1002-1005.

Lorio, P.L. 1966. *Phytophthora cinnamomi* and *Pythium* species associated with loblolly pine decline in Louisiana. Plant Disease Reporter 50: 596-597.

May, J.T. 1984. Southern Pine Nursery Handbook. U.S. Department of Agriculture, Forest Service, Southern Region.

Stumpff, N.J. and D.B. South. 1991. Benomyl root dips adversely affect first-year performance of stored loblolly pine seedlings. Southern Journal of Applied Forestry 15(3) 133-137.

Sun, X. 1996. Biological control of Pythiaceous fungi causing death of longleaf pine seedlings during cold storage. PhD dissertation, Louisiana State University: 135 pp.

Venator, C.R. 1984. Evidence of the presence of a lifting window for loblolly pine nursery seedlings. In Proc: Southern Nursery Conference-Eastern Session, Asheville, NC: 184-190.

Wakeley, P.C. 1954. Planting the southern pines. USDA Agricultural Monograph No.18: 233 p.

CHAPTER 3

EFFECTS OF *PYTHIUM* AND PEAT MOSS ON LOBLOLLY, LONGLEAF, SLASH, AND SHORTLEAF PINE SURVIVAL AFTER COLD STORAGE

3.1. Abstract

Cold storing bareroot pine (*Pinus* spp.) seedlings for as little as one week in November to mid-December has been shown to reduce seedling survival. In contrast, container-grown seedling survival is not affected when stored in November and December for longer durations. As bareroot seedlings are lifted from nursery beds, root systems can become damaged and wounded. *Pythium* spp. commonly found in nursery soils may utilize the wounds sustained during the lifting process and infect seedling roots during storage. The cool, moist environment in cold storage may be conducive for *Pythium* growth and result in poor seedling survival after outplanting. Container-grown seedling roots are grown within a plug of peat-based media and are more protected from wounding during extraction. The absence of wounded roots, and the presence of peat moss, may be why container-grown seedlings have increased storability and survival over bareroot seedlings.

To determine the effect that peat moss and root wounding had on seedling survival in cold storage, a number of experiments were conducted using bareroot and

container-grown seedlings. Bareroot loblolly pine (*Pinus taeda*) seedlings were inoculated with either *P. dimorphum* or *P. irregulare*, cold stored for either 0, 4, 8, or 12 weeks with or without peat moss, and monitored for survival. Peat moss did not increase seedling survival when bareroot seedlings were inoculated with *Pythium*, and more *Pythium* was recovered from seedling roots stored in peat moss than without. *Pythium irregulare* reduced survival of longleaf (*Pinus palustris*) and shortleaf pine (*Pinus echinata*) grown in peat moss and perlite, respectively, while wounding seedling roots decreased longleaf and slash (*Pinus elliottii*) pine survival. Peat moss was not effective at increasing survival or reducing *Pythium* populations when seedlings were inoculated with *Pythium* prior to storage. Wounding roots increased seedling mortality but only when seedlings were grown in peat moss.

3.2. Introduction

Prior to being shipped for outplanting, pine (*Pinus* spp.) seedlings are commonly held in cold storage after lifting from nursery beds. Occasionally, long-term (> 1 week) cold storage of bareroot seedlings lifted from October to mid-December results in poor outplanting survival (Kahler and Gilmore 1961; Dierauf 1976; Hebb 1982; Venator 1984). In contrast, container-grown seedling survival is not affected when stored in November and December for similar durations (Pickens and Robbins, unpublished data).

At lifting, bareroot and container-grown seedling root systems are treated differently, which may be one reason for the differences in seedling storability and outplanting survival observed between the two stock types. The greatest difference is that bareroot seedling roots are wounded during lifting by the process itself or if soils are

saturated or frozen (May 1984). The wounded roots then serve as infection sites for soilborne fungi, particularly the "water molds". *Pythium* is commonly found in nursery soils and is known for causing "damping-off" of seed and young germinants or "feeder root disease", which affects roots that are important for nutrient and water absorption (Kelley and Oak 1989). After infected seedlings are placed in storage, the cool temperatures (1 to 5°C) and moist conditions may provide an ideal environment for *Pythium* to multiply. Studies have shown that *Pythium* can be recovered from cold stored bareroot longleaf pine seedlings with populations increasing with storage time (Barnett et al. 1988; Jones et al. 1992; Sun 1996). To determine the role *Pythium* plays, experiments that applied the fungicides benomyl and metalaxyl alone or in combination to seedling roots increased seedling. survival when compared to seedlings that did not receive fungicides (Barnett et al. 1988; Jones et al. 1992). Their studies suggest that adding fungicides to seedling roots prior to storage, seedling survival can improve, perhaps by controlling detrimental fungi like *Pythium*.

In contrast to bareroot culture, container-grown seedlings are hand-lifted, and root systems are protected within a plug of peat moss-based media, which lessens the chance of root wounding and exposure to *Pythium* in the soil. However, *Pythium* can find its way into container seedling root plugs. *Pythium* is often introduced into container media by sowing contaminated seed (Dumroese et al. 1988), through irrigation (Landis 1989), and from substrate residue remaining on containers after use (Dumroese et al. 2002). However, *Pythium* can remain dormant until saturated or poorly drained cavities cause spore activity (Landis 1989).

As far as disease control, peat moss has been shown to contain organisms and/or offer properties that are capable of suppressing pathogenic fungi. For one study, a light-colored Swedish sphagnum peat was the most effective at suppressing *Pythium* spp., but suppressiveness was not evident when the peat was either heated above 60ºC or treated with benomyl (Wolffhechel 1988). The sterilization of the media implied that the suppressiveness was tied to antagonistic fungi present in peat moss. In another study, the specific fungi involved with suppression of *Pythium ultimum* Drechsler was with *Trichoderma* spp. in cucumber (*Cucumis sativas*) (Thrane et al. 2000) and tomato (*Lycopersicon esculentum*) (Gravel et al. 2006). In addition, other organisms in the genera *Cryptococcus*, *Rhodotorula*, and *Bullera* have been recovered from peat moss and shown to suppress *Pythium sylvaticum* Campbell and Hendrix (Hunter et al. 2006). Supressiveness has also been observed in nurseries. Ten weeks after inoculation with *Pythium vexans* de Bary, container-grown Fraser-fir (*Abies fraseri*) exhibited no disease symptoms, however, the pathogen was recovered from asymptomatic seedlings after extraction (Ivors et al. 2008). Therefore, it is possible for *Pythium* to persist within a container seedling root plug and not cause disease symptoms.

The lack of wounded roots and the presence of peat moss may be two reasons seedlings grown in containers have increased storability and subsequent survival over bareroot seedlings stored at the same time. To determine the effects that peat moss and/or wounding may play in seedling survival, two separate experiments were conducted to test the null hypotheses that: 1) packing bareroot loblolly pine (*Pinus taeda*) seedling roots in a peat-mix and inoculation with *Pythium* will not affect seedling survival after outplanting and 2) container media has no effect on loblolly, longleaf

(*Pinus palustris*), slash (*Pinus elliottii*), and shortleaf (*Pinus echinata*) pine seedling survival when inoculated with *Pythium*.

3.3. Materials and Methods

3.3.1. Packing and Storing Bareroot Seedlings in Peat-Mix

3.3.1.1. *Pythium* inoculum

Pythium dimorphum Hendrix and Campbell and *Pythium irregulare* Buisman were obtained from American Type Culture Collection (ATCC®, Manassas, VA) and were aseptically transferred to oatmeal agar (Kim et al. 2005). From the advancing margin of the mycelium, three 0.5 cm disks of each *Pythium* spp. were transferred to oatmeal agar to use as inoculum. Prior to inoculation, 1,190 g of oatmeal and 400 ml of distilled water were combined in two autoclavable bags, mixed thoroughly, and autoclaved. The sterilized oats were allowed to cool for 24 h and one autoclaved bag received five agar plates of *P. dimorphum* while the other bag received five agar plates of *P. irregulare*. The oatmeal-*Pythium* inoculum was mixed every 12 h and stored at room temperature for 11 days prior to seedling root inoculations (Figure 3.1).

3.3.1.2. Seedling Inoculations

Seven-month-old bareroot loblolly pine seedlings were obtained from Rayonier's Glennville Regeneration Center in Glennville, GA on October 31, 2008. Prior to inoculations, the seedlings remained in cold storage (4-5°C) for 3 days. Inoculation of seedling roots involved dipping seedlings into a bucket filled with 11 liters of water (controls) or water plus 200 g of oatmeal/*Pythium* inoculum (Figure 3.1). Buckets were

emptied, rinsed, and filled with a new oatmeal inoculum mixture after each seedling replication was inoculated. To simulate a container system, seedling roots were either packed with or without a peat-mix. Seedlings were placed on parchment paper to which 3,785 cm^3 of peat-mix was added (Figure 3.1), and seedlings were then rolled in the parchment paper while seedling tops remained exposed (Figure 3.1). The peat-mix was a greenhouse potting media (SunGro Sunshine Mix #8 Professional Growing Mix™), that consisted of 70-80% Canadian sphagnum peat moss, coarse grade perlite, vermiculite, dolomitic limestone, gypsum, and a wetting agent. The experiment consisted of six seedling treatments: *P. dimorphum* oatmeal inoculum with or without peat moss and *P. irregulare* oatmeal inoculum with or without peat moss. Control seedlings received no *Pythium* and were stored with or without peat-mix. Four replications of each seedling treatment were used (30 seedlings/rep), and each bundle (replication) was placed in a 49 liter plastic bag and put in cold storage (4-5ºC) for either 4, 8, or 12 weeks.

Figure 3.1. A. *Pythium* oatmeal inoculum. B. Inoculating loblolly pine seedlings in the *Pythium* oatmeal water mixture. C. Promix spread onto parchment paper. D. Loblolly pine seedlings being packed in a peat moss bundle.

3.3.1.3. Seedling Survival

To control weed competition prior to outplanting, the herbicides Arsenal® (imazapyr) and Oust® (sulfometuron-methyl) were applied at rates of 107 g ai/ha (active ingredient per hectare) and 112 g ai/ha, respectively, on a sandy loam site near Auburn University. After each storage period, seedlings were outplanted in a completely randomized block design at 0.3 X 0.3 m spacing (Figure 3.2). Each block represented 17 m^2 of planting area with 2,160 loblolly pine seedlings outplanted. Seedling survival was monitored for 4 months, and a seedling was considered dead when foliage was no longer green.

3.3.1.4. *Pythium* populations

To quantify *Pythium* populations, roots from three random seedlings from each replication/treatment were assayed after each storage period. After rinsing excess soil from the roots, both lateral and fine feeder roots (to about 5 cm from the taproot) were removed. Sub-samples were surface sterilized in a 10% bleach solution and rinsed two times in sterile, distilled water. After sterilization, twenty 2.5-cm root segments were randomly chosen and placed onto *Pythium* selective media (Feng and Dernoeden 1999). Each loblolly pine replication had 50 cm of roots on two plates (ten 2.5-cm root pieces/plate). *Pythium* populations were determined by counting the number of colony forming units (CFUs) on the selective media.

Figure 3.2. The outplanting site near Auburn University.

3.3.2. Container Seedlings Grown in Peat-Mix vs. Perlite

3.3.2.1. Seedling Production

Loblolly, slash, and shortleaf pine seed were obtained from the Georgia Forestry Commission in Macon, GA; longleaf pine seed from International Forest Company in Moultrie, GA and placed in a freezer for storage. Before sowing, seed were placed in a container of aerated water at room temperature for 24 h, removed, patted dry, and placed in plastic bags for stratification at 4-5°C for either 43 days (loblolly, slash, and shortleaf pine) or 10 days (longleaf pine). In a greenhouse at Auburn University, the stratified seed were sown into Ray Leach "Cone-tainer"™ stubby cells (volume of 115 cm^3, diameter of 4 cm, and depth of 14 cm) on April 2, 2009. Cells were placed in trays with a capacity to hold 98 cells (528 seedlings/m^2) that were filled with either a standard greenhouse potting media (SunGro Sunshine Mix #8 Professional Growing Mix™) or a 100% perlite (Sunshine Coarse Premium Grade Perlite™). Beginning on April 28, all seedlings were fertilized with Miracle Gro® (azalea/camellia/rhododendron) 30-10-10 (30N:4P:8K) water soluble fertilizer. Seedlings in perlite received weekly applications of water (pH = 2.0-3.0) acidified with phosphoric acid to lower the neutral pH (pH =7.0).

3.3.2.2. *Pythium* Inoculum

Pythium dimorphum Hendrix and Campbell and *Pythium irregulare* Buisman were transferred to oatmeal agar and used as inoculum. Six-hundred ml of autoclaved distilled water was poured into a blender and one plate of *P. dimorphum* was added and allowed to blend for one min (Figure 3.3). This process was repeated four times to

produce a total of 3,000 ml of *P. dimorphum*/water inoculum slurry. The same steps were used to make 3,000 ml of *P. irregulare*/water inoculum slurry.

3.3.2.3. Container-grown Seedling Inoculations

To determine the effect of media on seedling survival, loblolly, longleaf, slash, and shortleaf pine seedlings grown in either peat moss or perlite received one of six treatments (Table 3.1). Prior to inoculations, 4-month-old seedlings were top-pruned to a height of 15 cm. To minimize cross contamination of *Pythium* species, seedlings of each pine species were arranged in trays that contained *P. dimorphum*, *P. irregulare*, or controls (Figure 3.3). Each tray was then placed in a modified 40 gallon plastic bag that had the bottom cut open and 12 slits (5 cm) along the length to allow ventilation. Seedlings were placed in a cooler (4-5ºC) for either 0 (remained in greenhouse), 4, or 8 weeks. Five replications (trays) of each *Pythium* treatment (15 total) were represented for each storage period. In all, 900 each of loblolly and slash pine, 796 longleaf pine, and 727 shortleaf pine seedlings were used in the experiment.

3.3.2.4. Seedling Survival

After each storage period, seedlings were returned to the greenhouse, placed in a completely randomized block design, and survival was monitored for 4 months.

Table 3.1. Inoculation treatments for loblolly, longleaf, slash, and shortleaf pine grown in both peat moss and perlite media.

Treatment #	Treatment
1	2 mL *P. dimorphum* + root wounding
2	2 mL *P. dimorphum* without root wounding
3	2 mL *P. irregulare* + root wounding
4	2 mL *P. irregulare* without root wounding
5	No *Pythium* + root wounding
6	No *Pythium* without root wounding

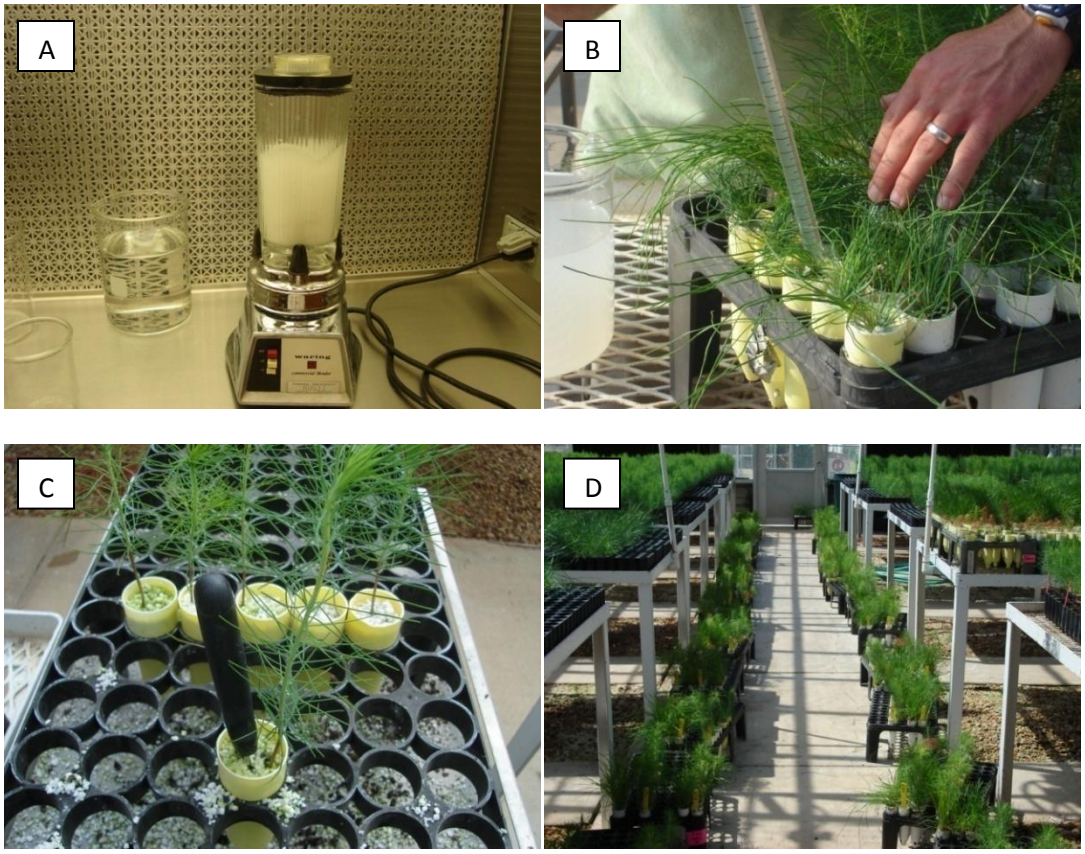

Figure 3.3. A. *Pythium*/water inoculum slurry in a blender. B. Inoculating seedlings with the *Pythium*/water inoculum slurry. C. Wounding seedling roots with a knife. D. Arranging seedlings into trays that were put into cold storage.

3.3.3. Statistical analyses

Analyses were conducted using a General Linear Model (GLM) in SAS statistical software (9th ed., SAS Institute, Cary, NC). An experimental unit consisted of 30 loblolly pine seedlings (Experiment 1) or 5 loblolly and slash pine, 4 or 5 longleaf pine, and 2, 3, or 4 shortleaf pine seedlings (Experiment 2). Means of each experimental unit for each dependant variable were analyzed using Analysis of Variance (ANOVA) in a factorial design ($\alpha = 0.05$). Data for each pine species were analyzed separately, and contrast analyses were performed using combined levels of each *Pythium* treatment.

3.4. Results

3.4.1. Packing and Storing Bareroot Seedlings in Peat-mix

The presence of peat-mix on *Pythium*-inoculated seedlings did not increase seedling survival (Table 3.2). However, bareroot loblolly pine roots challenged with either *P. dimorphum* or *P. irregulare* had less seedling survival (P = 0.0154), and was less when stored in peat-mix (16%) than without peat-mix (27%) (Table 3.3). The presence of *Pythium* reduced loblolly pine survival (20-23%) over non-inoculated controls (32%) (Table 3.3). Time in storage also affected seedling survival as survival at 4 weeks was 46% and 7% at 12 weeks for all 6 treatments (P = 0.0001) (Table 3.3). Although the interaction was not significant (P = 0.2933), as storage time increased, the survival of seedlings inoculated with *Pythium* and stored in peat-mix decreased. Overall, survival was 29%, 15%, and 4% at 4, 8, and 12 weeks, respectively, when compared to non-inoculated seedlings stored in peat-mix, 59%, 33%, and 9%, respectively (Table 3.3).

The presence of peat-mix affected the number of *Pythium* CFUs recovered from seedling roots and was dependent upon the treatment (P = 0.0019) (Table 3.2). Overall, higher levels of *Pythium* were recovered from loblolly pine stored in peat-mix than without peat-mix. Between the *Pythium* species used, more *P. dimorphum* CFUs were recovered from seedlings stored in peat-mix (Table 3.4). As storage time increased for seedlings without peat-mix, fewer *Pythium* CFUs were recovered (Table 3.4). After 12 weeks, seedlings stored in peat-mix had 15 CFUs compared to 12 CFUs for those stored without peat-mix, while non-inoculated (control) seedlings with or without peat-mix had < 1 CFU (Table 3.4).

Table 3.2. Analysis of variance for loblolly pine survival and *Pythium* colony forming units (CFUs).

		P > F	
	DF	Survival (%)	*Pythium* (CFUs)
Replication	3	0.2792	0.1063
Pythium	2	0.0154	0.0001
Storage	2	0.0001	0.0055
Media	1	0.0953	0.0001
*Pythium**Media	2	0.1013	0.0007
*Pythium**Storage	4	0.4254	0.0567
Storage*Media	2	0.0509	0.1481
*Pythium**Media*Storage	4	0.2933	0.0019
Control vs. *P. dimorphum*	(1)	0.0050	0.0001
Control vs. *P. irregulare*	(1)	0.0455	0.0001
P. dimorphum vs. *P. irregulare*	(1)	0.3800	0.8617
Error		51	123

Table 3.3. Loblolly pine survival for 4, 8, and 12-week stored (4-5°C) seedlings four months after outplanting.

Treatment[z]	Rep	Survival (%) 4 wks	8 wks	12 wks	Total
	1	20	20	30	
Control	2	23	17	7	
No peat moss	3	83	40	3	
	4	60	30	17	
	Mean	47	27	14	29
	1	67	33	10	
Control	2	43	23	11	
Peat moss	3	70	50	10	
	4	57	27	3	
	Mean	59	33	9	34
	1	43	13	7	
P. dimorphum 200 g	2	23	23	7	
No peat moss	3	87	7	7	
	4	60	13	17	
	Mean	53	14	10	26
	1	10	10	3	
P. dimorphum 200 g	2	25	7	7	
Peat moss	3	33	13	7	
	4	27	13	0	
	Mean	24	11	4	13
	1	43	43	3	
P. irregulare 200 g	2	73	3	7	
No peat moss	3	40	7	3	
	4	90	17	0	
	Mean	62	18	3	28
	1	37	27	0	
P. irregulare 200 g	2	33	17	7	
Peat moss	3	47	17	0	
	4	13	10	7	
	Mean	33	18	4	18
LSD[y]		*(22)*	*(11)*	*(5)*	*(8)*

[z] = *Pythium* inoculation treatments took place on November 3, 2008
[y] = least significant difference ($\alpha = 0.05$)

Table 3.4. Number of colony forming units (CFUs) from loblolly pine roots after 4, 8, and 12 weeks of storage (4-5°C).

Treatment[z]	Rep	*Pythium* (CFUs) 4 wks	8 wks	12 wks	Total
	1	0.5	0	0	
Control	2	1	0	0	
No peat moss	3	0	0	0	
	4	0	0	0	
	Mean	0.4	0	0	0.1
	1	0.5	1	0	
Control	2	0	0	0	
Peat moss	3	0.5	1.5	0.5	
	4	2	0	0.5	
	Mean	0.8	0.6	0.3	0.6
	1	8.5	12	9	
P. dimorphum 200 g	2	16.5	7.5	8	
No peat moss	3	21.5	14.5	7	
	4	9.5	8	5.5	
	Mean	14	10.5	7.4	10.6
	1	13	15.5	19.5	
P. dimorphum 200 g	2	16.5	23.5	15	
Peat moss	3	10	16.5	19	
	4	15	14	19	
	Mean	13.6	17.4	18.1	16.4
	1	16	19	11	
P. irregulare 200 g	2	14	5.5	9.5	
No peat moss	3	13	21.5	12.5	
	4	10.5	11.5	7.5	
	Mean	13.4	14.4	10.1	12.6
	1	16.5	16.5	10	
P. irregulare 200 g	2	15.5	14.5	16.5	
Peat moss	3	19	14.5	7	
	4	14.5	17	8	
	Mean	16.4	15.6	10.4	14.1
LSD[y]		*(2.4)*	*(1.7)*	*(2.0)*	*(1.4)*

[z] = *Pythium* inoculation treatments took place on November 3, 2008

[y] = least significant difference ($\alpha = 0.05$)

3.4.2. Container Seedlings Grown in Peat-mix vs. Perlite

3.4.2.1. Loblolly Pine

Wounding roots on seedlings grown in peat-mix did not affect seedling survival (Table 3.5). Survival for seedlings grown in peat-mix challenged with *Pythium* was 95% and 93% for non-stored and 8-week stored seedlings, respectively, whereas survival was 100% and 95%, respectively, for those seedlings grown in perlite (Table 3.6). Storage length reduced seedling survival (P = 0.0158) (Table 3.5) with seedlings in perlite and peat-mix at 99% at 4 weeks and 95% at 8 weeks (Table 3.6). Survival of non-inoculated loblolly pine was 96% at 8 weeks for seedlings grown in either peat-mix or perlite (Table 3.6).

3.4.2.2. Longleaf Pine

Peat-mix as a growing media did not increase seedling survival when challenged with *P. irregulare* prior to storage (P = 0.027) (Table 3.5). Survival of *P. irregulare*-inoculated seedlings in peat-mix was 62% compared to 82% for non-inoculated seedlings after 8 weeks (Table 3.7). Survival of non-stored *Pythium*-inoculated longleaf pine grown in peat-mix was 78% and was 98% and 76% at 4 and 8 weeks of storage, respectively (Table 3.7). Survival of non-inoculated seedlings grown in peat-mix was 96% at 4 weeks and 82% at 8 weeks (Table 3.7). Inoculated seedlings grown in perlite had 95%, 90%, and 94% survival after 0, 4, and 8 weeks of storage, respectively. Non-inoculated seedling survival was 100%, 90%, and 95% for the same storage periods (Table 3.7).

Wounding seedling roots reduced longleaf pine seedling survival in the presence of *Pythium*. Survival of wound-inoculated longleaf pine grown in peat-mix was 74% and 68% when stored for 0 and 8 weeks, respectively, compared to 92% and 78% for wounded non-inoculated longleaf pine at the same storage periods (Table 3.7). Survival of wounded *Pythium*-inoculated seedlings grown in perlite was 95% for 0 and 8-week stored seedlings, respectively, whereas wounded non-inoculated seedling survival was 100% and 90% in perlite for the same storage periods (Table 3.7).

3.4.2.3. Slash Pine

Peat-mix had no affect on slash pine survival after seedlings were inoculated with *Pythium*. However, wounding seedling roots did reduce slash pine survival after storage ($P = 0.0375$) (Table 3.5). Survival of 0 and 8-week stored wounded seedlings grown in peat-mix were both 89% compared to 96% and 92% for wounded seedlings grown in perlite at the same storage periods (Table 3.8). Overall, wounding seedling roots grown in peat-mix and perlite reduced survival to 93% compared to 96% for non-wounded roots (Table 3.8).

3.4.2.4. Shortleaf Pine

Pythium irregulare reduced shortleaf pine survival ($P = 0.0482$) (Table 3.9). However, peat-mix was effective at increasing shortleaf pine survival over those grown in perlite as storage time increased ($P = 0.0001$) (Table 3.5). *Pythium*-inoculated seedling survival was 86% and 72% at 4 and 8 weeks, respectively, when grown in peat-mix while shortleaf pine survival in perlite was 64% and 38%, respectively (Table 3.9). Survival of

non-inoculated shortleaf pine grown in peat-mix was 83% at 4 weeks and 90% at 8 weeks, while survival of non-inoculated seedlings in perlite was 63% and 58% at the same storage periods, respectively (Table 3.9).

Table 3.5. Analysis of variance for container-grown loblolly, longleaf, slash, and shortleaf pine survival.

		P > F			
		Survival (%)			
	DF	Loblolly	Longleaf	Slash	Shortleaf
Replication	4	0.8949	0.1413	0.3916	0.2567
Pythium	2	0.4091	0.0804	0.8545	0.0625
Wound	1	0.0752	0.0457	0.0375	0.3834
Media	1	0.1538	0.0007	0.7647	0.0001
Storage	2	0.0158	0.0103	0.1847	0.0001
*Pythium**Wound	2	0.4091	0.7759	0.6531	0.0976
*Pythium**Media	2	0.8795	0.1130	0.7469	0.6220
*Pythium**Storage	4	0.1979	0.0135	0.5068	0.1188
Wound*Media	1	0.1538	0.7373	0.7647	0.7220
Wound*Storage	2	0.5446	0.9528	0.3135	0.9480
Media*Storage	2	0.2108	0.0001	0.1093	0.0001
*Pythium**Media*Storage	4	0.4385	0.0003	0.7887	0.8529
*Pythium**Wound*Media	2	0.8795	0.8555	0.5712	0.4591
Wound*Media*Storage	2	0.0570	0.2141	0.1847	0.9630
*Pythium**Wound*Media*Storage	8	0.7271	0.8407	0.3654	0.7962
Control vs *P. dimorphum*	(1)	0.1900	0.1519	0.7139	0.0794
Control vs *P. irregulare*	(1)	0.6613	0.0270	0.5825	0.0482
P. dimorphum vs *P. irregulare*	(1)	0.3814	0.4286	0.8545	0.8195
Error		140	140	140	138

Table 3.6. Loblolly pine survival for 0, 4, and 8-week stored seedlings after four months in a greenhouse.

		Survival (%)							
		Peat-mix				Perlite			
Treatment[z]	Rep	0 wks	4 wks	8 wks	All	0 wks	4 wks	8 wks	All
	1	100(5)[y]	100 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
	2	80 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
Control	3	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
No wound	4	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	5	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	Mean	96	100	100	99	100	100	92	97
	1	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	2	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
Control	3	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
Wound	4	80 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
	5	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	Mean	96	100	92	96	100	100	100	100
	1	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
	2	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
P. dimorphum	3	100 (5)	80 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
No wound	4	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
	5	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	Mean	100	96	96	97	100	100	96	99
	1	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	2	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
P. dimorphum	3	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
Wound	4	80 (5)	100 (5)	60 (5)		100 (5)	80 (5)	100 (5)	
	5	100 (5)	100 (5)	60 (5)		100 (5)	100 (5)	80 (5)	
	Mean	96	100	80	92	100	96	92	96
	1	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	2	80 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
P. irregulare	3	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
No wound	4	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	5	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
	Mean	96	100	100	99	100	100	96	99
	1	40 (5)	100 (5)	100 (5)		100 (5)	80 (5)	100 (5)	
	2	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
P. irregulare	3	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
Wound	4	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	5	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	Mean	88	100	96	95	100	96	96	97
LSD[x]		*(17)*	*(5)*	*(14)*	*(8)*	*(0)*	*(7)*	*(12)*	*(4)*

[z] = *Pythium* treatments took place on August 20, 2009; [y] = seedlings per replication are in parenthesis
[x] = least significant difference ($\alpha = 0.05$)

Table 3.7. Longleaf pine survival for 0, 4, and 8-week stored seedlings after four months in a greenhouse.

		Survival (%)							
		Peat-mix				Perlite			
Treatment[z]	Rep	0 wks	4 wks	8 wks	All	0 wks	4 wks	8 wks	All
	1	100(5)[y]	100 (5)	75 (4)		100 (4)	100 (4)	100 (4)	
	2	100 (5)	100 (5)	100 (4)		100 (4)	100 (4)	100 (4)	
Control	3	100 (5)	100 (5)	100 (4)		100 (4)	75 (4)	100 (4)	
No wound	4	100 (5)	100 (5)	50 (4)		100 (4)	100 (4)	100 (5)	
	5	100 (5)	80 (5)	100 (4)		100 (4)	100 (4)	100 (4)	
	Mean	100	96	85	94	100	95	100	98
	1	100 (5)	100 (5)	50 (4)		100 (4)	100 (4)	100 (4)	
	2	100 (4)	100 (5)	100 (4)		100 (4)	75 (4)	100 (4)	
Control	3	100 (5)	100 (5)	100 (4)		100 (4)	75 (4)	75 (4)	
Wound	4	60 (5)	80 (5)	40 (5)		100 (4)	75 (4)	100 (4)	
	5	100 (5)	100 (5)	100 (4)		100 (4)	100 (4)	75 (4)	
	Mean	92	96	78	89	100	85	90	92
	1	100 (5)	100 (5)	100 (5)		75 (4)	100 (4)	100 (4)	
	2	50 (4)	100 (5)	100 (5)		100 (4)	75 (4)	100 (4)	
P. dimorphum	3	60 (5)	100 (5)	100 (5)		100 (4)	100 (4)	100 (4)	
No wound	4	60 (5)	100 (5)	80 (5)		100 (4)	100 (4)	25 (4)	
	5	100 (5)	100 (5)	80 (5)		100 (4)	100 (4)	100 (4)	
	Mean	74	100	92	89	95	95	85	92
	1	80 (5)	80 (5)	100 (5)		100 (4)	100 (4)	75 (4)	
	2	80 (5)	100 (5)	100 (5)		75 (4)	75 (4)	100 (4)	
P. dimorphum	3	80 (5)	100 (5)	40 (5)		100 (4)	100 (4)	100 (4)	
Wound	4	80 (5)	100 (5)	100 (5)		100 (4)	100 (4)	75 (4)	
	5	20 (5)	100 (5)	100 (5)		75 (4)	100 (4)	100 (4)	
	Mean	68	96	88	84	90	95	90	92
	1	80 (5)	80 (5)	40 (5)		75 (4)	100 (4)	100 (4)	
	2	100 (5)	100 (5)	60 (5)		100 (4)	100 (4)	100 (4)	
P. irregulare	3	80 (5)	100 (5)	80 (5)		100 (4)	100 (4)	100 (4)	
No wound	4	100 (5)	100 (5)	100 (5)		100 (4)	100 (4)	100 (4)	
	5	80 (5)	100 (5)	100 (5)		100 (4)	75 (4)	100 (4)	
	Mean	88	96	76	87	95	95	100	97
	1	80 (5)	100 (5)	20 (5)		100 (4)	75 (4)	100 (4)	
	2	80 (5)	100 (5)	60 (5)		100 (4)	100 (4)	100 (4)	
P. irregulare	3	60 (5)	100 (5)	60 (5)		100 (4)	75 (4)	100 (4)	
Wound	4	80 (5)	100 (5)	20 (5)		100 (4)	75 (4)	100 (4)	
	5	100 (5)	100 (5)	80 (5)		100 (4)	50 (4)	100 (4)	
	Mean	80	100	48	76	100	75	100	92
LSD[x]		*(23)*	*(10)*	*(32)*	*(16)*	*(11)*	*(17)*	*(21)*	*(10)*

[z] = *Pythium* treatments took place on August 20, 2009; [y] = seedlings per replication are in parenthesis
[x] = least significant difference ($\alpha = 0.05$)

Table 3.8. Slash pine survival for 0, 4, and 8-week stored seedlings after four months in a greenhouse.

		Survival (%)							
		Peat-mix				Perlite			
Treatment[z]	Rep	0 wks	4 wks	8 wks	All	0 wks	4 wks	8 wks	All
	1	100(5)[y]	100 (5)	100 (5)		100 (5)	80 (5)	100 (5)	
	2	100 (5)	80 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
Control	3	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
No wound	4	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
	5	80 (5)	80 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	Mean	96	92	96	95	100	96	96	97
	1	100 (5)	100 (5)	80 (5)		100 (5)	80 (5)	100 (5)	
	2	100 (5)	100 (5)	80 (5)		100 (5)	80 (5)	100 (5)	
Control	3	80 (5)	100 (5)	100 (5)		80 (5)	100 (5)	100 (5)	
Wound	4	60 (5)	100 (5)	100 (5)		100 (5)	80 (5)	80 (5)	
	5	80 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	Mean	84	100	92	92	96	88	96	93
	1	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
	2	100 (5)	100 (5)	100 (5)		100 (5)	80 (5)	100 (5)	
P. dimorphum	3	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
No wound	4	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
	5	100 (5)	100 (5)	100 (5)		80 (5)	100 (5)	100 (5)	
	Mean	100	100	92	97	96	96	100	97
	1	100 (5)	100 (5)	80 (5)		80 (5)	80 (5)	80 (5)	
	2	60 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
P. dimorphum	3	100 (5)	100 (5)	100 (5)		80 (5)	100 (5)	100 (5)	
Wound	4	80 (5)	100 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
	5	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	80 (5)	
	Mean	88	100	92	93	92	96	88	92
	1	80 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
	2	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
P. irregulare	3	100 (5)	100 (5)	100 (5)		100 (5)	80 (5)	100 (5)	
No wound	4	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	80 (5)	
	5	100 (5)	100 (5)	100 (5)		60 (5)	100 (5)	100 (5)	
	Mean	96	100	96	97	92	96	96	95
	1	100 (5)	100 (5)	60 (5)		100 (5)	80 (5)	100 (5)	
	2	80 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
P. irregulare	3	100 (5)	100 (5)	100 (5)		100 (5)	100 (5)	100 (5)	
Wound	4	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	60 (5)	
	5	100 (5)	100 (5)	80 (5)		100 (5)	100 (5)	100 (5)	
	Mean	96	100	84	93	100	96	92	96
LSD[x]		*(15)*	*(6)*	*(15)*	*(8)*	*(13)*	*(12)*	*(14)*	*(7)*

[z] = *Pythium* treatments took place on August 20, 2009; [y] = seedlings per replication are in parenthesis
[x] = least significant difference ($\alpha = 0.05$)

Table 3.9. Shortleaf pine survival for 0, 4, and 8-week stored seedlings after four months in a greenhouse.

		Survival (%)							
		Peat-mix				Perlite			
Treatment[z]	Rep	0 wks	4 wks	8 wks	All	0 wks	4 wks	8 wks	All
	1	100(4)[y]	100 (4)	100 (4)		100 (3)	100 (3)	67 (3)	
	2	75 (4)	100 (4)	100 (4)		100 (3)	67 (3)	100 (3)	
Control	3	100 (4)	75 (4)	100 (4)		100 (3)	100 (3)	0 (3)	
No wound	4	100 (4)	100 (4)	75 (4)		100 (3)	67 (3)	100 (3)	
	5	75 (4)	75 (4)	100 (4)		100 (3)	33 (3)	——	
	Mean	90	90	95	92	100	73	67	81
	1	100 (4)	25 (4)	75 (4)		100 (3)	33 (3)	67 (3)	
	2	25 (4)	100 (4)	75 (4)		100 (3)	67 (3)	33 (3)	
Control	3	50 (4)	75 (4)	100 (4)		100 (3)	67 (3)	0 (3)	
Wound	4	100 (4)	75 (4)	100 (3)		100 (3)	67 (3)	100 (3)	
	5	100 (4)	100 (4)	75 (4)		100 (3)	33 (3)	——	
	Mean	75	75	85	78	100	53	50	69
	1	67 (3)	100 (5)	40 (5)		100 (3)	67 (3)	33 (3)	
	2	100 (4)	60 (5)	80 (5)		67 (3)	33 (3)	33 (3)	
P. dimorphum	3	100 (4)	100 (5)	100 (5)		100 (3)	33 (3)	33 (3)	
No wound	4	80 (5)	80 (5)	60 (5)		67 (3)	67 (3)	67 (3)	
	5	75 (4)	80 (5)	60 (5)		100 (3)	67 (3)	33 (3)	
	Mean	84	84	68	79	87	53	40	60
	1	50 (4)	60 (5)	80 (5)		100 (3)	33 (3)	50 (2)	
	2	50 (4)	100 (5)	60 (5)		100 (3)	100 (3)	33 (3)	
P. dimorphum	3	100 (4)	60 (5)	100 (5)		67 (3)	100 (3)	100 (3)	
Wound	4	100 (5)	100 (5)	80 (5)		100 (3)	100 (3)	67 (3)	
	5	100 (4)	80 (5)	60 (5)		100 (2)	67 (3)	0 (3)	
	Mean	80	80	76	79	93	80	50	74
	1	50 (4)	80 (5)	60 (5)		100 (3)	100 (3)	33 (3)	
	2	100 (3)	80 (5)	100 (4)		100 (3)	67 (3)	67 (3)	
P. irregulare	3	100 (4)	100 (5)	80 (5)		100 (3)	67 (3)	33 (3)	
No wound	4	75 (4)	100 (5)	60 (5)		100 (3)	33 (3)	33 (3)	
	5	100 (4)	60 (5)	100 (5)		100 (3)	67 (3)	0 (3)	
	Mean	85	84	80	83	100	67	33	67
	1	75 (4)	100 (5)	20 (5)		100 (3)	67 (3)	33 (3)	
	2	50 (4)	80 (5)	100 (4)		100 (3)	67 (3)	33 (3)	
P. irregulare	3	100 (4)	100 (5)	100 (4)		67 (3)	67 (3)	0 (3)	
Wound	4	100 (4)	100 (5)	80 (5)		100 (3)	67 (3)	0 (3)	
	5	75 (4)	100 (5)	20 (5)		100 (3)	0 (3)	75 (4)	
	Mean	80	96	64	80	93	54	28	58
LSD[x]		*(30)*	*(25)*	*(30)*	*(16)*	*(15)*	*(33)*	*(39)*	*(21)*

[z] = *Pythium* treatments took place on August 20, 2009; [y] = seedlings per replication are in parenthesis
[x] = least significant difference (α = 0.05)

3.5. Discussion

3.5.1. Packing and Storing Bareroot Seedlings in Peat-mix

The use of peat-mix as a packing medium to control the negative effects of *Pythium* in storage did not affect seedling survival after outplanting. Therefore, in these trials it appears that something other than peat is why container seedlings survive better than bareroot seedlings in storage. One possibility could be due to the type of peat media used. A standard greenhouse potting media was used (SunGro Sunshine Mix #8 Professional Growing Mix™), which consisted of 70-80% sphagnum peat moss. When peat moss was used as a packing material, nursery managers used 100% sphagnum peat moss (Wakeley 1954) and decreases in outplanting survival were not reported. Barnett and others (1988) reported a three-fold increase in longleaf pine survival (64%) when roots were packed in 100% sphagnum peat moss compared to clay slurry (18%) after 3 weeks of storage. In their study, the suppression of *Pythium* spp. by antagonistic fungi with the 100% peat may be one factor for increases in longleaf pine survival. Unfortunately, in our study, *Pythium* suppression was not observed and may have been because the media did not contain 100% sphagnum. In the southern U.S., nursery managers grow container seedlings in media that consists of about 80% sphagnum peat moss (pers. comm., Dr. Tom Starkey, Research Fellow, Auburn University), which is higher than the 50% used in the Pacific Northwest (pers. comm., Dr. Kaston Dumroese, Research Plant Physiologist, U.S.D.A. Forest Service). Because container-grown seedling survival is not affected in storage with < 100% peat, there may be other factors affecting bareroot seedling survival. One possible factor may be the lack of mycorrhizal roots. Mycorrhizae are symbiotic fungi that grow on seedling feeder roots, which

increase the root surface area and assist in the uptake of water and nutrients (Landis et al. 1989). In addition, mycorrhizae can provide protection against pathogens as a physical barrier and/or with antibiotic production (Marx 1972). During lifting, bareroot seedlings lose many of their mycorrhizal roots, whereas container-grown seedling roots maintain a mycorrhizal association in an undisturbed root plug.

Studies have reported suppresiveness to *Pythium* spp. when *Trichoderma* spp. (Wolffhechel 1988; Thrane et al. 2000; Scheuerell et al. 2005; Gravel et al. 2006), basidiomycetous yeasts (Hunter et al. 2006), *Pseudomonas* spp. (Boehm et al. 1993) and *Streptomyces* spp. (Wolffhechel 1988) were present in peat. In addition, *Trichoderma* has been effective at controlling *Pythium in vitro* (Sun 1996). Other studies have tested the effects of benomyl for controlling storage fungi (Kais and Barnett 1984; Barnett et al. 1988; Stumpf and South 1991; Jones et al. 1992; South and Lowenstein 1994; Hallgren and Ferris 1995; Brissette et al. 1996), but *Trichoderma* has shown resistance to the fungicide (Ahmad and Baker 1987). Future seedling storage trials should test the hypothesis that *Pythium* and seedling survival are not affected when fungicides and peat moss containing fungicide resistant *Trichoderma* spp. are incorporated into bareroot pine seedling packing medium.

In contrast with other studies that examined *Pythium*, more *Pythium* CFUs were recovered from seedlings grown in peat-mix than without. In addition to lacking antagonistic properties, the increase in *Pythium* may reflect a stimulatory effect from the oatmeal used in the inoculum. At the time of outplanting, mycelia was observed on seedling roots that may have overwhelmed any suppressiveness. For example, studies that mixed a peat moss and oatmeal, without adding *Pythium*, reported a higher disease

incidence on cucumber (*Cucumis sativus*) over cucumber grown in a mixture of peat, oatmeal, and *Pythium ultimum* Drechsler (Wolffhechel 1988). Their results indicate a positive effect on disease control caused by the combination of peat moss, oatmeal, and naturally occurring *Pythium*.

3.5.2. Container Seedlings Grown in Peat-mix vs. Perlite

When inoculated with *P. irregulare*, the type of media used to grow seedlings had an effect on longleaf pine survival. Longleaf pine survival in peat-mix was 82% compared to non-inoculated seedlings (92%) (LSD = 10). Shortleaf pine survival was less when grown in perlite. For both loblolly and shortleaf pine, seedling age at inoculation (4 months) or genotype may be factors for the decreases in seedling survival. For example, nine-month-old container longleaf and shortleaf pine inoculated with *Pythium* had similar outplanting survival as non-inoculated seedlings (Chapter 2). Hart and Endo (1981) reported mortality after inoculation of 2 and 4-week-old celery (Tall Utah 52-70 R cultivar) with *Fusarium oxysporum* f. sp. *apii*, whereas 6 and 8-week-old plants were not killed. The authors, after observing disease symptoms within 8-10 days, suspect that infection occurred via apices of the younger roots (2 and 4-weeks-old), which is when new roots began to develop. In our study, the younger seedling roots may have been more susceptible to either the wounding (longleaf) or the *Pythium* (shortleaf).

Media type did not affect either loblolly or slash pine seedling survival after cold storage with *Pythium*. It has been well documented that antagonistic fungi such as *Trichoderma* are present in peat moss (Wolffhechel 1988), and the fungus can control *Pythium* (Sun 1996). For this reason it is thought that container-grown seedlings are

more storable than bareroot seedlings. The possibility that antagonistic fungi present in the peat-mix suppressed any *Pythium* that entered container seedling root plugs either through irrigation or from contaminated containers may explain the similar loblolly and slash pine survival. *Pythium* was mixed only with water in the inoculum and not with oatmeal. Therefore, antagonistic fungi may have had an advantage over *Pythium* in the absence of a food source for growth.

The ability of antagonistic fungi to control disease does not explain why loblolly, longleaf, and slash pine were unaffected by *Pythium* when grown in perlite. One factor could be the pH of the perlite media. Perlite does not contain either nutrients or antagonistic fungi (Landis 1990). To better utilize available nutrients, peat moss pH should be at 5.5. Perlite pH ranges from 6.0-8.0 (Landis 1990). Damping-off pathogens such as *Pythium* are more active in neutral to alkaline soils and can be controlled by lowering the soil pH with acid solutions (Kelley and Oak 1989). Wolffhechel (1988) evaluated several peat sources and reported a *Pythium*-inoculated sphagnum peat moss at pH 5.3 to have less disease than those at pH 6.0 and 6.3. Hendrix and Campbell (1973) suggest that at higher substrate pH levels, reductions in host plant vigor occur, which increases the susceptible time frame for infection. To ensure similar seedling sizes as seedlings grow in peat-mix, seedlings grown in perlite were irrigated with acidified water (pH 2.0-3.0) to increase the utilization of nutrients. By doing so, an unfavorable pH environment for *Pythium* may have been created which reduced its infection potential.

3.5.3. Summary

The peat moss-based media used in this study did not improve outplanting survival after cold storage. Therefore, the null hypothesis that peat-mix does not affect seedling survival after outplanting was not rejected.

Inoculated longleaf and shortleaf pine grown in peat-mix had lower survival than non-inoculated seedlings, thus, *Pythium* is detrimental to seedling survival. Loblolly, longleaf, and slash pine grown in perlite had similar survival to seedlings grown in peat-mix. The perlite pH may have been low due to acidified irrigation, resulting in an unfavorable environment for fungal growth. The second null hypothesis that inoculations with *Pythium* and growing longleaf, loblolly, slash, and shortleaf pine in peat-mix or perlite does not affect seedling survival after cold storage was rejected based on lower survival of longleaf and shortleaf pine with *Pythium irregulare*.

Future experiments could test *Pythium* populations and seedling survival after inoculating bareroot pine seedling roots with *Pythium* grown in an inoculum other than oatmeal. Also, studies using 100% sphagnum peat moss and peat moss at different levels of decomposition and varying pH levels as bareroot seedling packing media and container substrates are necessary.

3.6. Acknowledgements

The author would like to thank Kelly Dougherty at Rayonier's Glennville Regeneration Center in Glennville, GA for providing loblolly pine seedlings and Wayne Bell at International Forest Company in Moultrie, GA and Russ Pohl at the Georgia Forestry Commission in Macon, GA for providing pine seed used in the experiments. Special thanks go out to Tommy Hill, Marietjie Quicke, Barry Brooks, Patrick Jernigan, Dr. Tom Starkey, and Rena Miller for assistance with the experiments.

3.7. Literature Cited

Ahmad, J.S. and R. Baker. 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77: 182-189.

Barnett, J.P., J.C. Brissette, A.G. Kais, and J.P. Jones. 1988. Improving field survival of southern pine seedlings by treating with fungicides before storage. Southern Journal of Applied Forestry 12: 281-285.

Boehm, M.J., L.V. madden, and H.A.J. Hoitink. 1993. Effect of organic matter decomposition level on bacterial species diversity and composition in relationship to *Pythium* damping-off severity. Applied and Environmental Microbiology 59(12): 4171-4179.

Brissette, J.C., J.P. Barnett, and J.P. Jones. 1996. Fungicides improve field performance of stored loblolly and longleaf pine seedlings. Southern Journal of Applied Forestry 20(1): 5-9.

Dierauf, T.A. 1976. Fall planting and storage using non-dormant seedlings. In: Proc. Southeastern Area Nurseryman's Conference-SC Eastern: 31-36.

Dumroese, R.K., R.L. James, D.L. Wenny, and C.J. Gilligan. 1988. Douglas-fir seed treatments: effects on seedborne organisms and germination. In: Landis, T.D., tech. coords., Proc. Combined Meeting of the Western Forest Nursery Association, GTR-RM-167, Fort Collins, CO, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 155-160.

Dumroese, R.K., R.L. James, and D.L. Wenny. 2002. Hot water and copper coatings in reused containers decrease inoculum of *Fusarium* and *Cylindrocarpon* and increase Douglas-fir seedling growth. HortScience 37(6): 943-947.

Feng, Y. and P.H. Dernoeden. 1999. *Pythium* species associated with root dysfunction of creeping bentgrass in Maryland. Plant Disease 83: 516-520.

Gravel, V., C. Martinez, H. Antoun, and R.J. Tweddell. 2006. Control of greenhouse tomato root rot[*Pythium ultimum*] in hydroponic systems, using plant-growth-promoting microorganisms. Canadian Journal of Plant Pathology 28: 475-483.

Hallgren, S.W. and D.M. Ferris. 1995. Benomyl applied to roots improves second-year survival and growth of shortleaf pine. Southern Journal of Applied Forestry 19(1): 36-41.

Hart, L.P. and R.M. Endo. 1981. The effect of length of exposure to inoculum, plant age, root development, and root wounding on Fusarium yellows of celery. Phytopathology 71: 77-79.

Hebb, E.A. 1982. Cold storage of Choctawhatchee sand pine seedlings: Effect of lifting date and length of storage. Southern Journal of Applied Forestry 6(4): 229-231.

Hendrix, F.F., Jr. and W.A. Campbell. 1973. *Pythiums* as plant pathogens. Annual Review of Phytopathology 11: 77-98.

Hunter, P.J., G.M. Petch, L.A. Calvo-Bado, T.R. Pettitt, N.R. Parsons, J.A.W. Morgan, and J.M. Whipps. 2006. Differences in microbial activity and microbial populations of peat associated with suppression of damping-off disease caused by *Pythium sylvaticum*. Applied and Environmental Microbiology 72(10): 6452-6460.

Ivors, K.L., Z.G. Abad, and D.M. Benson. 2008. Evaluating the pathogenicity of *Pythium vexans* isolates from Fraser fir in North Carolina. Online. Plant Health Progress doi: 10.1094/PHP-2008-1006-01-RS.

Jones, J.P., D.J. Pantone, J.P. Barnett, and J.C. Brissette. 1992. The relationship between fungal population development and root damage of cold-stored longleaf pine seedlings. Louisiana State University Agricultural Center, Bulletin No. 832: 23 p.

Kahler, L.H. and A.R. Gilmore. 1961. Field survival of cold stored loblolly pine seedlings. Tree Planters' Notes, Issue 45: 15-16.

Kais, A.G. and J.P. Barnett. 1984. Longleaf pine growth following storage and benomyl root-dip treatment. Tree Planters' Notes 35(1): 30-33.

Kelley, W.D. and S.W. Oak. 1989. Forest Nursery Pests. In: Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, Jr., R.S., and Harvey, T.V, Tech. Coords., USDA Forest Service, Agricultural Handbook No. 680: 184 pp.

Kim, Y.K., C.L. Xiao, and J.D. Rogers. 2005. Influence of culture media and environmental factors on mycelia growth and pycnidial production of *Sphaeropsis pyriputrescens*. Mycologia 97(1): 25-32.

Landis, T.D., R.W. Tinus, S.E. McDonald, and J.P. Barnett. 1989. The Container Tree Nursery Manual, Volume 5, The Biological Component: Nursery Pests and Mycorrhizae. Agricultural Handbook 674. Washington D.C., USDA Forest Service: 171 p.

Landis, T.D. 1990. Containers and growing media, Vol. 2, The Container Tree Nursery Manual. Agricultural Handbook 674, Washington D.C., U.S.D.A. Forest Service: 41-85.

Marx, D.H. 1972. Ectomycorrhizae as biological deterrents to pathogenic root infections. Phytopathology 10: 429-454.

May, J.T. 1984. Southern Pine Nursery Handbook. U.S. Department of Agriculture, Forest Service, Southern Region.

Scheuerell, S.J., D.M. Sullivan, and W.F. Mahaffee. 2005. Suppression of seedling damping-off caused by *Pythium ultimum*, *P. irregulare*, and *Rhizoctonia solani* in container media amended with a diverse range of Pacific Northwest compost sources. Phytopathology 95(3): 306-315.

South, D.B. and N.J. Lowenstein. 1994. Effects of Viterra® root dips and benomyl on root growth potential and survival of longleaf pine seedlings. Southern Journal of Applied Forestry 18(1): 19-23.

Stumpff, N.J. and D.B. South. 1991. Benomyl root dips adversely affect first-year performance of stored loblolly pine seedlings. Southern Journal of Applied Forestry 15(3) 133-137.

Sun, X. 1996. Biological control of Pythiaceous fungi causing death of longleaf pine seedlings during cold storage. PhD dissertation, Louisiana State University: 135 p.

Thrane, C, D.F. Jenson, and A. Tronsmo. 2000. Substrate colonization, strain competition, enzyme production *in vitro*, and biocontrol of *Pythium ultimum* by *Trichoderma* spp. isolates P1 and T3. European Journal of Plant Pathology 106: 215-225.

Venator, C.R. 1984. Evidence of the presence of a lifting window for loblolly pine nursery seedlings. In Proc: Southern Nursery Conference-Eastern Session, Asheville, NC: 184-190.

Wakeley, P.C. 1954. Planting the southern pines. USDA Agricultural Monograph No.18: 233 p.

Wolffhechel, H. 1988. The suppressiveness of sphagnum peat to *Pythium* spp. Acta Horticulturae 221: 217-222.

CHAPTER 4

EFFECTS OF *PYTHIUM* AND COLD STORAGE ON THE ROOT GROWTH POTENTIAL OF LONGLEAF, LOBLOLLY, AND SLASH PINE SEEDLINGS

4.1. Abstract

Survival and root growth potential (RGP) of bareroot pine (*Pinus* spp.) seedlings tends to be lower when seedlings are lifted and held in cold storage (> 1 wk) from October to early December. There is speculation that the combination of root wounding at the time of lifting, *Pythium* presence in the soil, and the cool, moist conditions in cold storage may encourage fungal growth that results in seedling mortality after outplanting. The effect of these factors was investigated on longleaf pine (*Pinus palustris*), loblolly pine (*Pinus taeda*), and slash pine (*Pinus elliottii*). Seedlings were inoculated with either *Pythium dimorphum* or *Pythium irregulare*, cold stored for 3 weeks, and then placed in a hydroponic system (aerated aquariums) in a greenhouse. Seedling root collar diameter (RCD) and the number of new roots > 0.5 cm were measured on longleaf pine. The number, length, volume, surface area, and diameter of new roots were quantified on loblolly and slash pine. Seedling survival was recorded 4 months after outplanting on loblolly and slash pine. *Pythium* did not reduce longleaf pine RGP but *P. dimorphum* did reduce RCD. Both *Pythium* species reduced RCD, the length, surface area, volume, and

number of new roots in slash pine. *Pythium irregulare* reduced the number of new roots for loblolly pine. Despite lower RGP, *P. irregulare* did not affect loblolly pine survival but reduced slash pine survival when compared to non-inoculated seedlings. *Pythium dimorphum* and *P. irregulare* reduced RCD of longleaf and slash pine and RGP of loblolly and slash pine when cold stored for 3 weeks.

4.2. Introduction

Cold storage of pine (*Pinus* spp.) seedlings is a common practice in forest tree nurseries when seedling demand is either low or when outplanting conditions are poor. Occasionally, lifting bareroot seedlings from October to mid-December can result in poor survival after long-term (> 1 week) cold storage (Kahler and Gilmore 1961; Dierauf 1976; Hebb 1982; Venator 1984). However, bareroot seedling survival improves when seedlings are lifted and stored from mid-December to January (Kahler and Gilmore 1961; Dierauf 1976).

A key component of seedling survival after outplanting depends on the production of new seedling roots. Root growth potential (RGP) is defined as the ability of a seedling to initiate and grow new roots within a prescribed time period in an environment that is optimum for root growth (Simpson and Ritchie 1996). Thus, root growth potential is a measure of seedling quality based on new root production and can be used to determine seedling performance potential before outplanting. Methods to measure RGP include either soil, hydroponic, or aeroponic culture (Rietveld 1989). The number and/or size of new white roots produced in these systems are then used to quantify RGP (Figure 1.1). Generally, seedlings that produce new roots are considered to be of higher quality. For

Figure 4.1. A. Longleaf pine seedling with no new white root growth. B. Longleaf pine seedling with many new white roots.

example, RGP of loblolly pine (*Pinus taeda*) steadily declined after 12 and 9 weeks of storage for October and November-lifted seedlings, respectively, but improved after 9 weeks of storage in January (Dewald and Feret 1988). January and late December-lifted shortleaf pine (*Pinus echinata*) seedlings had the greatest RGP after 4 weeks of storage and seedling survival was greatest for seedlings lifted and stored concurrently for the same duration (Hallgren and Tauer 1989). In addition, (South 1999) coined the term "December dip", which described unexplained seedling mortality observed in loblolly pine outplantings during the month of December. Decreases in loblolly pine survival were more prevalent after cold storage and may have been linked to a decrease in seedling RGP (South 1999).

The environment in cold storage can provide ideal conditions for soilborne fungi, particularly "water molds" such as *Pythium*, which can cause fine feeder root mortality. *Pythium* is commonly found in nursery soils and infect the roots that are essential for water and nutrient absorption (Kelley and Oak 1989). Bareroot seedling roots are typically wounded during lifting, and the wounds can serve as infection sites for *Pythium*. After seedlings are placed in cold storage, conditions may be conducive for fungal growth and root infection, leading to outplanting failure. Jones and others (1992) detected *Pythium* on bareroot longleaf pine (*Pinus palustris*) seedlings held in storage for 6 weeks. Treating longleaf seedlings with combinations of the fungicides metalaxyl and benomyl resulted in > 90% survival, while those that did not receive fungicides had < 20% survival (Jones et al. 1992). Thus, the addition of a fungicide may have eliminated any soil-borne fungi present on seedling roots. Barden and Feret (1986) also found that when loblolly pine seedlings were kept dry before storage, root growth was greater over a

20-week storage period when compared to seedlings dipped in water. Thus, a moist environment in cold storage may be suitable for fungal growth and increase disease. The ability to place bareroot seedlings in cold storage from November to mid-December could extend the traditional planting season (winter) and insure that lifting operations end before spring (Garber and Mexal 1980).

The objective of these trials was to quantify the effects that *Pythium* had on RGP, diameter growth, and survival of longleaf, loblolly, and slash pine (*Pinus elliottii*) seedlings after long-term cold storage. These studies were designed to test the null hypothesis that RGP and seedling survival are not affected by *Pythium* inoculation and cold storage.

4.3. Materials and Methods

4.3.1. *Pythium* Inoculum

The fungi used in all experiments, *Pythium dimorphum* Hendrix and Campbell and *Pythium irregulare* Buisman were obtained from American Type Culture Collection (ATCC®, Manassas, VA). Each *Pythium* strain was aseptically transferred to oatmeal agar (Kim et al. 2005). From the advancing margin of the fungal mycelium, three 0.5 cm disks of each *Pythium* spp. were transferred to oatmeal agar for use as seedling inoculum. Prior to inoculation, 1,190 g of oatmeal (Quaker Oats®) and 400 ml of distilled water were added to two autoclavable bags, mixed thoroughly, and autoclaved. The sterilized oats were allowed to cool for 24 h and one oatmeal bag received three plates of *P. dimorphum* and the other bag received three plates of *P. irregulare*. The oatmeal/*Pythium* inoculum was mixed every 12 h and stored at room temperature for 10 days prior to root inoculations.

4.3.2. Seedling Inoculations

Two separate experiments were conducted using: 1) bareroot longleaf pine (2008) and 2) bareroot loblolly and slash pine (2009), that were obtained from Smurfit-Stone Corporation's Rock Creek Nursery near Brewton, AL. Prior to inoculations, longleaf pine remained in cold storage (4-5ºC) for 6 weeks and loblolly and slash pine for 8 weeks at Auburn University. Seedling roots were dipped into a bucket filled with 11 liters of water (controls) or 11 liters of water + the oatmeal/*Pythium* inoculum. Seedlings were subjected to five inoculation treatments: 50 g and 200 g of *P. dimorphum* oatmeal inoculum, 50 g and 200 g of *P. irregulare* oatmeal inoculum. Controls received no inoculum. Three replications of each treatment were inoculated and the experimental unit contained either 30 seedlings (longleaf pine) or 15 seedlings (loblolly and slash pine). Buckets were emptied, rinsed, and filled with a fresh inoculum mixture after each seedling inoculation. Inoculated seedlings were placed in 49 liter plastic bags and put in cold storage (4-5ºC) for three weeks. In all, 450 longleaf pine and 225 each of loblolly and slash pine seedlings were used in each experiment.

4.3.3. Longleaf Pine Root Growth Potential

After 3 weeks in storage, treated longleaf pine seedlings were placed in an aerated hydroponic system as described by (Palmer and Holen 1986). Each of the 15 aquariums contained 30 seedlings in a system that allowed seedling roots to be suspended in water (Figure 1.2). Each aquarium was an experimental unit and was placed in a randomized

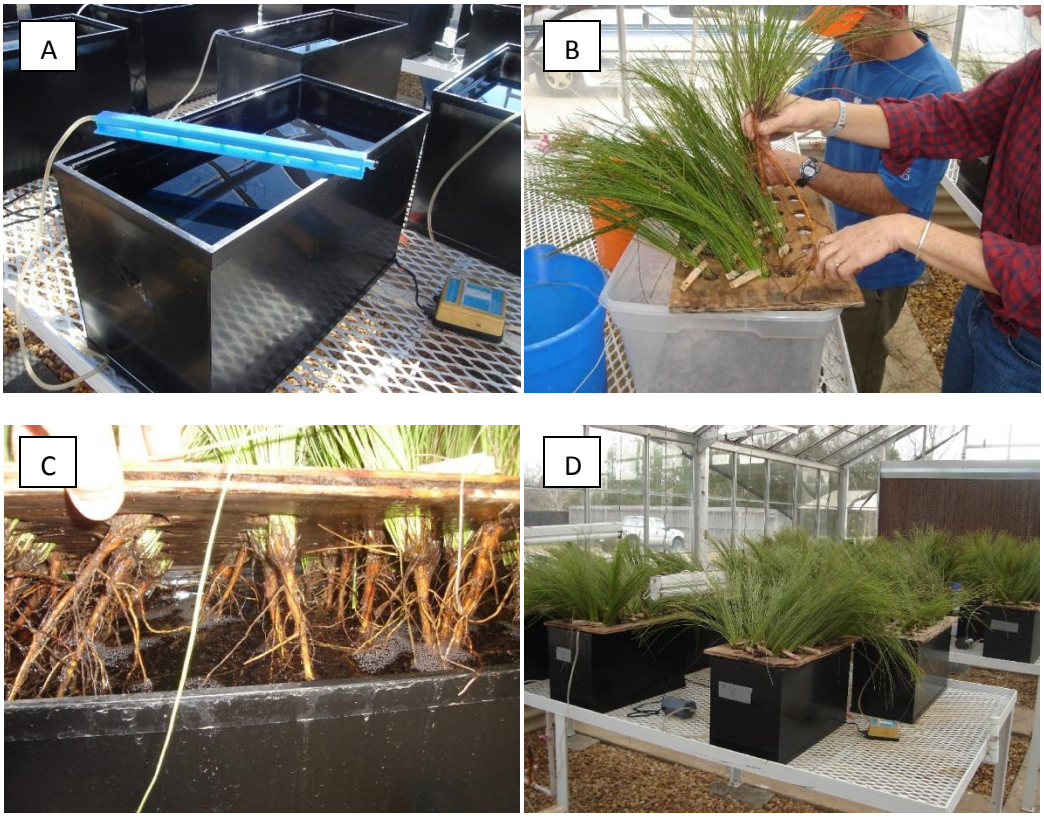

Figure 4.2. Hydroponic system for measuring root growth potential. A. Aquarium (38 L), aerator, and pump. B. Seedlings being placed in a fitted aquarium cover. C. Seedling roots suspended in aerated water. D. Several hydroponic systems on greenhouse benches.

complete block design on three greenhouse tables. As each seedling was placed in the aquarium, the root collar diameter (RCD) was measured to the nearest 0.01 mm. After 60 days in the hydroponic system, seedling RCD was re-measured, seedling survival recorded, and the number of new roots > 0.5 cm counted by hand.

4.3.4. Loblolly and Slash Pine Root Growth Potential

Loblolly and slash pine seedlings used a similar oatmeal inoculation, cold storage treatments and hydroponic methods for testing RGP as for longleaf pine. One aquarium was used for each experimental unit for loblolly and slash pine (15 seedlings of each species in the same aquarium). Seedling RCD was measured on day 1 and 28. At day 28, seedling survival was recorded and RGP quantified by measuring the number, length, volume, surface area, and diameter of new white root tips using a WinRhizo™ root scanner and computer software (Regent Instruments, Inc., Quebec City, Quebec, Canada). After scanning, seedlings were returned to the aquariums, and on day 34, were outplanted into sand near Auburn University in a randomized complete block design at 0.3 x 0.3 m spacing and monitored for survival.

4.3.5. Statistical Analyses

Analyses of means were conducted using a General Linear Model (GLM) in SAS statistical software (9th ed., SAS Institute, Cary, NC). An experimental unit consisted of 30 longleaf, 15 loblolly, or 15 slash pine seedlings. Means of each experimental unit for each dependant variable were analyzed using Analysis of Covariance (ANCOVA), where initial RCDs (before placing seedlings in the hydroponic system) were included to

account for any initial difference in seedling size (South et al. 1989). Contrast analyses were performed using combined levels of each *Pythium* treatment with data for each pine species analyzed separately.

4.4. Results

4.4.1. Longleaf Pine

The inoculation of *Pythium* on longleaf roots prior to 3 weeks of storage did not affect seedling RGP as measured by new seedling roots (Table 4.1). Although not significant (P = 0.0569), more new roots were produced on seedlings inoculated with *P. irregulare* than by *P. dimorphum* (Table 4.2). While RGP was unaffected, the RCD of all *Pythium*-inoculated seedlings decreased (shrunk) over the course of 60 days with the greatest diameter reduction occurring on seedlings inoculated with 200 g of *Pythium*/oatmeal inoculum (Table 4.2). *Pythium dimorphum* caused significant reductions in RCD when contrasted against non-inoculated seedlings (Table 4.1). Non-inoculated seedlings were the only seedlings to increase in RCD during the experiment (Table 4.2). Longleaf pine survival was not affected by *Pythium* or storage treatments (Table 4.1).

4.4.2. Loblolly Pine

Inoculation of loblolly pine with *P. dimorphum* and *P. irregulare* reduced the number of new root tips (Table 4.3). Non-inoculated seedlings had an average of 80 new roots, while seedlings inoculated with *P. dimorphum* and *P. irregulare* had an average of 51 and 38 new roots, respectively (Table 4.4). Aside from new root production, *P.*

dimorphum did not affect any other RGP measurement (Table 4.3). In contrast, *P. irregulare* reduced root length and surface area but did not affect either root volume or diameter (Table 4.3). *Pythium* had no affect on loblolly pine RCD (Table 4.3).

Seedling survival was not affected by root inoculation with *Pythium* species or amount of inoculum used (Table 4.5). Despite reductions in RGP by the *Pythium* treatments, only three of the 225 loblolly pine seedlings died during the RGP trial, and four months after outplanting, seedling survival was similar for all seedlings (Table 4.5). Seedlings that received 50 g of *P. dimorphum* had 46% survival compared to 43% for non-inoculated seedlings, and seedlings inoculated with 200 g of *Pythium* had the lowest survival (< 16%), yet all non-significant (Table 4.6).

Table 4.1. Analysis of covariance for longleaf pine seedling root growth potential (RGP) and root collar diameter (RCD) and analysis of variance for survival in 2008.

		P > F			
	DF	New roots	RCD after (mm)	RCD growth (mm)	Survival (%)
Covariate	1	0.1930	0.0001	0.7680	——
Replication	2	0.1678	0.0063	0.0063	0.7552
Treatment	4	0.2599	0.0386	0.0386	0.4625
Control vs *P. dimorphum*	(1)	0.8564	0.0097	0.0097	0.7151
Control vs *P. irregulare*	(1)	0.1663	0.1009	0.1009	0.4363
P. dimorphum vs *P. irregulare*	(1)	0.0569	0.6553	0.6553	0.1806
Error	7				

Table 4.2. Longleaf pine root growth potential (RGP), root collar diameter (RCD), and survival after 60 days in the aquariums in 2008.

Treatment[z]	Rep	New roots (#)	RCD before (mm)	RCD after (mm)	RCD growth (mm)	Survival (%)
Control	1	8	9.68	9.68	0.00	90
	2	44	9.98	10.31	0.33	90
	3	5	10.15	10.39	0.24	66
	Mean	20	9.94	10.12	0.19	82
P. dimorphum 50 g	1	12	9.01	8.49	-0.52	86
	2	21	9.81	9.81	0.00	63
	3	3	9.01	9.02	0.01	70
	Mean	13	9.28	9.11	-0.17	73
P. dimorphum 200 g	1	17	10.13	9.49	-0.65	90
	2	14	9.35	9.10	-0.25	90
	3	9	9.72	9.76	0.04	73
	Mean	14	9.74	9.45	-0.29	84
P. irregulare 50 g	1	19	8.51	8.14	-0.37	80
	2	35	8.27	8.30	0.04	100
	3	35	8.12	8.45	0.31	100
	Mean	30	8.30	8.29	-0.01	93
P. irregulare 200 g	1	11	8.61	8.31	-0.30	73
	2	16	8.28	7.95	-0.33	90
	3	29	9.66	9.53	-0.13	93
	Mean	19	8.85	8.60	-0.25	85
LSD[y]		(21)	(0.9)	(0.9)	(0.3)	*(23)*

[z] = *Pythium* inoculation treatments took place on January 19, 2008

[y] = least significant difference ($\alpha = 0.05$)

Table 4.3. Analysis of covariance for loblolly pine seedling root growth potential in 2009.

		P > F						
Factor	DF	New roots	Root length (cm)	Root surface area (cm^2)	Root volume (cm^3)	Root diameter (mm)	RCD after (mm)	RCD growth (mm)
Covariate	1	0.8748	0.4191	0.2038	0.1078	0.2201	0.0004	0.4879
Replication	2	0.1271	0.2717	0.3518	0.3788	0.0364	0.3964	0.3964
Treatment	4	0.0493	0.1381	0.2167	0.3407	0.7782	0.3108	0.3108
Control vs *P. dimorphum*	(1)	0.0241	0.0766	0.1716	0.3759	0.2421	0.1366	0.1366
Control vs *P. irregulare*	(1)	0.0053	0.0202	0.0397	0.0873	0.4209	0.0726	0.0726
P. dimorphum vs *P. irregulare*	(1)	0.2159	0.2957	0.2527	0.2311	0.5895	0.6216	0.6216
Error	7							

Table 4.4. Loblolly pine root growth potential (RGP) and root collar diameter (RCD) after 28 days in the aquariums in 2009.

Treatment[z]	Replication	New roots	Root length (cm)	Root surface area (cm^2)	Root volume (cm^3)	Root diameter (mm)	RCD before (mm)	RCD after (mm)	RCD growth (mm)
Control	1	98	29.60	6.09	0.10	0.67	4.49	4.74	0.25
	2	52	12.92	2.78	0.05	0.75	4.27	4.43	0.15
	3	90	31.58	6.32	0.11	0.71	4.39	4.47	0.09
	Mean	80	24.67	5.06	0.09	0.71	4.38	4.55	0.16
P. dimorphum 50 g	1	63	13.79	2.81	0.05	0.67	4.29	4.37	0.09
	2	28	6.78	1.38	0.03	0.96	4.25	4.09	-0.16
	3	66	13.11	2.74	0.05	0.69	4.46	4.55	0.09
	Mean	52	11.16	2.31	0.04	0.77	4.33	4.34	0.00
P. dimorphum 200 g	1	62	24.76	5.77	0.11	0.75	4.70	4.77	0.07
	2	62	25.98	7.24	0.17	0.87	4.59	4.77	0.18
	3	29	5.78	1.62	0.04	0.89	4.63	4.71	0.08
	Mean	51	18.81	4.88	0.11	0.84	4.64	4.75	0.11
P. irregulare 50 g	1	66	20.97	4.41	0.08	0.62	4.49	4.64	0.15
	2	30	6.44	1.29	0.02	0.75	4.19	4.28	0.09
	3	33	5.84	1.29	0.03	0.89	4.57	4.49	-0.08
	Mean	43	11.09	2.33	0.04	0.75	4.42	4.47	0.05
P. irregulare 200 g	1	37	9.55	2.00	0.03	0.69	4.35	4.29	-0.06
	2	36	10.25	2.89	0.07	0.88	4.71	4.83	0.13
	3	26	5.19	1.47	0.04	0.83	4.53	4.43	-0.09
	Mean	33	8.33	2.12	0.05	0.80	4.53	4.52	-0.01
LSD[y]		*(31)*	*(14.67)*	*(3.44)*	*(0.07)*	*(0.16)*	*(0)*	*(0.21)*	*(0.21)*

[z] = *Pythium* inoculation treatments took place on March 5, 2009; [y] = least significant difference ($\alpha = 0.05$)

Table 4.5. Analysis of variance for loblolly and slash pine seedling survival in 2009.

		P > F			
		Loblolly Pine		Slash Pine	
Factor	DF	Survival 1[z]	Survival 2[y]	Survival 1	Survival 2
Replication	2	0.0256	0.0949	0.1507	0.3759
Treatment	4	0.4609	0.3438	0.0659	0.0168
Control vs *P. dimorphum*	(1)	0.4774	0.3406	1.0000	0.1716
Control vs *P. irregulare*	(1)	0.4774	0.1774	0.0298	0.0040
P. dimorphum vs *P. irregulare*	(1)	1.0000	0.5841	0.0121	0.0158
Error	7				

[z] = survival after 28 days in hydroponic culture
[y] = survival 4 months after outplanting

Table 4.6. Loblolly and slash pine survival as affected by *Pythium* in 2009.

		Loblolly Pine		Slash Pine	
Treatment[z]	Rep	Survival 1[y]	Survival 2[x]	Survival 1	Survival 2
Control	1	100	20	100	33
	2	100	46	100	80
	3	93	64	100	93
	Mean	97	43	100	68
P. dimorphum 50 g	1	100	40	100	53
	2	100	20	100	13
	3	93	78	100	13
	Mean	97	46	100	26
P. dimorphum 200 g	1	100	20	100	46
	2	100	26	100	53
	3	100	0	100	93
	Mean	100	15	100	64
P. irregulare 50 g	1	100	40	80	0
	2	100	33	66	0
	3	93	50	100	0
	Mean	97	41	82	0
P. irregulare 200 g	1	100	6	86	7
	2	100	6	80	0
	3	100	13	100	33
	Mean	100	8	88	13
LSD[w]		*(4)*	*(33)*	*(14)*	*(41)*

[z] = *Pythium* inoculation treatments took place on March 5, 2009
[y] = survival after 28 days in hydroponic culture
[x] = survival 4 months after outplanting
[w] = least significant difference ($\alpha = 0.05$)

4.4.3. Slash Pine

Both *Pythium dimorphum* and *P. irregulare* reduced the number of new white roots, root length, root surface area, and root volume of slash pine seedlings (Table 4.7). Non-inoculated seedlings had 174 new roots, whereas *P. dimorphum and P. irregulare*-inoculated seedlings had 81 and 25 new roots, respectively (Table 4.8). While not significant, all RGP characteristics of *P. irregulare*-inoculated slash pine were numerically less than those of *P. dimorphum*-inoculated slash pine. Overall, RGP decreased as the level of *Pythium* inoculum increased (Table 4.8).

The root collar diameter of slash pine was reduced when seedlings were inoculated with *P. irregulare* (Table 4.7). There was a two-fold reduction in RCD (-0.17 mm) compared to non-inoculated seedlings (+0.15 mm) (Table 4.8). Non-inoculated seedlings had three times more diameter growth than seedlings inoculated with 200 g of *P. dimorphum* (Table 4.8). The RCD of seedlings inoculated with *P. dimorphum* was less, but was similar to non-inoculated slash pine seedlings ($P = 0.1021$).

Slash pine mortality did occur on seedlings inoculated with *P. irregulare* (Tables 4.5 and 4.6). During the 28-day RGP trial, *P. irregulare*-inoculated seedling survival was 85% compared to 100% for *P. dimorphum*-inoculated seedlings (Table 4.6). Four months following outplanting, seedling survival for *P. irregulare*-inoculated seedlings (7%) decreased compared to *P. dimorphum*-inoculated seedlings (45%) (Table 4.6). Seedlings had less RGP when slash pine was inoculated with *P. irregulare* at 50 g (16 new roots) and 200 g (35 new roots), and the lack of root production was consistent with low seedling survival (0% and 13%, respectively). However, even though RGP of slash

pine inoculated with *P. dimorphum* was less than non-inoculated seedlings, seedling survival was not affected (P = 0.1716).

Table 4.7. Analysis of covariance for slash pine seedling root growth potential in 2009.

		P > F						
Factor	DF	New roots	Root length	Root surface area	Root volume	Root diameter	RCD after	RCD growth
Covariate	1	0.7595	0.6918	0.7592	0.8215	0.1072	0.0068	0.4012
Replication	2	0.1209	0.0923	0.0550	0.0463	0.6994	0.5162	0.5162
Treatment	4	0.0022	0.0018	0.0017	0.0028	0.2262	0.0619	0.0619
Control vs *P. dimorphum*	(1)	0.0039	0.0024	0.0031	0.0073	0.2100	0.1021	0.1021
Control vs *P. irregulare*	(1)	0.0002	0.0002	0.0001	0.0002	0.9962	0.0126	0.0126
P. dimorphum vs *P. irregulare*	(1)	0.0124	0.0157	0.0095	0.0084	0.1346	0.1383	0.1383
Error	7							

Table 4.8. Slash pine root growth potential (RGP) and root collar diameter (RCD) after 28 days in the aquariums in 2009.

Treatment[z]	Rep	New roots	Root length (mm)	Root surface area (cm^2)	Root volume (cm^3)	Root diameter (mm)	RCD before (mm)	RCD after (mm)	RCD growth (mm)
Control	1	172	79.08	22.38	0.52	0.92	4.78	4.87	0.09
	2	142	62.90	17.26	0.39	0.85	4.87	5.05	0.18
	3	208	83.08	20.16	0.41	0.79	4.69	4.87	0.17
	Mean	174	75.02	19.93	0.44	0.85	4.78	4.93	0.15
P. dimorphum 50 g	1	134	52.45	15.83	0.41	0.97	4.68	4.83	0.15
	2	18	8.14	2.41	0.06	1.09	4.60	4.37	-0.23
	3	50	6.12	1.72	0.04	0.87	4.64	4.43	-0.21
	Mean	67	22.34	6.66	0.17	0.98	4.64	4.54	-0.10
P. dimorphum 200 g	1	115	58.63	15.35	0.34	0.83	4.56	4.62	0.06
	2	80	27.14	7.17	0.16	0.82	4.83	4.89	0.05
	3	89	24.61	7.23	0.18	0.90	4.86	4.89	0.03
	Mean	95	36.79	9.92	0.22	0.85	4.75	4.80	0.05
P. irregulare 50 g	1	15	2.62	0.51	0.01	0.61	4.45	4.28	-0.17
	2	17	3.50	0.92	0.02	0.94	5.03	4.79	-0.25
	3	15	1.12	0.33	0.01	0.99	4.93	4.63	-0.31
	Mean	16	2.42	0.59	0.01	0.85	4.80	4.56	-0.24
P. irregulare 200 g	1	40	10.88	2.81	0.06	0.79	4.75	4.72	-0.03
	2	16	1.28	0.36	0.01	0.92	4.89	4.61	-0.29
	3	48	10.43	2.71	0.06	0.82	4.51	4.55	0.05
	Mean	35	7.53	1.96	0.04	0.84	4.72	4.63	-0.09
LSD[y]		*(57)*	*(26)*	*(6.75)*	*(0.16)*	*(0.19)*	*(0)*	*(0.26)*	*(0.26)*

[z] = *Pythium* inoculation treatments took place on March 5, 2009

[y] = least significant difference ($\alpha = 0.05$)

4.5 Discussion

Inoculating longleaf, loblolly, and slash pine seedlings with *P. dimorphum* and *P. irregulare* and storing them for 3 weeks affected root growth potential, root collar diameter, and seedling survival but varied by tree species tested. Reductions in slash pine survival after outplanting reflected reductions in RCD in the RGP trials. In contrast, *P. dimorphum* (200 g) reduced longleaf pine RCD by 0.48 mm, but survival was similar to non-inoculated seedlings. Loblolly pine RCD was unaffected by *Pythium* and outplanting survival was similar to non-inoculated seedlings. Dewald and Feret (1988) reported no change in loblolly pine RCD for seedlings lifted from October to February and stored for up to 12 weeks. In contrast, 4 weeks of storage reduced RCD of shortleaf pine (*Pinus echinata*) (Hallgren and Tauer 1989). Genotypic variations between pine species might account for the differences in RCD growth that have been recorded either after storage (Dewald and Feret 1988; Hallgren and Tauer 1989) or after testing RGP of stored seedlings using a hydroponic system.

Pythium did not affect new root production on longleaf pine. In fact, non-inoculated seedlings had more seedlings (44%) with no new roots compared to *P. irregulare*-inoculated seedlings (33%) (data not shown). It is possible that prior to applying treatments (storage), seedling RGP was affected by naturally occurring *Pythium*, resulting in lower RGP of the control seedlings. Seedlings selected as controls could have been infected with *Pythium* during the lifting process and during storage, the fungal spores were able to germinate and multiply in storage.

These trials were the first to test longleaf pine RGP in the presence of *Pythium*. Jones and others (1992) recovered *Pythium* from stored longleaf pine roots, but they did

not associate the pathogen's presence to a measure of RGP. However, Sun (1996) inoculated longleaf pine with *P. dimorphum* before storage and reported a seedling survival < 3%, but he also did not test RGP.

Slash pine survival was less for *P. irregulare*-inoculated seedlings, which paralleled the decreases in RGP observed in the hydroponic system. Decreases in loblolly pine survival were not observed, despite reductions in RGP attributed to both *Pythium* spp. A RGP of 4 to 5 new roots was sufficient to provide adequate first year loblolly pine seedling survival (80-90%) (Feret et al. 1985). In our studies, 33 to 52 new roots for *Pythium*-inoculated loblolly pine resulted in seedling survival similar to non-inoculated seedlings (80 roots). Loblolly and slash pine seedlings inoculated with *P. dimorphum* were unaffected by the reduced RGP and had similar seedling survival as non-inoculated seedlings. Poor seedling survival and growth after outplanting can be caused by the limitations of seedling roots to access water and nutrients (Morris and Campbell 1991). It might be possible that sufficient amounts of water and nutrients were available to seedlings after outplanting, but slash pine inoculated with *P. irregulare* could not access enough of the resources, which caused reductions in survival.

Pythium dimorphum was first isolated from diseased loblolly pine roots in Louisiana (Hendrix and Campbell 1971) and since, has been isolated by Ho (1986) and Asiegbu and others (1996). Root inoculations with *P. dimorphum* reduced shoot height of Norway spruce (*Picea abies*) seedlings, and the pathogen was recovered often from benomyl-treated seedlings (Borja 1995). Seedling survival decreased and the percentage of *P. dimorphum* recovered increased when longleaf pine seedlings were inoculated with increasing amounts of inoculum and stored for 4 weeks (Sun 1996). In our trial, *P.*

dimorphum reduced longleaf pine RCD but not seedling survival after 60 days in the aquariums.

Adding benomyl (5% ai + clay slurry) to stored seedlings reduced the number and length of loblolly pine root tips, yet seedling survival remained near 100% (Barnett et al. 1988). Barnett and others (1988) assumed *Pythium* was the primary pathogen on roots before storage, and benomyl was able to control the pathogen and improve RGP. Unfortunately, benomyl is no longer available and further testing of seedling survival and RGP, fungicides, and *Pythium* may be warranted. Determining fungicide efficacy in storage could yield insight to the behavior of *Pythium* spp. and seedling survival and RGP after exposure to varying storage periods. These results are the first time that outplanting survival of slash pine has been associated with a disease of *P. irregulare* and indicates that slash pine is more sensitive to injury from *Pythium* than loblolly pine.

4.5.1. Summary

Inoculations with *P. dimorphum* and *P. irregulare* did not affect longleaf pine RGP but they reduced RCD. *Pythium* reduced loblolly and slash pine RGP, and after outplanting, only *P. irregulare*-inoculated slash pine with reduced RGP experienced decreases in survival. In contrast, despite reductions in RGP, *P. dimorphum*-inoculated loblolly and slash pine survived similarly to non-inoculated seedlings. The null hypothesis that seedling RGP and survival are not affected by *Pythium* inoculation and cold storage was rejected.

4.6 Acknowledgements

The author would like to thank Doug Shelburne at Smurfit-Stone's Rock Creek Nursery in Brewton, AL for providing the seedlings used in the experiments. Special thanks are awarded to Barry Brooks, Marietjie Quicke, and Patrick Jernigan for their assistance.

4.7. Literature Cited

Asiegbu, F.O., A. Lonneborg, and M. Johansson. 1996. Chitin and glucans detected in the cell walls of *Pythium dimorphum*-an oomycetous fungus. European Journal of Forest Pathology 26: 315-321.

Barden, C.J. and P.P. Feret. 1986. Root growth potential of loblolly pine seedlings held in cold storage under differing moisture treatments. In: Schroeder, R.A., ed., Proc. of Southern Forest Nursery Association Conference, July 22-24, 1986, Pensacola, FL: 218-223.

Barnett, J.P., J.C. Brissette, A.G. Kais, and J.P. Jones. 1988. Improving field survival of southern pine seedlings by treating with fungicides before storage. Southern Journal of Applied Forestry 12: 281-285.

Borja, I. 1995. Root dieback and defense responses in Norway spruce seedlings infected with the fungal pathogen, *Pythium dimorphum*. PhD Dissertation, Agricultural University of Norway: 126 p.

DeWald, L.E. and P.P. Feret. 1988. Changes in loblolly pine seedling root growth potential, dry weight, and dormancy during cold storage. Forest Science 34(1): 41-54.

Dierauf, T.A. 1976. Fall planting and storage using non-dormant seedlings. In: Proc. Southeastern Area Nurseryman's Conference-SC Eastern: 31-36.

Feret, P.P., R.E. Kreh, and L.E. DeWald. 1985. Root growth potential of stored loblolly pine seedlings. In: Shoulders, E. ed., Proc. of the third biennial southern silvicultural research conference, Atlanta, GA, Gen. Tech. Rep. SO-54. New Orleans, LA: USDA Forest Service, Southern Forest Experiment Station: 18-24.

Garber, M.P. and J.G. Mexal. 1980. Lift and storage practices: their impact on successful establishment of southern pine plantations. New Zealand Journal of Forest Science 10(1): 72-82.

Hallgren, S.W. and C.G. Tauer. 1989. Root growth potential, first-year survival, and growth of shortleaf pine seedlings show effects of lift date, storage, and family. Southern Journal of Applied Forestry 13: 163-169.

Hebb, E.A. 1982. Cold storage of Choctawhatchee sand pine seedlings: Effect of lifting date and length of storage. Southern Journal of Applied Forestry 6(4): 229-231.

Hendrix, F.F., Jr. and W.A. Campbell. 1971. A new species of Pythium with spiny oogonia and large chlamydospores. Mycologia 63: 978-982.

Ho, H.H. 1986. *Pythium dimorphum* from rhododendron. Mycopathologia 93: 141-145.

Jones, J.P., D.J. Pantone, J.P. Barnett, and J.C. Brissette. 1992. The relationship between fungal population development and root damage of cold-stored longleaf pine seedlings. Louisiana State University Agricultural Center, Bulletin No. 832: 23p.

Kahler, L.H. and A.R. Gilmore. 1961. Field survival of cold stored loblolly pine seedlings. Tree Planters' Notes, Issue 45: 15-16.

Kelley, W.D. and S.W. Oak. 1989. Forest Nursery Pests. In: Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, Jr., R.S., and Harvey, T.V, tech. coords., USDA Forest Service, Agricultural Handbook No. 680: 184 pp.

Kim, Y.K., C.L. Xiao, and J.D. Rogers. 2005. Influence of culture media and environmental factors on mycelia growth and pycnidial production of *Sphaeropsis pyriputrescens*. Mycologia 97(1): 25-32.

Morris, L.A. and R.G. Campbell, 1991. Soil and site potential, p. 183-20. In: M.L. Duryea and P.M. Dougherty (eds.). Forest regeneration manual. Kluwer Academic Publishers, Norwell, MA.

Palmer, L. and I. Holen. 1986. The aquarium tester-a fast, inexpensive device for evaluating seedling quality. Tree Planters' Notes 37(3): 13-16.

Rietveld, W.J. 1989. Evaluation of three root growth potential techniques with tree seedlings. New Forests 3: 181-189.

Simpson, D.G. and G.A. Ritchie. 1996. Does RGP predict field performance? A debate. New Forests 13: 249-273.

South, D.B. H.S. Larsen, H.M. Williams, and J.N. Boyer.1989. Use of seedling size as a covariate for root growth potential studies. Auburn University Southern Forest Management Cooperative, Technical Note Number 34: 8p.

South, D.B. 1999. The December dip of loblolly pine. In: Landis, T.D., Barnett, J.P., tech. coords., National Proc: forest and conservation nursery associations –1998. Gen. Tech. Rep. SRS-25. Asheville, NC, USDA Forest Service, Southern Research Station: 14-17.

Sun, X. 1996. Biological control of Pythiaceous fungi causing death of longleaf pine seedlings during cold storage. PhD dissertation, Louisiana State University: 135 pp.

Venator, C.R. 1984. Evidence of the presence of a lifting window for loblolly pine nursery seedlings. In Proc: Southern Nursery Conference-Eastern Session, Asheville, NC: 184-190.

CHAPTER 5

PYTHIUM POPULATIONS IN BAREROOT NURSERY SOILS AND *IN VITRO* ANALYSIS OF PROLINE® FOR CONTROL OF *PYTHIUM* AND *BOTRYTIS* SPP.

5.1. Abstract

During lifting, root systems can be wounded, which may allow soil-borne fungi such as *Pythium* to infect seedlings during cold storage and cause seedling mortality after outplanting. While nursery soils are either fumigated prior to sowing or sprayed as needed with fungicides, successful control of *Pythium* is dependent on fungicide efficacy and management of nursery soils that discourages *Pythium* activity.

To determine if seedling survival was related to *Pythium* levels in the soil, bareroot nursery soils were sampled in November and January in two consecutive years and assayed using selective agar media. *Pythium* levels were not related to sampling date.

In an effort to control storage fungi, the fungicide Proline® was tested *in vitro* against *Pythium dimorphum, Pythium irregulare,* and *Botrytis cinerea*. *Pythium irregulare* growth was not affected, while Proline® was fungistatic to *B. cinerea* and fungicidal to *P. dimorphum*. Proline® may be a viable option for control of *P. dimorphum* but not for *P. irregulare* or *B. cinerea*.

5.2. Introduction

Pine seedlings are often held in cold storage for several days or even weeks before being shipped from forest tree nurseries. Long-term cold storage (> 1 week) from October to mid-December can decrease bareroot seedling survival compared to when seedlings are lifted and stored in January (Kahler and Gilmore 1961; Dierauf 1976; Hebb 1982; Venator 1984). During lifting, bareroot seedling roots are wounded (May 1984), and the wounds could serve as infection sites for soil-borne fungi, particularly the "water molds" such as *Pythium*. Once seedlings are placed in storage, the moist, cool (1-5°C) conditions may provide an environment conducive for *Pythium* growth that results in seedling mortality after outplanting.

Pythium is a common pathogen found in nursery soils and is associated with "damping-off" disease that can cause seed to either not germinate or emerging seedlings to buckle (damp-off) at the hypocotyl area and die (Kelley and Oak 1989). *Pythium* also causes "fine feeder root disease" which infects and kills seedling feeder roots that are essential for water and nutrient absorption (Kelley and Oak 1989). *Pythium* spores can move throughout the soil profile and nursery bed after rainfall or irrigation (Kelley and Oak 1989). Factors that can contribute to increased *Pythium* activity include altered soil drainage caused by the use of machinery (Dumroese and James 2005), low soil pH, and the use of contaminated equipment (Kelley and Oak 1989).

Another nursery pathogen, *Botrytis cinerea* Pers.: Fr, the causal agent of gray mold, can develop in the nursery and continue in cold storage (Srago and McCain 1989). Unlike *Pythium*, *B. cinerea* is a foliage pathogen that infects seedlings in the nursery via airborne spores (Srago and Mccain 1989), yet both pathogens require moist conditions to

grow. Improving air circulation and less frequent irrigation are two ways to prevent *B. cinerea* activity (Srago and McCain 1989).

In an effort to control *Pythium*, bareroot nursery soils are routinely fumigated every 2 to 3 years. Methyl bromide has been the most effective fumigant for years (Duniway 2002) but other chemicals such as chloropicrin, metam sodium, and dazomet have been used (Fraedrich and Dwinell 2003; Cram et al. 2007). Occasionally, these fumigants do not completely sterilize nursery beds, and patches of soil containing *Pythium* remain (Duniway 2002). *Pythium* can then increase and spread to nearby soil that was successfully sterilized.

In addition to soil fumigation, fungicides are often applied to control *Pythium*. Boyer and South (1984) surveyed 63 nursery managers and found that Fermate®, Captan®, and Benlate® were the most popular fungicides for controlling damping-off. Today, Captan®, Subdue®, and Aliette® are used (pers. comm., Dr. Tom Starkey, Research Fellow, Auburn University). These fungicides are usually applied to seedlings within weeks of germination and not at all when lifted (7-8 months-old). If *Pythium* is being transferred from nursery soils and into cold storage on infected seedling roots, perhaps a fungicide treatment prior to lifting would reduce outplanting mortality. Although not yet registered for use in forest seedling nurseries, the fungicide Proline® (prothioconazole) has shown promise for controlling fusiform rust, pitch canker (*Fusarium circinatum* Nirenberg and O'Donnell), and *Rhizoctonia* foliar blight (*Rhizoctonia solani* Kuhn) (Starkey and Enebak, In press). Since Proline® has been effective in controlling three major nursery diseases, it might be useful to prevent seedling diseases in cold storage.

To determine the *Pythium* populations in bareroot nursery soils and the effect that Proline® had on *Pythium* growth, two separate experiments were conducted. The null hypotheses tested: 1) *Pythium* populations are similar in soils taken from southern forest tree nurseries in November and January and 2) Proline®-amended agar has no effect on the survival of *Pythium dimorphum* Hendrix and Campbell, *Pythium irregulare* Buisman, and *Botrytis cinerea*.

5.3 Materials and Methods

5.3.1. Bareroot Nursery Soil Surveys

5.3.1.1. Soil Sampling

In November and January 2008/2009 (Year 1) and 2009/2010 (Year 2), soil collecting kits, containing a soil sampling box, collecting instructions, and return postage, were mailed to 28 bareroot seedling nurseries in the southern U. S. Soils were sampled from an operational nursery bed prior to lifting seedlings. First, the top 5 cm was removed and samples collected from the next 15-20 cm at every 20-30 m within the nursery bed. Samples were labeled with the tree species grown in the sample area, the date of sampling, and the date of the last soil fumigation (Year 2). Upon receipt at Auburn University, soils were placed in cold storage (4-5°C) until processed.

5.3.1.2. *Pythium* Soil Assays

Soil samples collected from the nurseries were assayed in May 2009 (Year 1) and March 2010 (Year 2). Soils were transferred from the sampling boxes into plastic bags and mixed thoroughly. From the soil sample, a 1 g sub-sample was added to a 250 mL flask containing 100 mL distilled water/agar solution (2 g Difco® agar/1000 mL distilled

water). Two flasks per soil sample were placed on a shaker for 20 min. From each flask, 13 drops (1 mL) of the soil solution was plated onto *Pythium* selective media (Feng and Dernoeden 1999). The dropper was sterilized in 20% bleach/water solution and rinsed in distilled water before plating the next sample. A total of ten plates (repetitions) and 10 mL of soil solution were assayed per soil sample. *Pythium* recovery was the number of colony forming units (CFUs) identified on the selective media. In cases where soils were assayed from the same nursery in both November and January, a two sample paired t-test was performed using SAS statistical software (9th ed., SAS Institute, Cary, NC). Data from Year 1 and Year 2 were analyzed separately.

5.3.2. Proline®-Amended Agar Study

Pythium dimorphum and *Pythium irregulare* were obtained from American Type Culture Collection (ATCC®, Manassas, VA) and aseptically transferred to oatmeal agar (Kim et al. 2005). From the advancing margin of the mycelium, three 0.5 cm disks of *P. dimorphum* and *P. irregulare* were transferred to oatmeal agar to use later in the study. *Botrytis cinerea* was obtained from pine seedlings infected in cold storage. Needles were surface sterilized in a 10% bleach/water solution and rinsed two times in sterile, distilled water. After sterilization, five 2.5-cm needle segments were randomly chosen and placed onto PDA. From the advancing margin of the mycelium, three 0.5 cm disks of *B. cinerea* were transferred to potato dextrose agar (PDA) (Difco®) to use later in the study.

Proline® fungicide was added to PDA after autoclaving but before pouring into plates. Three rates of the fungicide were used: 1.25 fl oz/ac (0.25x label rate), 2.5 fl oz/ac (0.5x), 5 fl oz/ac (1x), and a control (no fungicide). There were 13 plates (replications) of

each treatment per fungus species. Using a #4 cork borer, an 8 mm plug of *P. dimorphum, P. irregulare,* and *B. cinerea* were placed at the center of each agar plate, and the radial fungal growth was measured over a 10-day period. A plate was considered covered when radial growth reached 80 mm.

To determine if the treatments were fungicidal (killed the fungus) or fungistatic (stopped fungal growth), on day 11, the fungal plugs from the amended and control PDA plates were transferred to non-amended PDA plates. Radial growth was measured again for 9 days. Statistical analyses were conducted using a General Linear Model (GLM) in SAS statistical software (9th ed., SAS Institute, Cary, NC). Means of radial fungal growth in the Proline®-amended agar trials were analyzed using Analysis of Variance (ANOVA) in a factorial design.

5.4. Results

5.4.1. Bareroot Nursery Soil Surveys

5.4.1.1. Year 1

Of the 16 nurseries with *Pythium,* eight were collected in the fall (Table 5.1). Two nurseries (U and V) accounted for 74% of the total *Pythium* recovered in the fall (Table 5.2). Of 9 nurseries that had no *Pythium* in November, five were positive and four remained negative for *Pythium* in January (Table 5.2).

5.4.1.2. Year 2

Similar to Year 1 samples, two nurseries (A and X) accounted for 64% of the total *Pythium* in the fall (Table 5.3). Sixteen nurseries provided soil samples in both fall and

winter; six were negative for *Pythium* in each season and two did not change (Table 5.3). Of the other eight nurseries, one had lower levels of *Pythium* in the winter, while *Pythium* levels increased in the winter by 14,000 CFUs/mg soil in seven nurseries (Table 5.3).

Table 5.1. Number of soil survey responses and nurseries that had a positive or negative *Pythium* sample.

	Year 1		Year 2	
	Nov 2008	Jan 2009	Nov 2009	Jan 2010
Responding Nurseries	19	16	20	19
Nurseries with *Pythium*	8	8	9	13
Nurseries without *Pythium*	11	8	11	6

Table 5.2. Number of colony forming units (CFUs) per mg of soil in November 2008 and January 2009 (Year 1).

	CFU/mg soil	
Nursery	November 2008	January 2009
A	7,000	——
B	——	2,000
C*	0	5,000
D*	0	9,000
E*	0	3,000
F	0	——
H	0	——
I	13,000	——
J	1,000	——
K*	0	0
L*	0	1,000
M*	1,000	0
N*	0	0
O*	1,000	1,000
R*	0	0
U*	57,000	9,000
V	33,000	——
X*	0	0
Y*	0	10,000
Z*	9,000	0
BB	——	0
Total	122,000	41,000
Mean of (+) Nurseries	15,000	5,000

* = paired t-test results (T = 0.57; P > T = 0.5774; Error DF= 12)

Table 5.3. Number of colony forming units (CFUs) per mg of soil in November 2009 and January 2010 (Year 2).

	CFU/mg soil	Years	CFU/mg soil	Years
Nursery	Nov 2009	Last Fum[z]	Jan 2010	Last Fum
A	37,000	1	——	——
B	——	——	1,000	2
C*	0	1	0	1
D*	0	——	0	2
E*	0	1	4,000	2
F	0	1	——	——
G	9,000	0.5	——	——
H*	0	1	4,000	0.5
I*	0	1.5	0	——
K*	0	1	4,000	2
L*	3,000	1	12,000	1
M*	4,000	1.5	33,000	——
N*	0	3	11,000	2
O*	2,000	3	2,000	——
Q*	0	2	0	——
R*	7,000	1	8,000	——
T	——	——	11,000	1
U	——	——	11,000	2
W	4,000	1	——	——
X*	30,000	1	4,000	1
Y*	0	1	5,000	1
Z*	0	1	0	3
AA	8,000	1	——	——
BB*	0	1.5	0	——
Total	104,000		110,000	
Mean of (+) Nurseries	12,000		8,000	

[z] = # of years since last soil fumigation

* = paired t-test results (T = -0.97; P > T = 0.3492; Error DF = 15)

5.4.2. Proline®-Amended Agar Study

The effect of the Proline® on control of the fungi was dependent upon the species. By day 8, *P. dimorphum* had completely covered (80 mm) the non-amended PDA plates (Figure 5.1). However, after 10 days, *Pythium dimorphum* growth was not evident on any of the amended PDA plates (Figure 5.1). In contrast, *P. irregulare* was not affected by Proline® (Figure 5.2). *Pythium irregulare* growth on non-amended and Proline®-amended agar plates reached 80 mm by day 4 (Figure 5.2). Although, *B. cinerea* growth was minimal (< 6 mm) on amended agar (Figure 5.3), the fungicide resulted in *B. cinerea* to grow aerially instead of along the media surface (Figure 5.4). Growth of *P. dimorphum* and *P. irregulare* is shown in Figure 5.5.

On day 11, the plugs were removed from the amended agar and placed onto non-amended PDA. After 9 days on the agar plates, *P. dimorphum* did not grow indicating 100% control with Proline® (Figure 5.6). In contrast, Proline® was fungistatic to *B. cinerea* as fungal growth was > 45 mm on non-amended media (Figure 5.7), but plugs from non-amended agar grew more (LSD = 9) than isolates taken from amended agar. *Pythium irregulare* was not affected by any of the fungicide treatments and were not included in the re-isolation trial.

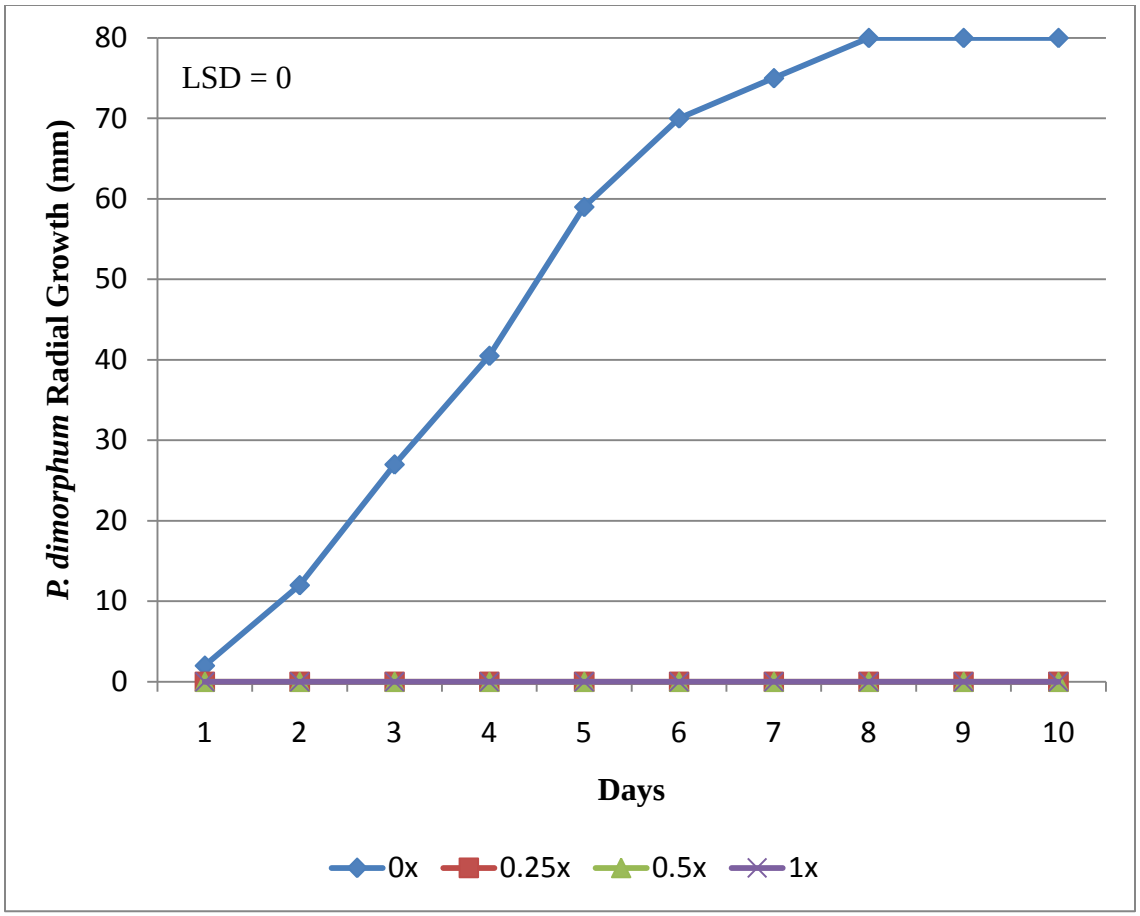

Figure 5.1. Radial growth of *Pythium dimorphum* on Proline®-amended agar. The least significance difference (LSD) value corresponds with radial growth on day 10.

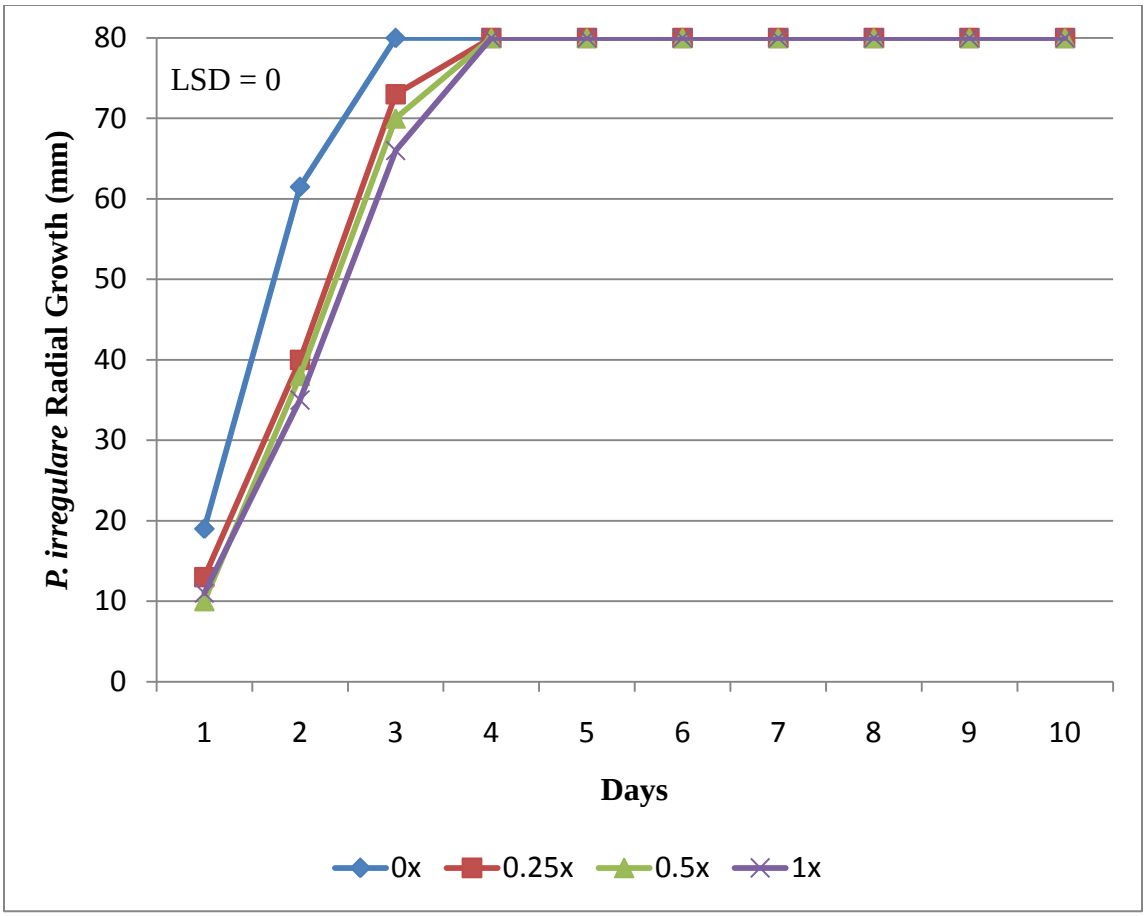

Figure 5.2. Radial growth of *Pythium irregulare* on Proline®-amended agar. The least significance difference (LSD) value corresponds with radial growth on day 10.

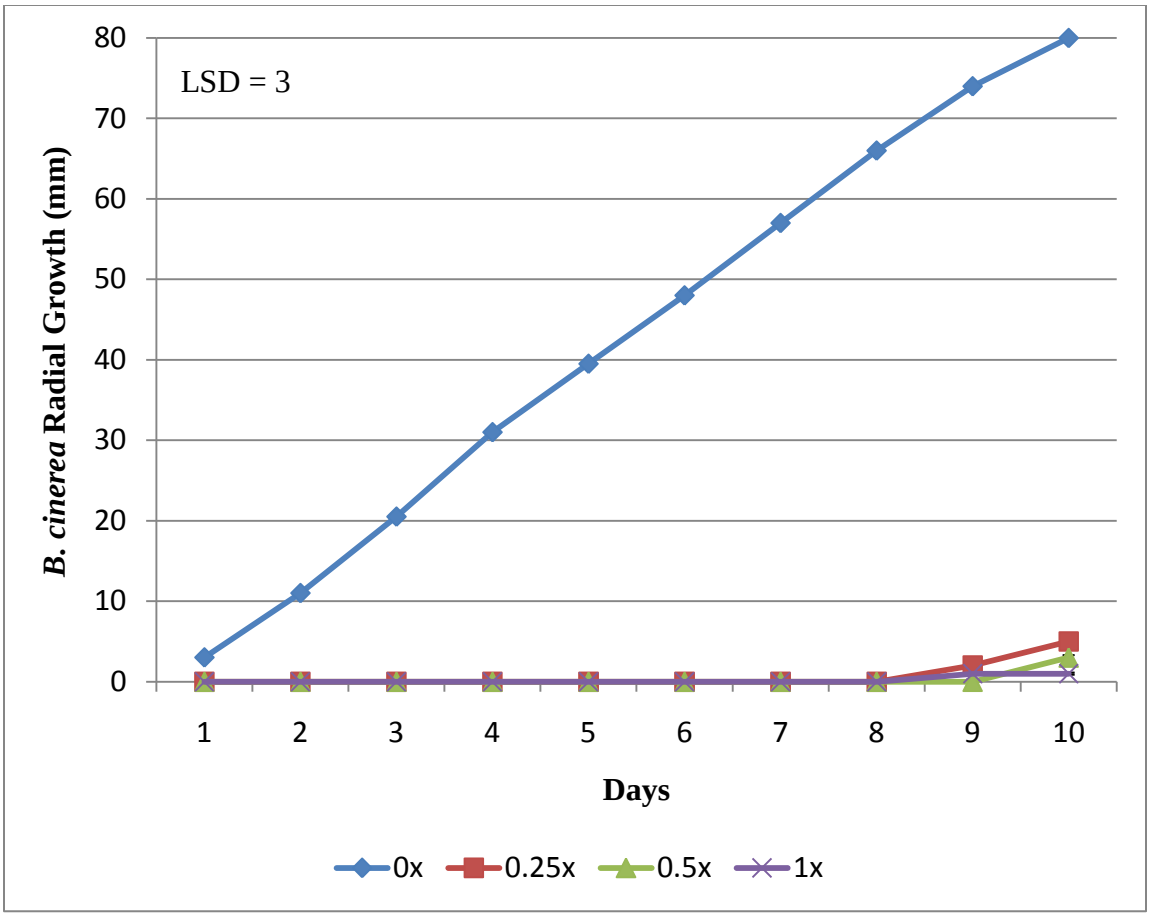

Figure 5.3. Radial growth of *Botrytis cinerea* on Proline®-amended agar. The least significance difference (LSD) value corresponds with radial growth on day 10.

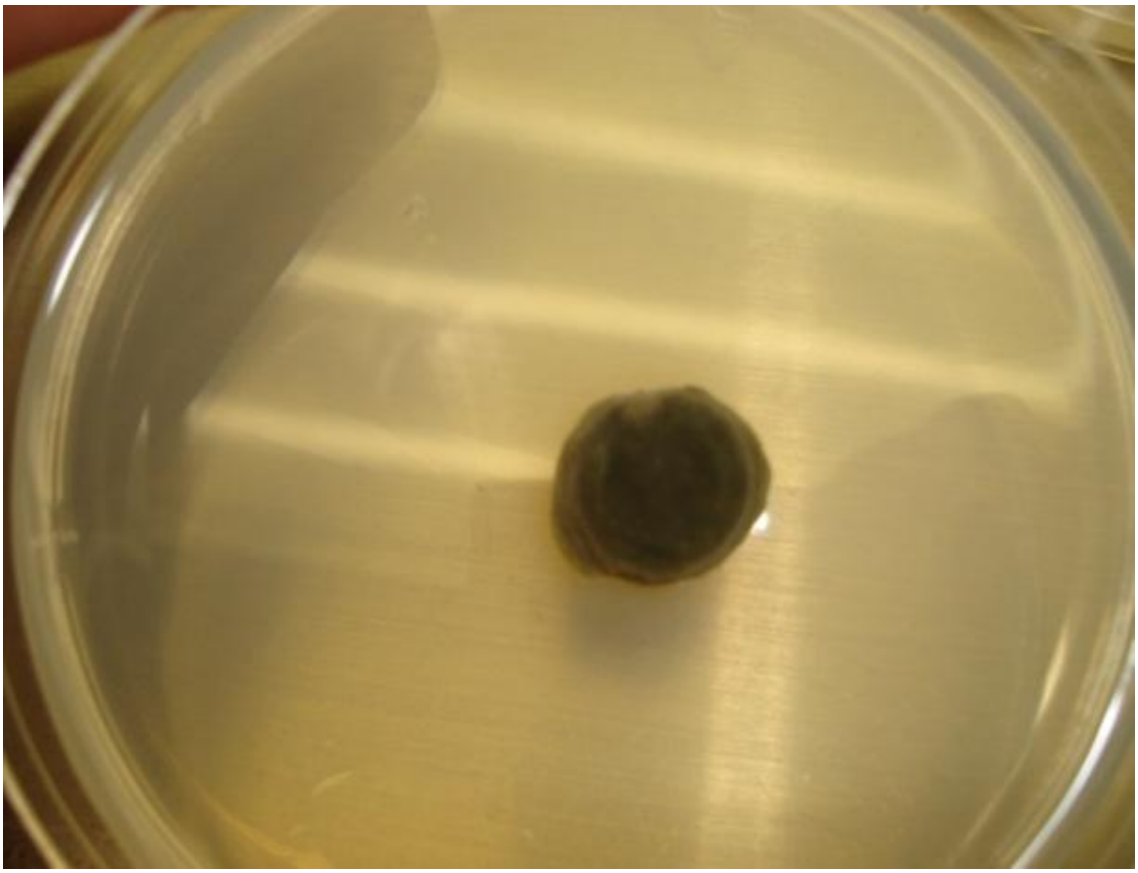

Figure 5.4. Aerial growth of *Botrytis cinerea*.

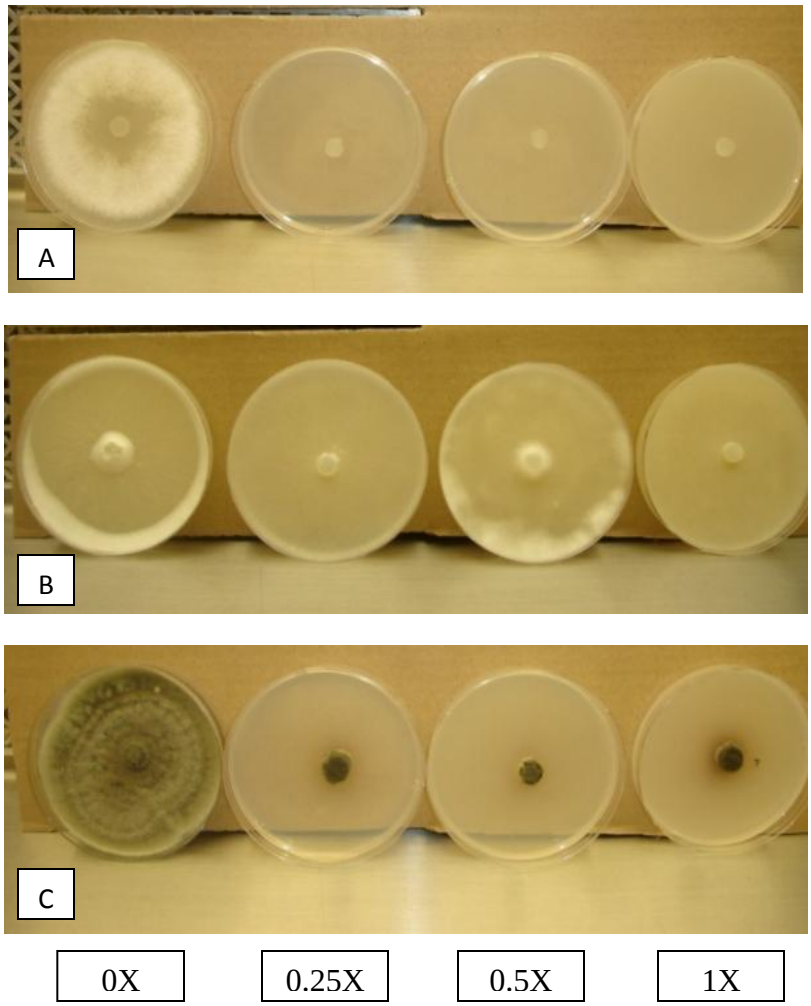

Figure 5.5. A. *Pythium dimorphum* growth on PDA agar amended with 0x, 0.25x, 0.5x, or 1x the labeled rate of Proline®. B. *Pythium irregulare* growth on PDA agar amended with 0x, 0.25x, 0.5x, or 1x the labeled rate of Proline®. C. *Botrytis cinerea* growth on PDA agar amended with 0x, 0.25x, 0.5x, or 1x the labeled rate of Proline®.

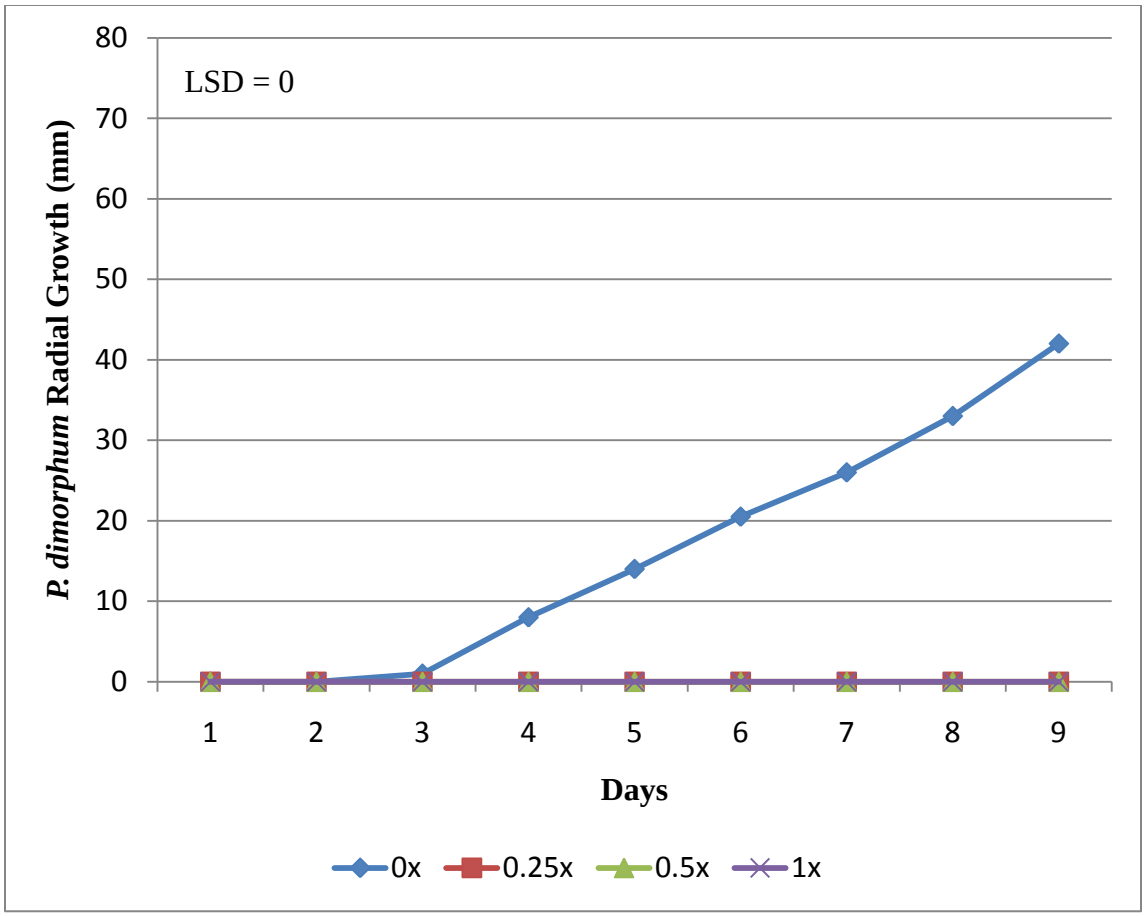

Figure 5.6. Radial growth of *Pythium dimorphum* that was re-isolated on non-amended potato dextrose agar (PDA). The least significance difference (LSD) value corresponds with radial growth on day 9.

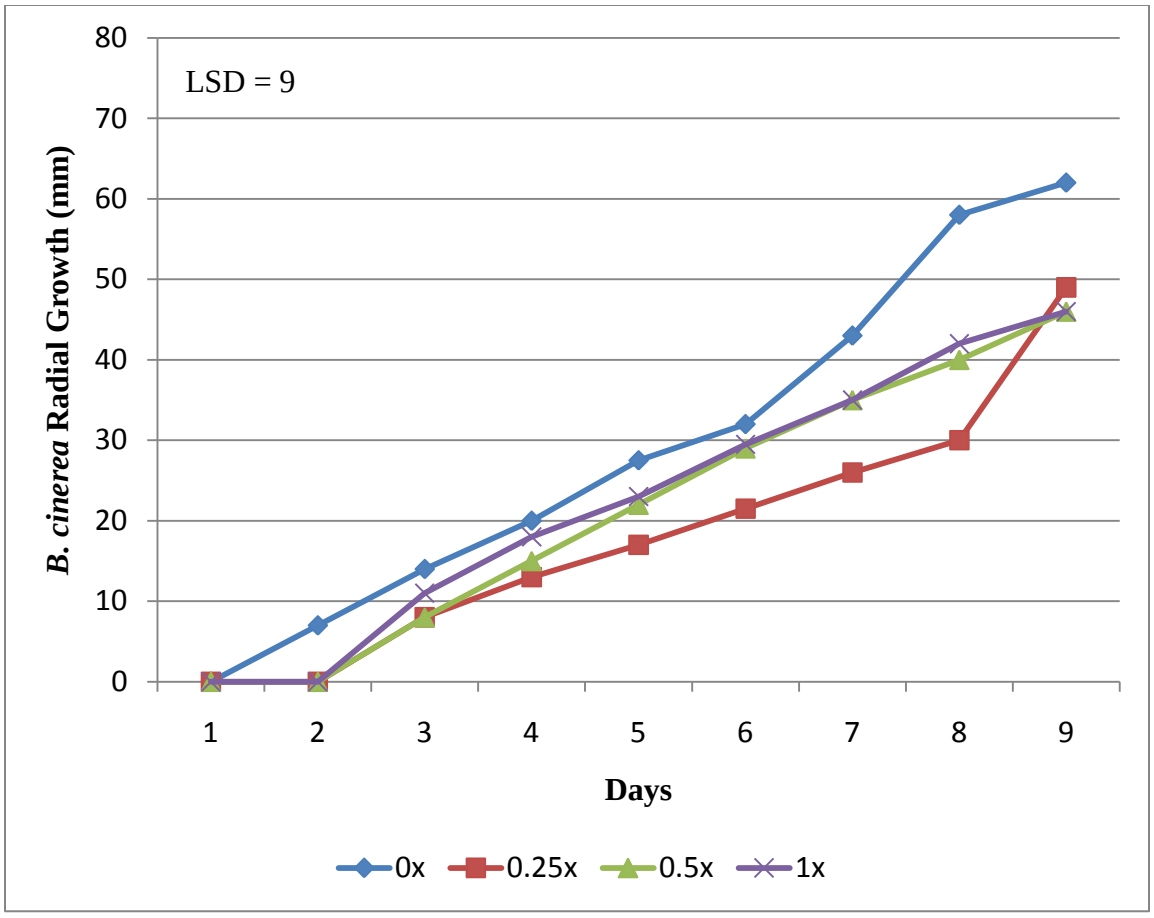

Figure 5.7. Radial growth of *Botrytis cinerea* that was re-isolated on non-amended potato dextrose agar (PDA). The least significance difference (LSD) value corresponds with radial growth on day 9.

5.5 Discussion

5.5.1. Bareroot Nursery Soil Surveys

Pythium populations in bareroot nursery soils were variable between the fall and winter seasons for both years. Because *Pythium* was present during the fall and winter, it is possible that seedling roots can become infected with *Pythium* during the fall lifting season. Other studies from soils sampled on two agricultural sites in two consecutive years have shown a steady increase in *Pythium* populations from September to December followed by a decrease from December to May with the highest levels reaching > 2,500 CFUs/g dry soil (Ali-Shtayeh et al.1986). The authors speculated that higher levels of *Pythium* in the fall may be caused by increases in soil organic material, lower levels of antagonistic fungi, or spore activation in favorable conditions.

One possibility for the variation in *Pythium* populations observed among the nurseries sampled could be the timing since the last soil fumigation. Surprisingly, more *Pythium* was recovered from soils sampled less than one year since fumigation. Fumigation time, type, and rates can differ between nurseries, which may result in variable control of *Pythium*. Spring fumigation using a standard 67% methyl bromide/33% chloropicrin combination (MC33) at 393 kg/ha, chloropicrin at 336 kg/ha (CP336), and a combination of metam sodium at 90 kg/ha and chloropicrin at 163 kg/ha (MS/CP) reduced *Pythium* spp. in April, but by November of the same year, *Pythium* populations were similar to those in non-fumigated soils (Cram et al. 2007). Similarly, a spring fumigation with MC33 at 360 kg/ha controlled *Pythium*, but populations rebounded by spring of the next year resulting in disease (Tanaka et al. 1986).

Another factor that contributes to *Pythium* survival is a suitable soil environment for the fungus. Cultural practices used to produce forest tree seedlings are well established and similar between nurseries. However, the methods used to implement these practices differ based on the idiosyncrasies of the nursery manager. *Pythium* requires high soil moisture conditions that can develop in areas with high soil compaction or improper field drainage. For example, *Pythium* CFUs increased in nursery bed depths of 12-24 cm when tilled with a moldboard plow but decreased at 0-15 cm depths when a disc was used (Juzwik et al. 1999). Sometimes, nursery personnel are unaware that routine seedling maintenance may promote *Pythium* activity, which could lead to subsequent seedling infection. Due to the ability of *Pythium* to survive and grow in storage (Chapter 2), the infection of even one seedling during lifting could cause *Pythium* to infect other seedlings in storage.

5.5.2. Proline®-Amended Agar Study

Proline® showed variable *in vitro* control of *Pythium* and *Botrytis* spp. when amended into agar media. *Pythium irregulare* was not affected by any rate of Proline® used, while *P. dimorphum* was controlled by all rates. *Pythium dimorphum* and *P. irregulare* have been shown to reduce bareroot seedling survival after storage (Chapter 2), and as reported here, *Pythium* is recovered from the soil throughout the lifting season. Therefore, even though Proline® was effective against *P. dimorphum,* it may not offer adequate control if seedlings are treated. Likewise, due to the fungistatic relationship with *B. cinerea*, Proline® applications would only slow fungal growth and could allow the possibility of carry-over into the field.

Studies have reported that Proline® controlled *Fusarium circinatum* and *Rhizoctonia solani* and reduced seedling disease in the field (Starkey and Enebak, In press). However, Proline® was ineffective at controlling *Fusarium sambucinum* Fuckel on passionfruit vines in New Zealand (Rheinlander et al. 2009), and it appears the fungicidal nature of Proline® may be species specific as seen with control differences between *P. dimorphum* and *P. irregulare* and *F. circinatum* (Starkey and Enebak, In press) and *F. sambucinum* (Rheinlander et al. 2009). Proline® has not been tested on pine seedlings for control of *B. cinerea*. Captan® controlled the fungus on lodgepole pine (*Pinus contorta*), while benomyl did not (James and Woo 1984). Captan® is still used in nurseries and may be useful in preventing *B. cinerea* prior to lifting. The use of fungicides to control *Pythium* damping-off after seedling germination is common, but the efficacy of fungicides remains unknown for controlling the dynamic levels of *Pythium* in the soil during the lifting season.

Future trials could test over-the-top applications of Proline® when seedlings are either outplanted into soil that is amended with *Pythium* or when seedling stems are inoculated with *Botrytis cinerea*. Results from such trials may be of more value in determining the effectiveness of Proline® as an over-the-top application to seedlings prior to lifting and storage. Also, studies that apply Proline® to seedlings that have been inoculated with *Pythium* after lifting but before storage may yield insight to the effect on seedling survival.

5.5.3. Summary

Pythium populations were variable in soils collected from bareroot nurseries in November and January. In Year 1, *Pythium* levels were higher in the fall, whereas higher levels were found in winter soils in Year 2. The variability of *Pythium* in the soil between seasons may be attributed to the lack of effective fumigation or nursery practices that promote an environment for the fungus. The results indicate that it is possible for *Pythium* to be in the soil during the fall and infect seedlings during lifting.

The fungicide Proline® was effective in controlling *Pythium dimorphum* but not *Pythium irregulare* or *Botrytis cinerea*. Due to inconsistent control between species, applying Proline® over-the-top of seedlings prior to lifting to prevent infection in cold storage would not be recommended.

5.6. Acknowledgements

The author would like to thank all of the nurseries in the Southern Forest Nursery Management Cooperative that took time to collect and send soil samples for analysis. Also, special thanks go to Dr. Tom Starkey and Marietjie Quicke for their assistance with the experiments.

5.7. Literature Cited

Ali-Shtayeh, M.S., L. Chee Len, and M.W. Dick. 1986. The phenology of *Pythium* (Peronosporomycetidae) in soil. Journal of Ecology 74: 823-840.

Boyer, J.N. and D.B. South. 1984. Forest nursery practices in the south. Southern Journal of Applied Forestry 8: 67-75.

Cram, M.M., S.A. Enebak, S.W. Fraedrich, L.D. Dwinell, and S.J. Zarnoch. 2007. Evaluation of fumigants, EPTC herbicide, and *Paenibacillus macerans* in the production of loblolly pine seedlings. Forest Science 53(1): 73-83.

Dierauf, T.A. 1976. Fall planting and storage using non-dormant seedlings. In: Proc. Southeastern Area Nurseryman's Conference-SC Eastern: 31-36.

Dumroese, R.K. and R.L. James. 2005. Root diseases in bareroot and container nurseries of the Pacific Northwest: epidemiology, management, and effects on outplanting performance. New Forests 30: 185-202.

Duniway, J.M. 2002. Status of chemical alternatives to Methyl Bromide for pre-plant fumigation of soil. Phytopathology 92(12): 1337-1343.

Feng, Y. and P.H. Dernoeden. 1999. *Pythium* species associated with root dysfunction of creeping bentgrass in Maryland. Plant Disease 83: 516-520.

Fraedrich, S.W. and L.D. Dwinell. 2003. An evaluation of Dazomet incorporation methods on soilborne organism and pine seedling production in southern nurseries. Southern Journal of Applied Forestry 27(1): 41-51.

Hebb, E.A. 1982. Cold storage of Choctawhatchee sand pine seedlings: Effect of lifting date and length of storage. Southern Journal of Applied Forestry 6(4): 229-231.

James, R.L. and J.Y. Woo. 1984. Fungicide trials to control Botrytis blight at nurseries in Idaho and Montana. Tree Planters' Notes 16: 4 p.

Juzwik, J., K.M. Gust, and R.R. Allmaras. 1999. Influence of cultural practices on edaphic factors related to root disease in *Pinus* nursery seedlings. Plant and Soil 207: 195-208.

Kahler, L.H. and A.R. Gilmore. 1961. Field survival of cold stored loblolly pine seedlings. Tree Planters' Notes, Issue 45: 15-16.

Kelley, W.D. and S.W. Oak. 1989. Forest Nursery Pests. In: Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, Jr., R.S., and Harvey, T.V, tech. coords., USDA Forest Service, Agricultural Handbook No. 680: 184 p.

Kim, Y.K., C.L. Xiao, and J.D. Rogers. 2005. Influence of culture media and environmental factors on mycelia growth and pycnidial production of *Sphaeropsis pyriputrescens*. Mycologia 97(1): 25-32.

Rheinlander, P.A., R.A. Fullerton, and P. Sale. 2009. Sustainable management of passionfruit diseases in New Zealand. Final Report 2687, The New Zealand Institute for Plant and Food Research, Auckland, New Zealand: 44 p.

Srago, M.D. and A.H. McCain. 1989. Forest Nursery Pests. In: Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, Jr., R.S., and Harvey, T.V, tech. coords., USDA Forest Service, Agricultural Handbook No. 680: 184 p.

Starkey, T.E. and S.A. Enebak. (In press). The use of prothioconazole to control forest nursery diseases of *Pinus* spp. In: Proc. Of the 7th Meeting of IUFRO Working party 7.03-04, USDA Forest Service, Southern Region, Forest Health Protection, Report 10-X: X-X.

Tanaka, Y., K.W. Russell, and R.G. Linderman. 1986. Fumigation effect on soilborne pathogens, mycorrhizae, and growth of Douglas-fir seedlings. In: Landis, T.D., ed., Proc. Western Forest Nursery Council, GTR-RM-137, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station: 147-152.

Venator, C.R. 1984. Evidence of the presence of a lifting window for loblolly pine nursery seedlings. In Proc: Southern Nursery Conference-Eastern Session, Asheville, NC: 184-190.

CHAPTER 6

CONCLUSIONS

The purpose of these studies was to answer two questions regarding the storability of southern pine (*Pinus* spp.) seedlings: 1) why have bareroot seedlings experienced poor survival following long-term cold storage during the months of October to mid-December and 2) why is container-grown seedling survival unaffected by lifting and long-term storage during the same period? A previous study by Sun (1996) reported that bareroot longleaf pine (*Pinus palustris*) survival decreased as the level of *Pythium dimorphum* applied to seedling roots increased prior to storage. These results led to the theory that *Pythium* may be infecting seedling roots through wounds sustained during lifting, which then multiply in cold storage, and cause seedling mortality after outplanting. This theory served as a baseline for the experiments in this dissertation, and the following are the conclusions of the experiments and prospects for future research direction.

In Chapter 2, seedling survival was evaluated by inoculating bareroot and container-grown seedlings with *Pythium* and storing them for up to 12 weeks. Bareroot longleaf pine survival decreased as a result of the *Pythium* inoculations but bareroot loblolly (*Pinus taeda*) and slash pine (*Pinus elliottii*) were not affected by the *Pythium*.

The results for longleaf pine mirrored that found by Sun (1996) with *P. dimorphum*, but our study was the first to link *Pythium irregulare* to seedling mortality after storage. Container-grown seedling survival was not affected by *Pythium* even after wounding roots. However, *Pythium* was recovered in higher numbers from inoculated wounded roots with *P. irregulare* recovered more frequently than *P. dimorphum* on loblolly and slash pine. The root wounding treatments were to mimic the wounding of bareroot seedling roots during lifting. Even though *Pythium* and wounded roots did not affect survival, results indicate that *P. dimorphum* and *P. irregulare* are able to utilize wounds and survive in storage. In the future, experiments that involve applying fungicides to seedlings before lifting, and to seedlings that have been inoculated with *Pythium* after lifting, may yield more insight into *Pythium* behavior in storage and subsequent seedling survival.

The effects of *Pythium* on seedling survival was further investigated in Chapter 3 by examining the possibility that antagonistic fungi in peat moss may suppress *Pythium* and allow container-grown seedlings to increase their storability. In the first of two trials, bareroot loblolly pine were inoculated with *Pythium*, packed with or without a peat-mix, and stored for up to 12 weeks. The presence of the peat-mix on inoculated roots did not improve seedling survival. Both *P. dimorphum* and *P. irregulare* reduced survival and mortality was more severe in the peat-mix treatments. While this study was done to simulate a container system where seedling roots grow within a plug of peat-based media, the results indicate that something other than peat may influence the increased storability of container-grown seedlings. One possibility could be the presence of mycorrhizae on seedling roots, which can provide a physical and chemical barrier against *Pythium* (Marx

1972). During lifting, bareroot seedlings may lose their mycorrhizal roots, whereas container-grown seedlings retain mycorrhizae in an undisturbed root plug. While seedling roots were being assayed for *Pythium* in these trials, mycorrhizal roots were frequently observed on container-grown seedlings but were absent on bareroot seedlings. Research involving *Pythium* and mycorrhizae on bareroot and container-grown seedling roots and their relationship in storage would be useful for understanding effects on survival.

In the second peat moss trial, container loblolly, longleaf, slash, and shortleaf pine (*Pinus echinata*) were grown in either a peat-mix or perlite media, roots were either wounded or not wounded, and seedlings were stored for up to 8 weeks. Perlite was used to represent growing media that was devoid of peat moss, and therefore, absent of any antagonistic fungi that could suppress *Pythium*. Loblolly and slash pine survival was not affected by *Pythium* in either media type, but *P. irregulare* reduced longleaf and shortleaf pine survival. Longleaf pine survival was less in the peat-mix, while shortleaf pine survival decreased in perlite as storage time increased. Seedling genetics may have affected the way roots responded to the treatments. Longleaf pine did not benefit from antagonistic fungi that may have been present in the peat-mix. Also, by inoculating young seedlings (8-weeks-old), both longleaf and shortleaf pine roots could have been more susceptible to *Pythium*. It must be reiterated that a media with 70-80% peat moss was used in the trials. Therefore, studies that test seedling survival in the presence of *Pythium* using 100% sphagnum peat moss at different levels of decomposition and pH levels as a packing media and container substrates are warranted.

Pythium is a fine feeder root pathogen, and since fine feeder roots are critical for seedling establishment after outplanting, Chapter 4 addressed the effects that *Pythium* may have on seedling root growth potential (RGP). A seedling's RGP is used to gauge seedling performance before outplanting and is based on new root growth within a certain time period. Inoculations with *P. dimorphum* and *P. irregulare* did not affect longleaf pine RGP but reduced loblolly and slash pine RGP after 3 weeks of cold storage. After outplanting, only *P. irregulare*-inoculated slash pine with reduced RGP experienced decreases in survival. In contrast, despite reductions in RGP, *P. dimorphum*-inoculated loblolly and slash pine survived at rates similar to non-inoculated seedlings. Survival after outplanting is dependent on roots accessing water and nutrients. It is possible that *P. irregulare*-inoculated slash pine could not uptake enough resources, which led to decreases in survival.

Interestingly, *Pythium* reduced longleaf and slash pine root collar diameters (RCD). This is the first report of decreases in seedling RCD as a result of *Pythium* in a hydroponic system for testing RGP. A larger RCD usually indicates a healthier seedling and has been correlated to increased growth and survival after outplanting (South et al. 1985). Comparing the RCDs of seedlings before and after storage (before outplanting or testing RGP) in similar inoculation trials may give an indication of the effect *Pythium* has on RCD in storage.

If bareroot seedlings are infected with *Pythium* during fall lifting, *Pythium* must be present in nursery soils. Therefore, in Chapter 5, soils from bareroot nurseries were assayed to quantify *Pythium* populations between the fall and winter seasons. *Pythium* populations varied among nurseries with more *Pythium* recovered from samples collected

in the fall of Year 1 and in the winter of Year 2. In Year 2, regardless of season, *Pythium* levels were higher in soils fumigated within the previous year. Thus, it is possible that *Pythium* could be in the soil during the lifting season of the same year in which soils were fumigated. However, even though *Pythium* may be present, its activity is dependent on a favorable environment. Depending on how nursery practices are implemented, the level of *Pythium* activity can differ from nursery to nursery. For instance, the type of equipment used (Juzwick et al. 1999), increases in organic matter (Ali-Shtayeh et al. 1986), and root exudation (Hendrix and Campbell 1973) after undercutting seedling roots could increase *Pythium* populations.

There are over 120 reported species of *Pythium* (Alexopoulos et al. 1996), and so far, only *Pythium dimorphum* and *Pythium irregulare* have been tested directly on seedling roots in cold storage. Before these trials, only *P. dimorphum* had been associated with seedling mortality after storage (Sun 1996). Other fungi have been recovered from stored seedlings (Jones et al. 1992), but until now, no other study had linked *P. irregulare* to seedling mortality after storage and outplanting. *Pythium irregulare* is known primarily as a damping-off pathogen (Hendrix and Campbell 1973) and, on one occasion, for causing Mexican weeping pine (*Pinus patula*) mortality in South Africa (Linde et al. 1994). In cases where seedlings were affected by one *Pythium* species over the other, *P. irregulare* consistently showed more virulence than *P. dimorphum*. However, both can be considered storage pathogens that can reduce the survival of bareroot longleaf pine, bareroot loblolly pine in the presence of peat moss, and RGP of bareroot loblolly and slash pine. In addition, *P. irregulare* can reduce the survival of bareroot slash pine and 8-week-old container-grown longleaf and shortleaf

pine. These results suggest that if *P. dimorphum* and *P. irregulare* are actively present in the soil at lifting, infect seedling roots through wounds, and remain on seedling roots in cold storage that reductions in seedling root growth potential and survival are possible.

6.1. Literature Cited

Alexopoulas, C.J., C.W. Mims, and M. Blackwell. 1996. Introductory Mycology. 4th edition, John Wiley and Sons, Inc.: 869 p.

Ali-Shtayeh, M.S., L. Chee Len, and M.W. Dick. 1986. The phenology of *Pythium* (Peronosporomycetidae) in soil. Journal of Ecology 74: 823-840.

Hendrix, F.F., Jr. and W.A. Campbell. 1973. Pythiums as plant pathogens. Annual Reviews Phytopathology 11: 77-98.

Jones, J.P., D.J. Pantone, J.P. Barnett, and J.C. Brissette. 1992. The relationship between fungal population development and root damage of cold-stored longleaf pine seedlings. Louisiana State University Agricultural Center, Bulletin No. 832: 23 p.

Juzwik, J., K.M. Gust, and R.R. Allmaras. 1999. Influence of cultural practices on edaphic factors related to root disease in *Pinus* nursery seedlings. Plant and Soil 207: 195-208.

Linde, C., G.H.J. Kemp, and M.J. Wingfield. 1994. *Pythium irregulare* associated with *Pinus* seedling death on previously cultivated lands. Plant Disease 78: 1002-1005.

Marx, D.H. 1972. Ectomycorrhizae as biological deterrents to pathogenic root infections. Phytopathology 10: 429-454.

South, D.B., J.N. Boyer, and L. Bosch. 1985. Survival and growth of loblolly pine as influenced by seedling grade: 13-year results. Southern Journal of Applied Forestry 9(2): 76-81.

Sun, X. 1996. Biological control of Pythiaceous fungi causing death of longleaf pine seedlings during cold storage. PhD dissertation, Louisiana State University: 135 p.

APPENDIX

EVALUATION OF SELECTIVE HERBICIDES FOR YELLOW NUTSEDGE CONTROL AND LOBLOLLY AND SLASH PINE SEEDLING TOLERANCE

A.1. Abstract

Yellow nutsedge (*Cyperus esculentus*) is one of the more troublesome weeds to control in conifer nurseries. Soil fumigation with methyl bromide has been used to control nutsedge for years but may not be available in the future. For this reason, identifying herbicides that can control yellow nutsedge but do not injure pine seedlings is a primary concern of nursery managers.

Yellow nutsedge tubers and loblolly (*Pinus taeda*) and slash pine (*Pinus elliottii*) seedlings were potted separately, and treated with three rates of the herbicides dimethenamid (Tower®), mesotrione (Callisto®), imazosulfuron (Valent-V10142), and halosulfuron (Sedgehammer®). Imazosulfuron and halosulfuron provided the best suppression of nutsedge, but imazosulfuron injured loblolly and slash pine. Slash pine diameter and height growth were reduced by imazosulfuron. Halosulfuron did not affect loblolly pine but it reduced slash pine height growth and the number of new leaders the following spring. Dimethenamid and mesotrione injured slash pine but provided some

suppression of nutsedge. Halosulfuron applied at 35 to 140 g ai/ha (active ingredient per hectare) over the top of loblolly pine (≥ 60 cm height and ≥ 8 mm diameter) can control yellow nutsedge without injuring pine seedlings.

A.2. Introduction

An effective weed control program in bareroot nurseries is critical for seedling production and eradicating troublesome weeds such as yellow nutsedge (*Cyperus esculentus*). Wakeley (1954) reported yellow nutsedge as being the most difficult weed to control, and in 1981, 29 nursery managers ranked it as one of the most difficult weeds to control in bareroot nurseries (Boyer and South 1984). Two decades later, it remains as a challenge for bareroot nursery managers.

Yellow nutsedge is a perennial weed that reproduces aggressively using rhizomes and tubers and can be found world-wide across many climates and soil types (Anderson 1999). A single plant can produce seven to nine tubers at the end of a rhizome, and one tuber has been reported to produce more than 1,900 plants and 6,900 tubers within a year (Defelice 2002). Yellow nutsedge is troublesome due to its prolific germination, and because it is spread throughout the nursery with mechanical equipment (Wakeley 1954).

Soil fumigation with methyl bromide is the primary method of reducing yellow nutsedge populations in bareroot nurseries. However, methyl bromide is considered an ozone-depleting chemical under the Montreol Protocol, and a phase-out of its use is pending (Cram et al. 2007). The potential elimination of methyl bromide has led to the testing of alternative soil fumigants, but nutsedge control with these products has been variable (Carey 2000). The lack of viable alternatives intensifies the need to identify herbicides that can effectively control nutsedge while not injuring seedlings. Trials using

glyphosate in fallow fields have resulted in nutsedge control (Fraedwich and Dwinell 2003) but injury can occur when it is applied directly to loblolly pine (*Pinus taeda*) (Haywood and Melder 1991).

A number of herbicides have suppressed the growth of yellow nutsedge. Applications of chlorimuron (12 g ai/ha), imazethapyr (70 g ai/ha), cloransulam (18 g ai/ha), rimsulfuron (18 g ai/ha), and imazomox (45 g ai/ha) suppressed potted yellow nutsedge compared to non-treated controls (Nelson and Renner 1999). Of these herbicides, rimsulfuron did not affect 8-week-old bareroot loblolly pine seedling density or growth (South and Hill 2009). Unfortunately, rimsulfuron is not registered for use in forest tree nurseries. Another herbicide, monosodium methanearsonate (MSMA), has been used to control yellow nutsedge (Blum et al. 2000). When applied at 1,680 and 2,240 g ai/ha over-the-top of bareroot loblolly pine, MSMA did not reduce seedling growth and increased seedling density (South et al. 2007). However, MSMA is not labeled for use in nursery seedbeds but may be used in non-crop areas along riser lines and in fallow fields (South et al. 2007).

The herbicide fomesafen (Reflex®) is registered in some southern states for use in pine nurseries and has partial activity as a preemergence or postemergence application (may not be acceptable for commercial weed control). South (1997b) reported that preemergence applications of fomesafen at 500 g ai/ha reduced loblolly pine seedling density in silt-loam soils but did not affect loblolly pine growth in coarse textured soils. The same rate applied as a postemergence treatment did not affect loblolly pine performance in either soil texture (South 1997b). Even though fomesafen is used operationally and methyl bromide is still available, controlling yellow nutsedge remains

challenging due to its ability to spread and reproduce. Repeated applications of glyphosate in fallow fields is an acceptable method for eradicating yellow nutsedge and preventing tubers from spreading into production areas (South 1984a; Fraedrich and Dwinell 2003).

Other herbicides are labeled for control of yellow nutsedge. Dimethenamid (Tower®) is a preemergence herbicide for use on conifers including Fraser-fir (*Abies fraseri*), Douglas-fir (*Pseudotsuga menziesii*), and western red cedar (*Thuja plicata*) but not during bud break or before soil has settled around root systems. Mesotrione (Callisto®) and halosulfuron (Sedgehammer®) are applied as postemergence applications and are labeled for use on corn or landscaped areas, respectively, but not in forest tree nurseries. Imazosulfuron (Valent-V10142) is a relatively new herbicide that is highly active at low rates but is not yet registered for use in the U.S. (Morrica et al. 2002).

The objective of this study was to evaluate yellow nutsedge control and tolerance of pine seedlings to selected herbicides. The null hypothesis tested was: the herbicides dimethenamid, mesotrione, imazosulfuron, and halosulfuron have no effect on the growth of yellow nutsedge, loblolly pine, and slash pine (*Pinus elliottii*) seedlings.

A.3. Materials and Methods

A.3.1. Trial 1

Nine-month-old bareroot loblolly and slash pine seedlings were lifted from Smurfit- Stone's Rock Creek Nursery near Brewton, AL in December 2007 and placed in cold storage (4-5ºC) at Auburn University. In February 2008, 140 seedlings of each pine species were transplanted into 3.8 L plastic containers filled with pinebark:sand (6:1)

(v:v), amended with 8.3 kg/m^3 of 18-6-12 (N-P-K) Polyon™ (8-9 month control-release fertilizer), 3.0 kg/m^3 of dolomitic lime, and 0.9 kg/m^3 of Micromax™. An additional 140 containers were filled with the same media on May 19 (for postemergence treatments) and June 10 (for preemergence treatments), and three yellow nutsedge tubers were planted 2.5 cm deep in each container. All containers were irrigated to allow settling. On June 10, using a CO_2 backpack sprayer calibrated to deliver a rate of 187 L/ha, 12 herbicide treatments (3 pre- and 9 postemergence) were applied over the top of nutsedge and pine seedlings, while two control treatments (1 pre- and 1 postemergence) received no herbicide (Table 7.1). Each of the 14 herbicide treatments consisted of 10 replications (containers). Nutsedge receiving postemergence herbicide treatments were about 20 cm tall. All plants were maintained under daily irrigation in full sun for the duration of the study.

Yellow nutsedge and pine seedling injury ratings were made using a scale from 1 to 10 (1 = no injury; 10 = dead). Ratings of 2-9 reflected a progressive increase in the amount of either chlorotic or brown foliage. Nutsedge injury was recorded 15, 30, and 60 days after treatment (DAT), and foliage was clipped on day 60 to measure fresh weights (g). At 90 DAT, nutsedge was clipped a second time to measure the fresh weight of re-growth. Nutsedge plants treated with dimethenamid were not rated, but weed control was assessed based on the fresh weight of foliage.

Slash and loblolly pine injury was recorded 15, 30, 60, and 90 DAT, and diameters (mm) and heights (cm) were measured prior to herbicide application and after 90 days. In May 2009 (329 DAT), the number of leaders and the lengths of the three tallest leaders (cm) were measured.

A.3.2. Trial 2

The herbicide experiment was repeated in August 2008 using 140 loblolly and 140 slash pine seedlings that were potted in February 2008. An additional 140 containers were filled with the same media on August 8 (for postemergence treatments) and August 28 (for preemergence treatments) and planted with three nutsedge tubers. Materials and methods were similar to the first trial with the following exception. Fresh weights of nutsedge foliage 60 DAT and of re-growth 90 DAT were not recorded. The nutsedge trial (without pine seedlings) was repeated a third time in September 2009 using similar methods, and injury ratings 15 and 30 DAT and foliage fresh weights 60 DAT were recorded.

Analyses were conducted using a General Linear Model (GLM) in SAS statistical software (9^{th} ed., SAS Institute, Cary, NC). Means of each dependant variable were analyzed using analysis of variance (ANOVA) in a factorial design ($\alpha = 0.05$). Contrast analyses were performed using combined rates of each herbicide treatment. Data for yellow nutsedge, loblolly pine, and slash pine were analyzed separately.

Table A.1. Yellow nutsedge and loblolly and slash pine herbicide treatments.

Herbicide	Active Ingredient	Rate	Active Ingredient (g/ha)
Tower®	dimethenamid	1X	1120
		2X	2240
		4X	4480
Callisto®	mesotrione	1X	108
		2X	215
		4X	430
Valent (V10142)	imazosulfuron	1X	841
		2X	1682
		4X	3364
Sedgehammer®	halosulfuron	1X	35
		2X	70
		4X	140
Control-pre	———	0X	———
Control-post	———	0X	———

A.4. Results

A.4.1. Yellow Nutsedge

Foliar injury from mesotrione, imazosulfuron, and halosulfuron treatments were similar at 15 DAT, but at 30 and 60 DAT, applications of imazosulfuron caused the most injury with a rating of 9.8 (Table 7.2) (Figure 7.1). Imazosulfuron killed 26 of 30 plants (data not shown). Foliage fresh weight of treated plants was 97% less than non-treated controls 60 DAT and little new growth (< 1 g) occurred on imazosulfuron-treated plants at 90 DAT (Table 7.2). Imazosulfuron controlled nutsedge in trials 2 and 3 with injury ≥ 6.7 at 30 DAT and foliage fresh weight 71% less than non-treated controls (Table 7.3).

Halosulfuron provided good control of nutsedge but less than with imazosulfuron. At 60 DAT, halosulfuron injury was 8.7 (Table 7.2) and 12 of 30 plants were killed (data not shown). Foliage fresh weight was 95% and 92% less than control plants at 60 and 90 DAT, respectively, which was similar to imazosulfuron treatments (Table 7.2). When repeated, halosulfuron controlled nutsedge similarly to the first trial (Table 7.3).

At 15 DAT, injury from mesotrione was similar to halosulfuron and imazosulfuron with an average rating of 4.3 (Table 7.2). However, at 30 and 60 DAT, nutsedge injury was greater with imazosulfuron and halosulfuron, while injury with mesotrione was less (Table 7.2). Mesotrione reduced nutsedge foliage by 60% and 68% at 60 and 90 DAT, respectively (Table 7.2). When repeated, similar trends were observed for mesotrione injury at 15 and 30 DAT, but foliage fresh weight increased by 10% (Table 7.3).

Dimethenamid reduced nutsedge foliage by 45% and 37% at 60 and 90 DAT, respectively (Table 7.2). Suppression of nutsedge foliage was better with dimethenamid

60 DAT compared to mesotrione but had the most re-growth 90 DAT than all postemergence treatments (Table 7.2).

Significant herbicide by rate interactions occurred with injury ratings at 15 and 60 DAT and with foliage fresh weights at 60 DAT. The 2X and 4X rates of imazosulfuron and 2X rate of halosulfuron caused more nutsedge injury at 15 DAT than the 1X rates (Table 7.4). Nutsedge injury was similar among all rates of mesotrione 15 DAT, but the 4X rate caused more injury 60 DAT (Table 7.4).

At 60 DAT, the 2X and 4X rates of dimethenamid reduced foliage fresh weights by 36% and 79%, respectively, while the same rates of mesotrione reduced fresh weights by 60% and 81%, respectively (Table 7.4). Reductions of nutsedge foliage were similar among all three rates of imazosulfuron and halosulfuron (Table 7.4). The 1X rates of imazosulfuron, halosulfuron, mesotrione, and dimethenamid reduced foliage fresh weights by 97%, 92%, 38%, and 20% respectively (Table 7.4).

Table A.2. Yellow nutsedge injury ratings and foliage fresh weights in Trial 1.

	Injury Rating[z]			Fresh Weight (g)	
Herbicide	15 DAT[y]	30 DAT	60 DAT	60 DAT	90 DAT[x]
Control-pre	———	———	———	183.6	24.3
Dimethenamid-pre	———	———	———	100.7	15.2
Mesotrione-post	4.3	4.4	4.1	144.4	6.4
Imazosulfuron-post	4.1	7.6	9.8	9.9	0.3
Halosulfuron-post	3.9	6.6	8.7	19.1	1.7
Control-post	1.0	1.1	1.6	359.4	20.1
LSD[w]	*(0.5)*	*(0.6)*	*(0.9)*	*(40.5)*	*(6.5)*
Rate					
0X	1.0	1.1	1.6	271.5	22.2
1X	3.7	5.6	6.8	102.3	7.6
2X	4.3	6.4	7.9	69.8	5.0
4X	4.2	6.5	8.0	33.5	5.1
LSD	*(0.5)*	*(0.6)*	*(0.9)*	*(30.3)*	*(4.9)*
Main Effects			P < F		
Herbicide	0.0001	0.0001	0.0001	0.0001	0.0001
Rate	0.0174	0.0006	0.0012	0.0001	0.4011
Herbicide*Rate	0.0133	0.5535	0.0384	0.0014	0.5048
Control-pre vs dimethenamid	———	———	———	0.0015	0.0135
Control-post vs mesotrione	0.0001	0.0001	0.0001	0.0001	0.0002
Control-post vs imazosulfuron	0.0001	0.0001	0.0001	0.0001	0.0001
Control-post vs halosulfuron	0.0001	0.0001	0.0001	0.0001	0.0001

[z] = herbicide injury ratings on 1-10 scale (1 = no injury, 10 = dead)
[y] = days after treatment on June 10, 2008
[x] = fresh weight of re-growth
[w] = least significant difference ($\alpha = 0.05$)

Table A.3. Yellow nutsedge injury ratings and foliage fresh weights for Trials 2 and 3.

	Repeat-2008		Repeat-2009		
	Injury Rating[z]		Injury Rating		Fresh Weight (g)
Herbicide	15 DAT[y]	30 DAT	15 DAT[x]	30 DAT	60 DAT
Dimethenamid-pre	———	———	———	———	———
Mesotrione-post	2.4	2.5	1.8	1.9	99.0
Imazosulfuron-post	5.6	8.1	3.3	6.7	25.6
Halosulfuron-post	4.6	7.0	2.7	5.3	35.2
Control-post	1.1	1.0	1.0	1.0	88.9
LSD[z]	*(0.7)*	*(0.6)*	*(0.7)*	*(0.9)*	*(29.4)*
Rate					
0X	1.1	1.0	1.0	1.0	88.9
1X	4.0	5.4	2.6	4.1	42.4
2X	3.8	5.4	2.5	4.6	37.0
4X	4.8	6.8	2.8	5.3	40.5
LSD	*(0.7)*	*(0.6)*	*(0.7)*	*(0.9)*	*(22.0)*
Main Effects			P > F		
Herbicide	0.0001	0.0001	0.0001	0.0001	0.0001
Rate	0.0040	0.0001	0.4359	0.0044	0.8802
Herbicide*Rate	0.0056	0.0051	0.3720	0.2010	0.8746
Control-pre vs dimethenamid	———	———	———	———	0.0023
Control-post vs mesotrione	0.0042	0.0010	0.0365	0.0921	0.0622
Control-post vs imazosulfuron	0.0001	0.0001	0.0001	0.0001	0.0001
Control-post vs halosulfuron	0.0001	0.0001	0.0021	0.0001	0.0001

[z] = herbicide injury ratings on 1-10 scale (1 = no injury, 10 = dead)
[y] = days after treatment on August 28, 2008
[x] = days after treatment on September 3, 2009
[w] = least significant difference ($\alpha = 0.05$)

Table A.4. Yellow nutsedge herbicide by rate interaction for injury and foliage fresh weights in Trial 1.

		Injury Rating[z]		Fresh Weight (g)
Herbicide	Rate	15 DAT[y]	60 DAT	60 DAT
Dimethenamid-pre	1X	———	———	146.8
	2X	———	———	117.4
	4X	———	———	37.9
Mesotrione-post	1X	4.4	3.0	221.1
	2X	4.5	3.9	143.9
	4X	4.0	5.4	68.4
Imazosulfuron-post	1X	3.3	9.5	12.5
	2X	4.1	10.0	8.4
	4X	4.8	10.0	8.7
Halosulfuron-post	1X	3.4	7.8	28.8
	2X	4.3	9.7	9.7
	4X	3.9	8.7	18.9
Control-pre	0X	———	———	183.6
Control-post	0X	1.0	1.6	359.4
LSD[x]		*(0.8)*	*(1.2)*	*(54.3)*

[z] = herbicide injury ratings on 1-10 scale (1 = no injury, 10 = dead)
[y] = days after treatment on June 10, 2008
[x] = least significant difference ($\alpha = 0.05$)

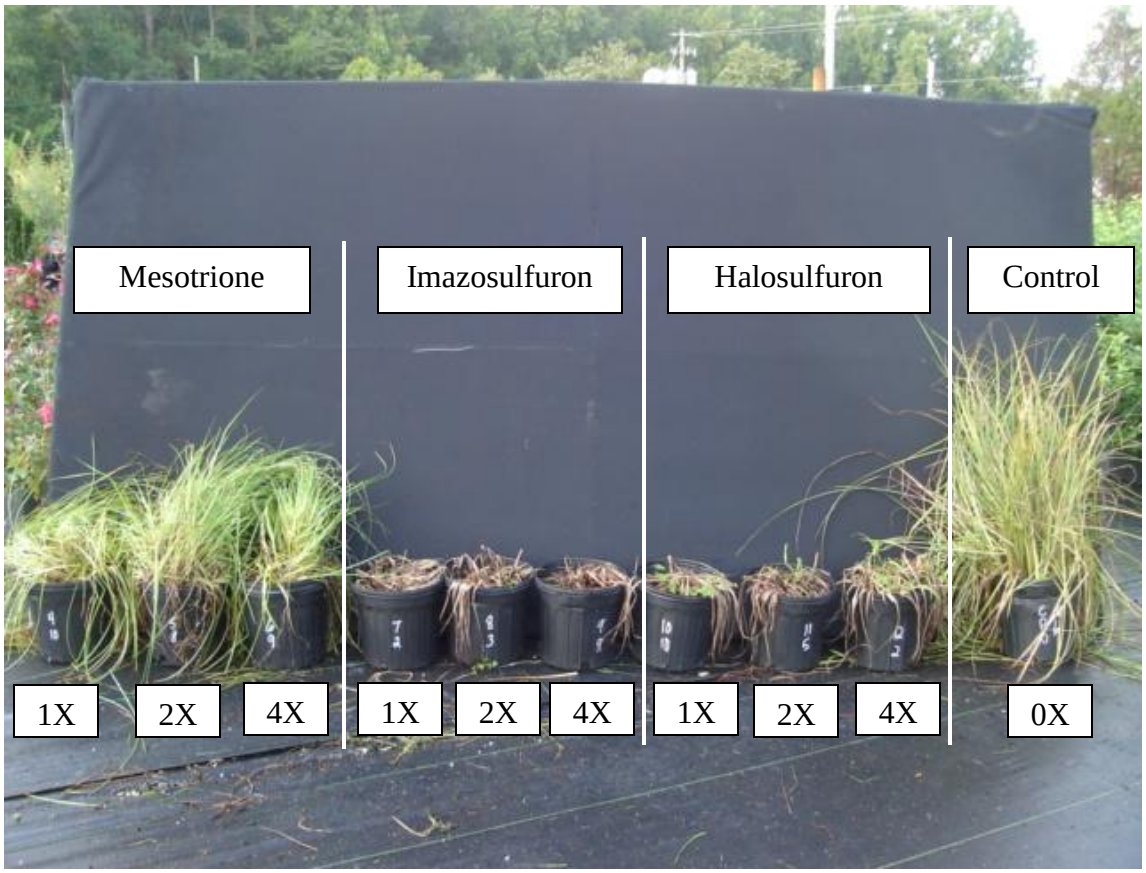

Figure A.1. Yellow nutsedge injury and growth 60 DAT with increasing rates of postemergence herbicides.

A.4.2. Loblolly Pine

Imazosulfuron was the only herbicide that injured loblolly pine. Slight injury was noted at 30 DAT (Table 7.5) as terminal foliage turned light yellow. Imazosulfuron did not affect diameter growth, but seedling height was reduced by 12 cm when compared to non-treated seedlings (Table 7.5). However, despite less height growth, new leaders were longer on imazosulfuron-treated seedlings in the spring (Table 7.5).

In Trial 2, leader lengths were reduced by imazosulfuron, and dimethenamid reduced diameter growth and the number of leaders (Table 7.6). Mesotrione and halosulfuron did not affect loblolly pine diameter and height growth, leader number, or leader lengths (Table 7.6). Foliar injury was not affected by any of the herbicide treatments (Table 7.6).

A.4.3. Slash Pine

Dimethenamid, mesotrione, and imazosulfuron caused slight injury to slash pine at 15 and 30 DAT and reduced diameter growth (Table 7.7). At 60 DAT, slash pine injury for the 1X and 2X rates was similar to that observed on non-treated seedlings (Table 7.7). By 90 DAT, more foliar injury was observed in imazosulfuron-treated seedlings than non-treated slash pine (Table 7.7). All postemergence treatments reduced slash pine height growth with the greatest reduction in imazosulfuron-treated seedlings (> 24 cm) (Table 7.7). In addition, imazosulfuron-treated seedlings had fewer leaders and less growth than non-treated seedling leaders the following spring (Table 7.7). Halosulfuron only reduced the number of leaders and did not cause injury or affect diameter and height growth (Table7.7).

Significant herbicide by rate interactions occurred with slash pine injury at 15, 60, and 90 DAT and seedling diameter, height, and leader growth. When applied at the 4X rate, dimethenamid caused injury 15 DAT, while foliar injury from imazosulfuron was highest at 15 and 90 DAT (Table 7.8). The injury from the 4X rate of imazosulfuron at 90 DAT was triple the amount of injury recorded at the 1X rate (Table 7.8).

Applications of dimethenamid at the 4X rate reduced slash pine diameter and leader length, while the 4X rate of mesotrione reduced diameter and height growth (Table 7.8). The 4X rate of imazosulfuron caused the largest decrease in diameter, height, and leader growth with reductions of 47%, 76%, and 84%, respectively, when compared to control seedlings (Table 7.8). Halosulfuron caused no foliar injury with any treatment, but height growth was reduced by all three rates and leader lengths by the 2X rate (Table 7.8).

In Trial 2, mesotrione was the only herbicide that injured slash pine at 15 and 30 DAT (Table 7.9). Herbicide treatments did not affect diameter, height, and leader growth, and all treatment effects were similar (Table 7.9).

Table A.5. Loblolly pine injury ratings, growth, and spring flush measurements in Trial 1.

	Injury Ratings[z]				Growth		Spring Flush	
	15	30	60	90	Diameter (mm)	Height (cm)	Leader (#)	Leader (cm)
Herbicide	DAT[y]	DAT	DAT	DAT	90 DAT	90 DAT	329 DAT	329 DAT
Control-pre	1.0	1.1	1.0	1.0	9.2	48.7	5.0	45.4
dimethenamid-pre	1.1	1.2	1.0*	1.0	8.2	50.8	4.7	49.8
mesotrione-post	1.2	1.1	1.0	1.0	9.2	51.2	5.1	51.1
imazosulfuron-post	1.1	1.4	1.0*	1.1	9.6	36.8	5.1	57.5
halosulfuron-post	1.0	1.3	1.0	1.0	9.4	49.3	5.1	51.1
Control-post	1.0	1.0	1.0	1.0	9.6	49.1	4.5	45.6
LSD[x]	*(0.17)*	*(0.24)*	*(0.07)*	*(0.14)*	*(1.0)*	*(7.6)*	*(0.7)*	*(9.4)*
Rate								
0X	1.0	1.1	1.0	1.0	9.4	48.9	4.7	45.5
1X	1.1	1.1	1.0	1.0	9.7	51.1	5.0	52.1
2X	1.0	1.2	1.0*	1.0	9.2	48.1	4.9	53.1
4X	1.1	1.4	1.0*	1.1	8.6	41.4	5.2	52.1
LSD	*(0.13)*	*(0.19)*	*(0.06)*	*(0.11)*	*(0.7)*	*(5.7)*	*(0.5)*	*(7.0)*
Main Effects					P > F			
Herbicide	0.2781	0.0026	0.7541	0.1522	0.0097	0.0001	0.3699	0.0837
Rate	0.1461	0.0043	0.5624	0.0767	0.0051	0.0007	0.3497	0.8827
Herbicide*Rate	0.2610	0.2234	0.2409	0.0201	0.4582	0.2025	0.0219	0.7836
Control-pre vs dimethenamid	0.4921	0.4879	0.4506	1.0000	0.0914	0.5983	0.5215	0.3300
Control-post vs mesotrione	0.0875	0.6436	1.0000	1.0000	0.4623	0.6097	0.1292	0.2534
Control-post vs imazosulfuron	0.3034	0.0031	0.4506	0.1256	0.9801	0.0057	0.1441	0.0153
Control-post vs halosulfuron	0.7311	0.0659	1.0000	1.0000	0.6733	0.9461	0.1254	0.2590

[z] = herbicide injury ratings on 1-10 scale (1 = no injury, 10 = dead); [y] = days after treatment on June 10, 2008

[x] = least significant difference ($\alpha = 0.05$); * = value was rounded down

Table A.6. Loblolly pine injury ratings, growth, and spring flush measurements in Trial 2.

	Injury Ratings[z]				Growth		Spring Flush	
	15	30	60	90	Diameter (mm)	Height (cm)	Leader (#)	Leader (cm)
Herbicide	DAT[y]	DAT	DAT	DAT	90 DAT	90 DAT	252 DAT	252 DAT
Control-pre	1.0	1.0	1.0	1.0	6.7	3.7	5.7	49.1
dimethenamid-pre	1.0*	1.0	1.0	1.0	5.3	-0.6	4.7	50.8
mesotrione-post	1.0	1.0	1.0	1.0	5.3	2.7	5.0	52.8
imazosulfuron-post	1.0*	1.0*	1.0*	1.0*	5.5	3.5	4.6	48.9
halosulfuron-post	1.0	1.0	1.0	1.0	5.6	3.4	5.0	57.1
Control-post	1.0	1.0	1.0	1.0	5.5	5.1	4.9	58.7
LSD[x]	*(0.08)*	*(0.06)*	*(0.06)*	*(0.06)*	*(1.0)*	*(6.5)*	*(0.8)*	*(8.4)*
Rate								
0X	1.0	1.0	1.0	1.0	6.1	4.4	5.3a	53.9a
1X	1.0*	1.0	1.0	1.0	5.5	2.8	4.9a	51.7a
2X	1.0	1.0	1.0	1.0	5.1	2.8	4.8a	54.9a
4X	1.0*	1.0*	1.0*	1.0*	5.7	1.2	4.8a	50.6a
LSD	*(0.06)*	*(0.4)*	*(0.4)*	*(0.04)*	*(0.7)*	*(4.9)*	*(0.6)*	*(6.3)*
Main Effects					P > F			
Herbicide	0.7541	0.5999	0.5999	0.5999	0.1577	0.5177	0.2403	0.0823
Rate	0.5624	0.3150	0.3150	0.3150	0.1363	0.6384	0.7811	0.2966
Herbicide*Rate	0.2409	0.3289	0.3289	0.3289	0.7046	0.2934	0.2258	0.4353
Control-pre vs dimethenamid	0.4506	1.0000	1.0000	1.0000	0.0104	0.2321	0.0355	0.7099
Control-post vs mesotrione	1.0000	1.0000	1.0000	1.0000	0.6728	0.5151	0.8320	0.2057
Control-post vs imazosulfuron	0.4506	0.2847	0.2847	0.2847	0.9547	0.6619	0.4799	0.0365
Control-post vs halosulfuron	1.0000	1.0000	1.0000	1.0000	0.8204	0.6418	0.8875	0.7413

[z] = herbicide injury ratings on 1-10 scale (1 = no injury, 10 = dead); [y] = days after treatment on August 28, 2008
[x]= least significant difference ($\alpha = 0.05$); * = value was rounded down

Table A.7. Slash pine injury ratings, growth, and spring flush measurements in Trial 1.

	Injury Ratings[z]				Growth		Spring Flush	
Herbicide	15 DAT[y]	30 DAT	60 DAT	90 DAT	Diameter (mm) 90 DAT	Height (cm) 90 DAT	Leader (#) 329 DAT	Leader (cm) 329 DAT
Control-pre	1.0	1.1	1.0	1.0	8.3	41.7	4.2	61.3
dimethenamid-pre	1.3	1.8	1.1	1.3	7.2	36.2	3.5	48.4
mesotrione-post	2.0	1.9	1.1	1.1	6.3	32.0	3.8	55.3
imazosulfuron-post	1.4	1.6	1.3	1.9	7.2	23.6	2.6	31.9
halosulfuron-post	1.1	1.3	1.2	1.1	8.5	35.8	3.8	51.8
Control-post	1.0	1.0	1.0	1.0	9.0	47.4	5.2	63.6
LSD[x]	*(0.28)*	*(0.39)*	*(0.30)*	*(0.49)*	*(1.1)*	*(6.8)*	*(1.0)*	*(12.5)*
Rate								
0X	1.0	1.1	1.0	1.0	8.7	44.6	4.7	62.4
1X	1.4	1.5	1.1	1.1	8.1	35.7	4.0	57.7
2X	1.4	1.5	1.1	1.2	7.9	34.1	3.3	45.5
4X	1.7	2.1	1.3	1.9	6.0	26.1	3.0	37.4
LSD	*(0.21)*	*(0.29)*	*(0.23)*	*(0.37)*	*(0.8)*	*(5.1)*	*(0.8)*	*(9.4)*
Main Effects					P > F			
Herbicide	0.0001z	0.0001	0.3913	0.0001	0.0001	0.0001	0.0003	0.0001
Rate	0.0032	0.0001	0.0371	0.0001	0.0001	0.0001	0.0177	0.0001
Herbicide*Rate	0.0216	0.0010	0.4500	0.0001	0.0079	0.0050	0.1823	0.0107
Control-pre vs dimethenamid	0.0497	0.0042	0.5551	0.3725	0.1059	0.1869	0.2172	0.0993
Control-post vs mesotrione	0.0001	0.0003	0.6939	0.8427	0.0002	0.0004	0.0229	0.3079
Control-post vs imazosulfuron	0.0189	0.0131	0.1171	0.0062	0.0117	0.0001	0.0001	0.0001
Control-post vs halosulfuron	0.5533	0.2355	0.3259	0.7659	0.4995	0.0063	0.0168	0.1318

[z] = herbicide injury ratings on 1-10 scale (1 = no injury, 10 = dead)
[y] = days after treatment on June 10, 2008
[x] = least significant difference ($\alpha = 0.05$)

Table A.8. Slash pine herbicide by rate interaction for seedling injury, growth, and spring flush growth in Trial 1.

		Injury Rating[z]			Growth		Spring Flush
		15	60	90	Diameter (mm)	Height (cm)	Leader (cm)
Herbicide	Rate	DAT[y]	DAT	DAT	90 DAT	90 DAT	329 DAT
dimethenamid-pre	1X	1.2	1.0	1.1	8.3	39.3	61.1
	2X	1.1	1.0	1.0	7.6	37.9	50.6
	4X	1.7	1.3	1.8	5.5	31.3	33.5
mesotrione-post	1X	2.1	1.1	1.1	6.7	32.2	57.6
	2X	1.9	1.0	1.0	7.1	38.9	55.3
	4X	2.0	1.1	1.1	5.1	25.6	53.3
imazosulfuron-post	1X	1.1	1.0	1.0	8.6	35.6	52.0
	2X	1.3	1.2	1.3	8.4	23.8	33.9
	4X	1.8	1.6	3.5	4.8	11.5	10.0
halosulfuron-post	1X	1.0	1.1	1.0	8.9	35.8	60.1
	2X	1.2	1.2	1.3	8.3	35.3	42.1
	4X	1.1	1.2	1.0	8.5	36.3	53.3
Control-pre	0X	1.0	1.0	1.0	8.3	41.7	61.3
Control-post	0X	1.0	1.0	1.0	9.0	47.4	63.0
LSD[x]		*(0.38)*	*(0.42)*	*(0.66)*	*(1.4)*	*(9.2)*	*(17.0)*

[z] = herbicide injury ratings on 1-10 scale (1 = no injury, 10 = dead)
[y] = days after treatment on June 10, 2008
[x] = least significant difference ($\alpha = 0.05$)

Table A.9. Slash pine injury ratings, growth, and spring flush measurements in Trial 2.

	Injury Ratings[z]				Growth		Spring Flush	
Herbicide	15 DAT[y]	30 DAT	60 DAT	90 DAT	Diameter (mm) 90 DAT	Height (cm) 90 DAT	Leader (#) 252 DAT	Leader (cm) 252 DAT
Control-pre	1.0	1.0	1.0	1.0	4.9	7.4	4.3	60.1
dimethenamid-pre	1.0	1.0	1.0	1.0	4.3	6.0	3.8	52.0
mesotrione-post	1.3	1.2	1.0*	1.0	4.7	5.3	4.3	55.1
imazosulfuron-post	1.0	1.0	1.0	1.0	5.8	4.0	4.5	59.1
halosulfuron-post	1.0	1.0	1.0	1.0	4.9	4.8	4.2	57.4
Control-post	1.0	1.0	1.0	1.0	5.1	8.0	3.9	52.5
LSD[x]	*(0.15)*	*(0.13)*	*(0.06)*	*(0.06)*	*(1.0)*	*(4.1)*	*(0.8)*	*(9.9)*
Rate								
0X	1.0	1.0	1.0	1.0	5.0	7.7	4.1	56.3
1X	1.1	1.0	1.0*	1.0*	5.3	5.0	4.5	60.9
2X	1.1	1.1	1.0	1.0	4.8	3.9	4.2	53.4
4X	1.1	1.1	1.0	1.0	4.5	6.2	4.0	53.5
LSD	*(0.11)*	*(0.09)*	*(0.04)*	*(0.04)*	*(0.8)*	*(3.0)*	*(0.6)*	*(7.4)*
Main Effects					P > F			
Herbicide	0.0001	0.0003	0.5999	0.5999	0.0131	0.4147	0.3573	0.4167
Rate	0.6158	0.5074	0.3150	0.3150	0.0682	0.2356	0.2500	0.0427
Herbicide*Rate	0.4834	0.6641	0.3289	0.3289	0.9205	0.7697	0.7254	0.1997
Control-pre vs dimethenamid	0.6859	1.0000	1.0000	1.0000	0.2387	0.5307	0.2875	0.1536
Control-post vs mesotrione	0.0015	0.0045	0.2847	0.2847	0.4463	0.2248	0.3616	0.6443
Control-post vs imazosulfuron	1.0000	1.0000	1.0000	1.0000	0.2387	0.0725	0.1721	0.2439
Control-post vs halosulfuron	1.0000	1.0000	1.0000	1.0000	0.7052	0.1554	0.4469	0.3900

[z] = herbicide injury ratings on 1-10 scale (1 = no injury, 10 = dead)
[y] = days after treatment on August 28, 2008
[x] = least significant difference ($\alpha = 0.05$)
* = value was rounded down

A.5. Discussion

Halosulfuron and imazosulfuron killed yellow nutsedge, but halosulfuron did so without injuring loblolly pine. Therefore, over-the-top applications of halosulfuron at 35 to 140 g ai/ha to large loblolly pine (i.e. ≥ 8 mm in diameter and ≥ 60 cm tall) can control yellow nutsedge in this container system. Imazosulfuron provided slightly better control of yellow nutsedge but it injured both loblolly and slash pine seedlings. However, when the study was repeated, imazosulfuron did not injure pine seedlings. Greater seedling tolerance may have been influenced by seedling size. In Trial 2, imazosulfuron-treated loblolly and slash pine seedlings were taller and had larger diameters than seedlings treated in Trial 1 (data not shown). Applying herbicides to smaller and younger seedlings increases the chance of seedling injury, which has been shown to be dependent on the herbicide used, species, and environmental conditions at the time of application (South 1984b).

Other studies have shown variable responses with different rates of halosulfuron on bareroot pine seedlings. South (1997a) reported that 8-week-old loblolly and slash pine did not exhibit injury or growth reductions with applications of halosulfuron at 30 g ai/ha. However, in another trial he reported reductions in root collar diameter, height, and biomass with the same rate of halosulfuron (South and Hill 2007). Addition of a surfactant (0.25% v/v) to halosulfuron applied at 69 g ai/ha reduced 8-week-old bareroot loblolly and slash pine growth (McNabb and Bergstrom 1999), whereas loblolly pine was not affected with a lower rate (15 g ai/ha) plus a surfactant (0.5%) (South and Hill 2007). Further testing of halosulfuron (with and without surfactants) at 35 to 140 g ai/ha are needed as over-the-top applications on younger loblolly and slash pine seedlings in

bareroot nurseries before this compound can be used operationally to control yellow nutsedge.

South and Hill (2009) reported that treatments with imazosulfuron reduced seed efficiency (number of plantable seedlings per pure live seed) as well as survival of 8-week-old bareroot loblolly pine seedlings. Their study, along with these results, indicates that it would not be advantageous to use imazosulfuron over-the-top of loblolly and slash pine seedlings. Doing so may hinder the nursery manager's objective of producing quality pine seedlings.

Nutsedge growth was reduced by dimethenamid and mesotrione, but control was not as effective as with either halosulfuron or imazosulfuron. In addition, both dimethenamid and mesotrione injured slash pine. Dimethenamid has caused reductions in 8-week-old loblolly pine height and diameter on a sandy site with low organic matter (South and Hill 2005). These results indicate that dimethenamid and mesotrione are marginal for controlling nutsedge in nurseries but could be used over-the-top when loblolly pines are grown in this container system. The results with halosulfuron are promising, but further testing on younger pine seedlings is recommended.

A.5.1. Summary

Treatments of halosulfuron suppressed yellow nutsedge and did not injure loblolly pine. Although imazosulfuron treatments suppressed nutsedge better than halosulfuron, this herbicide injured and reduced the growth of pine seedlings. Stunting was not observed when the study was repeated, which may have been due to treating larger seedlings. Applying an over-the-top application of halosulfuron at 35 to 140 g ai/ha to

loblolly pine that are ≥ 8 mm in diameter and ≥ 60 cm tall was the most promising treatment. The null hypothesis that dimethenamid, mesotrione, imazosulfuron, and halosulfuron have no effect on the tolerance level and growth of yellow nutsedge and loblolly and slash pine seedlings was rejected.

A.6 Acknowledgements

The author would like to thank Doug Shelburne at Smurfit Stone's Rock Creek Nursery in Brewton, AL for providing the loblolly and slash pine seedlings. A special thank you goes to Greg Creech, Chris Marble, Anna-Marie Murphy, Albert Van Hoogmoed, David Joyner, Jennifer Snyder, Casey Teel, Patrick Jernigan, Rena Miller, Jesse Creech, Wes Ezzell, Carey Cawthon, B.J. Haisten, Justin Phillips, Cody Keifer, Buck Malone, and Heather Fain for their assistance.

A.7. Literature Cited

Anderson, W.P. 1999. Perennial Weeds: characteristics and identification of selected herbaceous species. Iowa State University Press, Ames, Iowa: 228 p.

Blum, R.R., J. Isgrigg III, and F.H. Yelverton. 2000. Purple (*Cyperus rotundus*) and yellow nutsedge (*C. esculentus*) control in bermudagrass (*Cynodon dactylon*) turf. Weed Technology 14: 357-365.

Boyer, J.N. and D.B. South. 1984. Forest nursery practices in the south. Southern Journal of Applied Forestry 8(2): 67-75.

Carey, W.A. 2000. Fumigation with chloropicrin, metham sodium, and EPTC as replacements for methyl bromide in southern pine nurseries. Southern Journal of Applied Forestry 24(3): 135-139.

Cram, M.M., S.A. Enebak, S.W. Fraedrich, L.D. Dwinell, and S.J. Zarnoch. 2007. Evaluation of fumigants, EPTC herbicide, and *Paenibacillus macerans* in the production of loblolly pine seedlings. Forest Science 53(1): 73-83.

Defelice, M.S. 2002. Yellow nutsedge *Cyperus esculentus* L.-Snack food of the gods. Weed Technology 16: 901-907.

Fraedrich, S.W. and L.D. Dwinell. 2003. Broadcast applications of glyphosate control nutsedge at a South Georgia forest tree nursery. Southern Journal of Applied Forestry 27(3): 176-179.

Haywood, J.D. and T.W. Melder. 1991. Effectiveness of glyphosate mixed with soil-active herbicides. Research Note SO-365, USDA Forest Service, Southern Forest Experiment Station: 5 p.

McNabb, K. and T. Bergstrom. 1999. Application and timing trials with Manage® herbicide. Auburn University Southern Forest Nursery Management Cooperative, Research Report 99-7: 3 p.

Morrica, P., P. Fidente, S. Seccia, and M. Ventriglia. 2002. Degradation of imazosulfuron in different soils-HPLC determination. Biomedical Chromatography 16: 489-494.

Nelson, K.A. and K.A. Renner. 2002. Yellow nutsedge (*Cyperus esculentus*) control and tuber production with glyphosate and ALS-inhibiting herbicides. Weed Technology 16: 512-519.

South, D.B. 1984a. Southern Pine Nursery Handbook, Chapter 15-Weed Control. In: May, J., tech. coord., U.S. Department of Agriculture, Forest Service, Southern Region.

South, D.B. 1984b. Response of loblolly pine and sweetgum seedlings to oxyfluorfen. Canadian Journal of Forest Research. 14: 601-604.

South, D.B. 1997a. Halosulfuron-methyl: a herbicide with activity on nutsedge. Auburn University Southern Forest Nursery Management Cooperative, Technical Note 97-1: 11 p.

South, D.B. 1997b. Fomesafen: A herbicide for pine seedbeds. Southern Journal of Applied Forestry 21(3): 143-145.

South, D.B. and T. Hill 2005. Herbicide trials with flumioxazin and dimethenamid. Auburn University Southern Forest Nursery Management Cooperative, Research Report 05-02: 5 p.

South, D.B. and T. Hill. 2007. Shielded applications of sulfonylurea herbicides in loblolly pine seedbeds: Part III. Auburn University Southern Forest Nursery Management Cooperative, Research Report 07-3: 5 p.

South, D.B., S.A. Enebak, and T.E. Hill. 2007. Tolerance of young loblolly pine (*Pinus taeda*) seedlings to post-emergence applications of MSMA. New Zealand Journal of Forestry 52 (3): 28-35.

South, D.B. and T. Hill. 2009. Preliminary results from testing nutsedge herbicides in loblolly pine seedbeds. Auburn University Southern Forest Nursery Management Cooperative, Research Report 09-2: 4 p.

Wakeley, P.C. 1954. Planting the southern pines. USDA Agricultural Monograph No.18: 233 p.